Microbial fuel cell is emerging as a versatile technology: A review on its possible applications, challenges, and strategies to improve the performances

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SUMMARY

Microbial fuel cells are emerging as a versatile renewable energy technology. This is particularly because of the multidimensional applications of this eco-friendly technology. The technology depends on the electroactive bacteria, popularly known as exoelectrogens to simultaneously produce electric power and treat wastewater. The electrode modifications with nanomaterials such as gold nanoparticles, iron oxide nanoparticles or pre-treatment methods such as sonication and autoclave sterilization have shown promising results to enhance the MFC performance for electricity generation and wastewater treatment. The MFC technology has been also investigated for the removal of various heavy metals and toxic elements, and to detect the presence of toxic elements in wastewater. In addition, the MFCs can be modified into microbial electrolysis cells to generate hydrogen energy from various organic matter. This article provides a comprehensive and state-of-the-art review of possible

applications of the MFC technology. This also points out the various challenges that limit the MFC performance. Finally, this article identifies the strategies to improve MFC performance for different applications.

KEYWORDS: microbial fuel cell; electricity generation; wastewater treatment; bioremediation; biosensor; hydrogen production

1. INTRODUCTION

Depletion of non-renewable energy resources and environmental pollution are critical threats facing us. Extracting energy from organic or inorganic wastes can provide an efficient means to solve the energy and environmental problems simultaneously. Many anaerobic fermentation technologies have been combined with other purification techniques to generate alternative energy fuels such as hydrogen and methane [1-3]. However, a sustainable energy collection must include a diversity of carbon-neutral and renewable energy technologies. Microbial fuel cell (MFC) technology has attracted an increased number of researchers in the recent years due to its potential particularly for bioenergy production and wastewater treatment. This is reflected by the number of articles published in last five years that has increased successively from year to year, as shown in Fig. 1. MFC technology has become an attractive technology today because of its capability to convert the chemical energy present in organic/inorganic wastes into electrical energy. It links microbial metabolism with electrochemical reactions [3-5]. Consequently, the technology can be used for electricity generation, wastewater treatment, bioremediation of heavy metals/toxic compounds and other niche applications. The general principle of an MFC is given in Fig. 2. MFCs are the bioelectrochemical devices that typically consist of two chambers i.e. the anode chamber (anaerobic; contains an electrode, microorganisms and anolyte) and the cathode chamber (aerobic/anaerobic; an electrode, electron acceptor and a catalyst), separated by a proton exchange membrane (PEM) e.g., nafion [6-8]. The microorganisms are used as the biocatalysts to oxidize the substrate in the anode chamber, and have been denoted as the power house of MFCs. The electrons are transferred to the anodic (an electrode) surface, which are then directed to the cathode through an electrical connection [9, 10]. In the cathode, the electrons combine with protons and oxygen to form water. A catalyst e.g., platinum is generally used to catalyse the reduction reaction in the cathode; alternatively, a microorganism can also be used to replace such costly catalyst [11, 12].

The advantage of MFCs mainly lies in the use of microorganisms as the biocatalysts at the anode and the cathode chambers of MFCs. The exceptional characteristic of the microorganisms used in MFCs is their self-potential to mediate the electrons (generated from the oxidation of the substrates) from their outer cell membrane to the surface of an electrode (in anode) and to accept the electrons from the electrode surface (in cathode) to catalyse the reduction of electron acceptors e.g., oxygen reduction [5, 9, 12]. The microorganisms that contain a molecular machinery to transfer the electrons to an electron acceptor without any external assistance or to accept the electrons from the electrode surfaces are usually called as exoelectrogens. Due to this unique characteristic of exoelectrogens the MFC technology has been experimented for a number of applications. The most widely studied application of MFC technology is electricity generation. In the anode chamber of an MFC, the oxidation of organic matter by exoelectrogens results into a low redox potential while in the cathode chamber, reduction of an electron acceptor e.g., oxygen results into a higher redox potential. This difference in the redox potentials drives the electrons to flow from the anode to the cathode, which consequently results in bioelectricity generation. Many different designs have been utilized to produce electric current in various optimized parameters [10-12]. A pure culture (e.g., *G. sulfurreducens* and *Shewanella oneidensis*) or a mixed culture (from anaerobic sludge or primary wastewater) can be used to generate electric current [13-18]. Many attempts have been made to increase the electric output in MFCs. Out of these, anode surface modifications with nanomaterials and bacterial gene modification are the most prevalent approaches that have been employed to improve the MFC performances [19-21, 22]. For example, nitrogen doped carbon nanoparticles were coated on carbon cloth electrodes, which increased the power density more than three times as compared to untreated electrodes [21]. Alternatively, a synthetic flavin biosynthesis pathway from *Bacillus subtilis* was expressed in *S. oneidensis* MR-1, which secreted a very high amount of flavins than the wild type, consequently, increasing the power output ~13 folds as compared to wild *S. oneidensis* [22]. Because bacteria can degrade the organic matter present in the wastewater, the technology can be used to remove the pollutants and generate electricity from wastewater. Several wastewaters ranging from low-strength to high-strength have been utilized in MFCs for their treatment and electricity generation simultaneously [23-28, 29-34]. In addition, MFC can be modified into microbial electrolysis cell (MEC) to produce hydrogen gas, but unlike MFC, electricity is provided in the MEC to produce hydrogen [35]. Generally, a voltage of 0.2 to 0.8 V is required to reduce the protons to form hydrogen [10]. Such low voltage is easily achievable in the MFC. Therefore, an MFC can be used to supply the voltage to the MEC for hydrogen production.

The aim of this review article is to critically analyse the routes of MFC applications and the strategies to improve their performances. Many review articles have been published describing specific aspects of the MFCs such as the substrates used in MFCs [3], assessment of MFC configurations [1], and specific application of MFCs like wastewater treatment [4], and bioremediation [6]. However, that the current review provides a comprehensive understanding of the MFC applications, their basic principles, challenges and the strategies to improve their performances. The primary applications of MFCs i.e., electricity generation, wastewater treatment, bioremediation, biosensors, and hydrogen production have been covered. A special focus has been given to the strategies to improve the MFC performance, making the technology scalable in the real world to compete with commercialized green energy technologies.

2. THE 'MOLECULAR MACHINERY' OF EXOELECTROGENS

It is important to get an idea about the unique characteristic of MFC technology because of which this technology has become the centre of attraction among the renewable technologies. All the applications of MFC technology are particularly interesting because of the molecular machinery of the bacteria that helps in transferring the electrons to an electrode surface and vice-versa. The molecular machinery means the biomolecules, proteins or the genes that help to donate or accept the electrons between bacterial and electrode interface, which chiefly lies between the inner and the outer membrane of the bacteria. So far, only two bacteria namely, *Geobacter spp.* and *Shewanella spp.* have been extensively investigated to explore the extracellular electron transfer (EET) mechanisms. Two types of EET mechanisms have been confirmed in both the bacteria [5]. The first is direct electron transfer (DET) mechanism and the second is mediated electron transfer (MET) mechanism. The molecular machinery comprising the known pathways and hypothetical pathways is presented in Fig. 3.

G. sulfurreducens is the most studied and explored exoelectrogen in MFCs. It forms highly thick biofilms on the electrode surfaces and can utilize the various carbon sources as a substrate for bioenergy production. It has been found that *G. sulfurreducens* in its initial stages of biofilm formation relies on MET for electron transport. The exoelectrogen secretes flavin molecules such as riboflavin in the single layer biofilms. The riboflavin combines with outer membrane c-type cytochromes (OM c-Cyts) to make a complex that furthers the electron transfer to the electrode surface [5,22]. As the biofilm grows *G. sulfurreducens* adapts to DET for extracellular electron transport. In a multi-layered biofilm, *G. sulfurreducens* active adjacent to electrode surface utilizes OM c-Cyts (essentially OmcZ) for extracellular electron transfer while the bacteria respiring distant from the electrode produce conductive nanowires (type IV pili) that assist in transporting the electrons inside the biofilm and finally onto the electrode surface [5].

The other exoelectrogen studied extensively for MFC applications is *Shewanella oneidensis.* The bacterium is the most versatile exoelectrogen in the MFCs because it exhibits the potential to reduce a variety of electron acceptors [36, 37]. Earlier *S. oneidensis* MR-1 was thought to produce conductive nanowires like type IV pili of *G. sulfurreducens.* But it is now confirmed that *S. oneidensis* does not contain nanowires and these nanowires like structures are the extensions of periplasmic and outer membrane multiheme cytochromes associated with outer membrane vesicles [38]. This exoelectrogen secretes mainly two types of flavin molecules. The first is riboflavin (RF) and the second is flavin mononucleotide (FMN). These flavin molecules act as cofactors for the cytochromes such as OmcA and MtrC. It has been found that RF acts as a cofactor for OmcA while FMN contains the binding sites for MtrC. [39]. These complexes, RF-OmcA and FMN-MtrC further promote the electron transfer to the electrode surfaces [39]. The various known proteins or genes from different exoelectrogens involved in EET mechanisms are depicted in Fig. 3. To date, some proteins or genes are well known to participate in EET mechanisms that function in a specific pathway. However, the functional role of other proteins/genes in EET mechanisms is still under debate and demands a deep investigation to validate their role and ability to mediate the electrons transfer.

3. MFCs FOR ELECTRICITY GENERATION

The MFCs are chiefly used for the application of electric current generation and many efforts have been made to ameliorate the current density such as electrode modifications, MFCs designs, use of metal catalysts at the anode as well as at the cathode etc. [1, 6, 8, 9, 12]. Recent studies reporting high current densities even from reactors as small as 14 ml are encouraging [2]. Evidently, Bruce E. Logan and his colleagues at The Pennsylvania State University, United States America (USA) successfully ran a small fan using an MFC with a working volume of two litres, (http://www.engr.psu.edu/mfccam/). If a two litre - MFC can run a small fan, then we can conceptually expect higher current output from an MFC of higher volume capacity e.g., of 2000 litres or even more. But it is unlikely to be materialized in the near future because of obstacles including very high cost of the materials used in MFCs (electrodes, PEM), high internal resistance, costly catalysts (e.g., platinum) used in cathode for oxygen reduction, and limited availability of exoelectrogens in the environment. However, researchers from all around the world continue to contribute to the technology to make it a viable alternative for renewable energy generation.

The first step in MFCs towards current generation is the acclimatization of the exoelectrogens in the anode chamber and subsequent biofilm formation on the electrode surface (anode). Consequently, the exoelectrogens form a conductive biofilm on the anode surface. The biofilm thickness may be a few tens of micrometre, for example, ~30 µm or ~50 µm [36, 37]. Biofilm formation by exoelectrogens is a unique characteristic and differs from other bacteria or microorganisms. The development of biofilm on the electrode surface from the single bacterial cell is stimulated by the assembly of adhesins and extracellular matrix components [38, 39]. Later, some pivotal proteins specifically pili and outer membrane c-type cytochromes (OMC c-Cyts) e.g., OmcZ, OmcS etc. also promote the biofilm formation

[40, 41]. *Geobacter sulfurreducens* is unable to form biofilm in the absence of pili and OMC c-Cyts [40]. The formation of thick biofilm is taken as an important parameter in MFCs for efficient performance. Usually, optimal biofilm thickness is preferred in MFCs for higher current densities, as highly thick biofilms also confine the electron passage [41]. In addition, the selection of suitable bacterial inoculum (pure culture of mixed culture) with preferred substrate can be highly beneficial to extract more energy for the current generation. For example, *Geobacter sulfurreducens* can reduce acetate with ~100% electron recovery to generate electricity [42].

After the establishment of a suitable biofilm, the exoelectrogens transfer the metabolically generated electrons from their outer cell membrane to the anode surface. There are two known electron transfer mechanisms i.e. direct electron transfer (DET) and mediated electron transfer (MET), which have been observed in case of *Geobacter* species and *Shewanella* species [43-45]. In *Geobacter sulfurreducens*, DET involves OMC c-Cyts (e.g., OmcZ, OmcB) for the short-range electron transfer during the initial development of biofilms and pili (type IV) for long-range electron transfer in multilayer biofilms [17, 19]. In MET process flavin molecules such as riboflavin (RF) plays a key role in electron transfers [46]. In *Shewanella oneidensis*, the complex of cytochromes-flavins mediates the exocellular electron transfer mechanism. For example, flavin mononucleotide (FMN) acts as a cofactor for cytochrome MtrC and RF for cytochrome OmcA [47].

The transferred electrons on the anode surface are transported to the cathode surface via an electrical connection. The electrons at the cathode surface react with protons and an electron acceptor. If the electron acceptor is oxygen the end product will be water, resulting maximum open circuit voltage (OCV) at the cathode of ca. 0.805 V. Generally, the cathode surface is bound with a catalyst to increase the oxygen reduction rate. The most commonly used catalyst is platinum [1]. The carbon/platinum electrodes are commercially available with different concentrations of platinum e.g., carbon cloth with 0.2 mg/cm^2, 0.5 mg/cm^2. Alternatively, a microorganism can also be used for the oxygen reduction to make the fuel cell more cost-effective. Electron acceptors other than oxygen, such as ferricyanide, potassium permanganate are also useful alternatives [10].

The selection of exoelectrogens, substrate (electron donor), and the final electron acceptor are the pivotal factors in MFC technology. Different MFCs have used pure cultures as wells as mixed cultures for bioelectricity generation. Some examples of the MFC studies with pure cultures and mixed cultures are given in Table 1 and Table 2, respectively. The performance of similar MFCs with different inoculum can be compared to find which inoculum is more favourable to generate high power density. Some studies report that mixed cultures produce high power density than pure cultures [5]. However, a few other studies showed that pure cultures can also generate high current [40]. For example, in a continuous flow ministack MFC using carbon cloth for both the electrodes, fed with acetate, *G. sulfurreducens* produced higher power density than the mixed cultures using a similar reactor and operational conditions [40]. The study achieved a maximum power density of 1900 mW/m^2, which was approximately 21% more than the mixed cultures (sewage sludge inoculum) [40]. The selection of the inoculum in a particular growth phase (exponential phase) is also useful to attain high current in MFCs. It has been found that the bacteria in lag phase form thin biofilms and contain fewer amounts of c-type cytochromes while the bacteria in exponential phase form thicker biofilms and contain the higher number of c-type cytochromes, consequently generating higher electrical current [48]. Moreover, a selective inoculum of mixed culture referred as controlled inoculum (of known bacteria e.g. *Pseudomonas aeruginosa*, *Azospira oryzae*, *Acetobacter peroxydans* and *Solimonas* *variicoloris*) has shown to produce a higher power density than unknown inoculum [49]. A study from our group revealed that such controlled inoculum can produce 100% more power than anaerobic sludge (inoculum) in a double chamber MFC [49]. Further, some pre-treatment methods of inoculum can also be employed to enhance the power output in the MFCs [5].

The microbial community structure in an MFC is affected by the type of substrates used in the anode chamber, which could be simple substrates that are easily fermentable or complex substrates that are non-fermentable [3]. For example, acetate is commonly used in MFCs, and the exoelectrogens such as *Geobacter and Shewanella* spp. readily use acetate for electricity production [5]. Therefore, the abundant availability of acetate in the anode can exclude the effect of other fermentable bacteria. But wastewaters may contain simple as well as complex organic contents. Hence, pre-acclimation strategies can be employed to hydrolyse and ferment the wastewaters. For example, three pre-acclimation strategies were employed to evaluate the response of microbial community for electricity generation in an air-cathode MFC inoculated with anaerobic sludge from domestic wastewater [50]. In the first strategy, the MFC was pre-acclimated with glucose and acetate; in the second, with glucose before adding domestic wastewater and in the third strategy, the wastewater was directly used without any pre-acclimation [50]. The results revealed a great variation in the microbial community due to the pre-acclimation strategies. The MFC with first strategy was abundant with bacteria belonging to phylum *Chloroflexi* and genus *Gemmobacter* while the MFC pre-acclimated with second strategy contained predominantly *Enterobacter* and *Escherichia*. On the other hand, the MFC with third strategy was dominant with *Dechloromonas* and *Anaerolinaceae*. Moreover, the MFC with first strategy generated maximum current density and achieved maximum COD removal as compared to the other MFCs [50].

The researchers engaged in the MFC studies around the globe have endeavoured many innovative efforts to increase the power output of the fuel cells. Many of them are developing new MFC designs using different effective materials for the electrodes and membrane, operating MFCs at specific conditions (e.g., setting electrode potentials, maintaining pH of the electrolytes, pre-treatment of membranes and electrodes), treatment of the inoculum, and nanomodification of the electrodes. Some methods used to increase the electricity generation in the MFCs are discussed in the following section.

Electrode modification with metal catalyst or nanoparticles or chemical treatment has become a new trend to improve the performance of MFCs. The main purpose to modify the electrodes in MFCs is to increase the power outputs, in the anode by providing high surface area for the biofilm formation and to increase the exocellular electron transfer (EET) mechanisms. The cathode modifications are the centre of attraction to replace the highly costly platinum catalyst by cheaper catalysts of nearly or same catalytic properties [12]. Most of the studies regarding electrode modifications also claimed to decrease the internal resistance of the system as well as start-up time of the reactor. In the anode, different approaches have been employed to modify the electrodes to increase the power outputs either by simple modification methods such as heat-treated electrodes and nitrogen-doped electrodes or by some sophisticated tools such as by coating some highly effective catalysts (e.g., gold nanoparticles, graphene, carbon nanotubes (CNT) etc.) on the electrodes [51-55]. Interestingly, almost every kind of metal nanoparticles or other carbon nanoparticles have been used in the MFCs. Therefore, the researchers are now using electrode with different composite materials (e.g., CNT–gold–titania nanocomposites) to improve the performance [53]. Another effective method includes the use of nitrogen doped carbon nanoparticles to modify the electrode to enhance the EET mechanism. For example, nitrogen doped carbon nanoparticles were coated on carbon cloth electrodes in a two-chamber MFC inoculated with *Shewanella oneidensis* MR-1. The study revealed that the treated electrodes absorbed more electron mediators (flavins) secreted by the organism that subsequently increased the electron transfer rate. Consequently, the power density also increased more than three times as compared to untreated electrodes [55]. The anode can also be modified with metal or non-metal nanoparticles (with different morphologies as well) to influence the EET and thus the performance of the MFCs. In a study, CNT powder was directly added to the anode chamber to increase the biofilm growth of *G. sulfurreducens* in a two-chamber MFC using plain carbon paper as the electrode material in both the chambers [52]. The addition of CNT powder in the anode chamber reduced the internal resistance of the system as well as the start-up time of the MFC. The shortened start-up time could be attributed to the promotion of the bacterial adhesion to the electrode material with the addition of CNT powder in the anode chamber [52]. The performance of the anode can be further improved by using different morphologies of the material that can provide more active sites and enhance biocompatibility with the electrode material. In a double chamber MFC, the anode (carbon cloth) was modified with bamboo-like carbon nanotubes that produced ca. four times higher power density than the MFC using plain carbon cloth as the anode [52].

It is evident that Fe (III) oxide exhibits high affinity for c-type cytochromes such as OmcA and MtrC present on the outer surface of *Shewanella* species [38, 39]. Therefore, it is more favourable for the bacteria to mediate the electrons from its outer surface to Fe (III) oxide. Moreover, it has been also revealed that *Shewanella* species are more attractive to iron oxide surfaces [5]. In other words, iron oxide surfaces enhance the microbial growth and increase the extracellular electron transfer, increasing the biofilm metabolic activity which can be advantageous for improving the performance of MFC-centred applications. For example, Song et al., utilized graphene/ Fe_3O_4 nanocomposites coated carbon paper as the anode electrode to improve the bacterial activity in a two-chamber MFC inoculated with *Shewanella oneidensis* MR-1 [56]. The results showed that the start-up time of the MFC was significantly decreased with increase in Fe_3O_4 concentration, indicating a faster attachment of bacteria onto the anode surface, which can be attributed to the high affinity of outer membrane c-type cytochromes to iron oxide [56]. In addition, the MFC with modified anode achieved a maximum current density of 1800 mA/cm^2, which was ~6 times higher than the bare anode (carbon paper) [56]. In another study, Fe_3O_4-carbon cloth was used as an anode to examine the beverage wastewater treatment and electricity generation [57]. The MFC produced a maximum current density that was 100% higher than the bare cathode and a COD reduction of ~52% was achieved [57]. The iron oxide layers can be prepared on the electrode surfaces to make them more biocompatible for enhanced microbial growth and functions. For example, stainless steel electrodes can be heat-treated to generate a layer of iron oxide on its surface. Evidently, Guo et al., prepared heat-treated stainless steel electrodes which generated a layer of iron oxide as confirmed by X-ray photoelectron spectroscopy [58]. This modification further improved the biofilm formation and enhanced the extracellular electron transfer as expected. Consequently, the current density was significantly increased. The MFC generated a maximum current density of 1.5 mA/cm^2, which was seven times higher than the bare electrode [58]. Previously, stainless steel mesh was modified with flame synthesis of carbon nanostructures on its surface, which increased its BET surface by 300 times as compared to the bare stainless steel mesh electrode. The microscopy results revealed that the addition of carbon nanostructures onto stainless steel mesh enhanced the biofilm formation. As a result, the MFC with modified anode produced a power density of 187 mW/m^2, which was 60 times higher than the bare anode [59].

The cathode modification is chiefly focused on to replace the platinum by some other cost-effective catalysts [12, 60-62]. Cobalt oxide and manganese oxide have shown the potential to substitute the platinum in MFCs. Specifically, cobalt oxide (with other materials e.g., iron phthalocyanine or nickel) has been repetitively experimented as a cathode catalyst for oxygen reduction reaction (ORR) [12]. Such MFCs with modified cathode electrode produced effective results but slightly lower than the MFCs with platinum (as cathode catalyst). An MFC using cobalt oxide-iron phthalocyanine as a cathode catalyst for oxygen reduction produced a maximum power density of ca. 655 mW/m^2, which was 37% higher than the MFC with iron phthalocyanine, indicating the effective potential of oxygen reduction activity of cobalt oxide for ORR [61]. In contrast, the MFC with a carnation-like manganese dioxide coated cathode produced 1.5 times higher power density than the plain electrode [62]. Alternatively, some bacteria (pure cultures or even mixed cultures) have also been used as cathode catalyst for oxygen reduction but could not produce satisfactory electric outputs [24]. Moreover, the overpotential obtained for ORR was also higher in the study due to the poor bacterial activity at the cathode, neglecting the choice of biocathode in real large scale MFC applications.

The electricity generated from MFCs can be further used to power electric instruments or machines. MFCs have been successfully applied to operate robots. Such robots are usually termed as "Gastrobots", which means robots with a stomach. These kinds of robots can metabolize the natural food or can be sustained by water or air. These robots digest the substrate fuel and convert it into electricity, which is usually stored in the batteries fitted in the robots, making them an autonomous power system. Evidently, MFCs were utilized to power a robot named as "Gastronome". Gastronome is thought to be the first robot that utilized biomass driven energy conversion technology [63]. Gastronome was built by joining train-like three wheeled wagons, as shown in Fig. 4. A stack of six MFCs was used in the robot and Ni-Cd batteries were utilized, which were charged by the electric output of the MFCs [63]. Ecobot-II is another example of a robot that was completely driven by MFCs for environmental monitoring [64]. A picture of Ecobot-II is shown in Fig. 5. The robot was connected to a wireless transmitter that was further connected to a sensor (which can be for temperature, toxicity, humidity etc.) [64]. In addition, the robot was packed with eight MFCs and utilized raw foodstuffs such as rotten fruits as substrate fuel. The authors also claimed that Ecobot-II was the first robot in the world powered by MFCs that was utilized for environmental monitoring [64]. In an alternative study, the MFCs were successfully used to power wireless sensors to detect the changes in temperature. The diagram of the sensor and telemetry system powered by the MFC is given in Fig. 6. In this study, the MFC was connected with a highly efficient electronic circuitry to provide a stable power for wireless sensor [65]. The electricity produced by the MFC was further stored in a capacitor and was used to power the telemetry system. However, the voltage generated by the system was lower (2.1 V) than needed for a commercial electronic circuit (3.3 V). Therefore, a DC-DC converter was utilized to increase the potential and to power the transmitter that received the data from the sensor and transmitted to the receiver [65]. Further, Tender et al. demonstrated the application of MFC for the first time in the world to power a meteorological buoy [66]. They used benthic type of MFCs and the meteorological buoy to measure air temperature, pressure, relative humidity, and water temperature. The results from this study are shown in Fig. 7.

4. MFCs FOR WASTEWATER TREATMENT

The process of wastewater treatment involves safe disposal or recycling of water which is highly polluted or contains toxic substances. Wastewater discharged from different industries can be particularly hazardous. According to an astounding report by Lux Research, governments and water utilities across the world spent approximate $28 billion in year 2012 to develop their existing wastewater treatment infrastructure that provided a surplus global wastewater treatment capacity of 16.3 million cubic metres (m^3) per day. MFC technology has the potential to provide an effective platform for the treatment of highly polluted industrial wastewater or urban wastewater and can curb the financial expenditure, which can be further used for other development programs of a country.

In the late nineteenth century, Habermann and Pommer (1991) used MFCs for continuous treatment of wastewaters for nearly 5 years [67]. They used sodium sulphate solution (different concentrations (%, 0.5-5) as the electrolyte in the anode, sulphate reducing microorganisms such as *Proteus vulgaris, Escherichia coli, Pseudo-monas aeruoinosa* and *P. fluorescens,* and two types of wastewaters (sewage works effluent and landfill effluent). The results showed that the MFC achieved a COD reduction of 35% with sewage works effluent and 75% with landfill leachate [67]. In addition, a maximum anodic current density of 150 mA/cm^2 at a potential of -50 mV was also obtained in the demonstration [67].

In the later years, different types of wastewaters were used in MFCs for its treatment and bioenergy production [54-60, 68, 69, 70-75]. On one side of the picture, MFC technology can be used to treat the wastewater while on the other side, the wastewater can be used to provide substrate as the carbon source for the bacterial growth and hence for the end products of the oxidation process i.e. electrons and protons for sustainable bioelectricity generation [3]. Primary wastewater from an industry such as chocolate industry wastewater [29] or palm oil mill effluent (POME) [34] can be used to provide the inoculum or the biocatalysts for the substrate oxidation. Moreover, defined bacterial culture (pure or mixed) can be isolated from the wastewater that can be further used as inoculum for the MFCs [5]. The wastewater can be used as catholyte as well though it may contain some minerals that can act as electron acceptors [29]. Though our review is focused on the performance of MFCs for wastewater treatment, the next section of the article reviews some studies that demonstrated the efficiency of MFCs for wastewater treatment and some approaches employed to improve the wastewater treatment efficiency of the MFCs.

The effect of different parameters on MFC performance has been studied. These primarily include chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids, total dissolved solids, acidity etc. Usually, standard methods are adopted to evaluate the wastewater treatment efficiency of the MFCs. Typically, COD test is performed (or is sufficient) to examine the performance of MFC toward wastewater treatment. Some examples of MFC studies demonstrated for wastewater treatment are given in Table 3. The MFCs have achieved up-to 98% COD removal from wastewater [55, 56]. Almost all the studies demonstrated for wastewater treatment are coupled with the foremost application of MFCs i.e. electricity production.

Animal wastewaters contain high organic content and high concentrations of phosphate and nitrate in wastewater, the latter causing eutrophication of surface water. A few studies have demonstrated the use of animal wastewater in different MFCs for its treatment and bioenergy production. A study using swine wastewater in different MFCs (two chambered MFC and single chamber MFC) achieved maximum 92% COD removal and approximately 83% ammonia reduction after operation of the MFC for around 100 hours [26]. Another study treated animal carcass wastewater (ACW) with high organic content in an up-flow tubular MFC [68]. The disposed animal carcasses can be further hydrolysed with alkaline treatment (sodium hydroxide or potassium hydroxide) into smaller constituents like amino acids, sugars and minerals forming a sterile solution referred as ACW (of BOD-70 g/l, COD-105 g/l and ammonia-1 g/l). The maximum COD reduction obtained in the demonstration was more than 50% and the nitrate removal efficiency of MFC was nearly 80% [68].

Food wastewater or food industry wastewater is non-toxic but exhibits high BOD and is rich in sugars and starch as compared to other industrial wastewaters. A study using cereal wastewater in a double-chambered MFC achieved more than 95% COD removal. The initial COD of the feed wastewater was 595 mg/l [69]. The production of starch foodstuffs (for example, potato chips) in food industries requires great usage of water, consequently releases large quantities of wastewater to the environment. Such starch processing wastewater (SPW) comprises high contents of proteins, carbohydrates, cellulose, vitamins and other nutrients. An MFC demonstration used SPW to evaluate the treatment efficiency of a double chambered MFC. The MFC achieved 98% COD reduction after an operation of 140 days. This was accompanied by an ammonia-nitrogen removal efficiency of 91% [27]. In another study involving potato processing wastewater (PPW), 91% of COD reduction was achieved [33]. Similarly, another organic-rich, nontoxic wastewater i.e. chocolate wastewater was used in a double chambered MFC by Patil et al. [29]. The results showed that maximum 75% COD was removed after the MFC operation in batch-mode. The BOD removal and total solid removal was ca. 65% and 68%, respectively [29].

Conventional wastewater treatment techniques cannot effectively treat the wastewaters containing lignocellulosic biomass (e.g. cellulose, hemicellulose and lignin) However, Huang and Logan used paper recycling wastewater in a single chamber MFC (sMFC) for its treatment and electricity generation. The results suggested that the MFC, after nearly three weeks of operation, achieved more than 76% COD removal while ca. 96% of cellulose was removed by the bacteria [11]. This indicates that the microbial community in the MFC not only degraded the lignocellulose biomass and converted it to simpler sugars but also extracted energy from such wastewaters to generate electricity.

The brewery wastewater has been widely investigated in different MFCs for its treatment and bioenergy production. The brewery wastewater exhibits high COD, up to 5000 mg/l. Moreover, it contains high levels of carbohydrates or sugars that can be used as electron donors in the MFCs. Here we present two examples of the studies that used brewery wastewater in MFCs. In the first example, air cathode sMFC was used with different concentrations of the wastewater and was operated in fed-batch mode [32]. When the wastewater with less COD value was used in the MFC, low COD removal was obtained and vice-versa. When COD concentration was 84 mg/l and 1600 mg/l, the COD removal was ~58% and 98%, respectively [32]. In the second study, sMFC was operated in continuous mode with a hydraulic resistance time (HRT) of 2.13 hours. The wastewater was diluted with deionized water and the COD ranged between 600 mg/l and 660 mg/l. The sMFC achieved 43% and 46% COD removal, respectively [31].

The effect of temperature on treatment efficiency of MFCs was investigated by Ahn and Logan using air-cathode sMFC [69]. They operated the fuel cell (batch mode and continuous mode) at two different temperatures i.e. ambient temperature ($23 \pm 3^{\circ}$ C) and mesophilic temperature ($30 \pm 1^{\circ}$ C). The results showed that the % COD removal, as well as the COD removal rate was higher in the MFCs operated at mesophilic temperature than the ambient temperature. Moreover, ca. 10% more nitrogen removal was achieved from the MFCs operated at higher temperature. Overall, the MFCs in the fed-batch mode removed more than 2.5 times COD as compared to MFCs operated in continuous mode [70].

Treatment of wastewaters from different other mills (agro-industries and oil industries) have been also investigated in MFCs. Such wastewaters show high COD and are toxic. For example, cassava mill effluent can have a COD over 16000 mg/l and a cyanide concentration of ca. 86 mg/l [71]. A 30 L double chambered MFC achieved nearly 90% COD removal after 120 hours of operation [71]. Palm oil industries release large amount of highly toxic wastewater, referred to as palm oil mill effluent (POME). POME exhibits COD and BOD as high as 50000 and 25000 mg/l, respectively [34]. Cheng et al. treated POME in an upflow membrane less MFC (UML-MFC) coupling MFC and up-flow anaerobic sludge blanket (UASB) reactors. This integrated system achieved 96% COD and 94% nitrogen removal [34].

Usually, the MFCs produce more power density with wastewater of high COD values. However, the highly concentrated substrate can cause fouling of the PEM, resulting in the restriction of protons, which consequently leads to the accumulation of protons in the anode chamber (low pH) and less availability of protons in the cathode (high pH). Therefore, concentrated wastewaters are sometimes diluted to maintain proper functioning of the MFCs. Furthermore, some pre-treatment methods can be employed to change the physiochemical or biological properties of the wastewater for enhanced performance of the MFCs. For example, the wastewater can be autoclaved to kill the methanogens (the anaerobic bacteria that yield methane as a metabolic by-product) that otherwise use the organic matter to produce methane instead of protons and electrons. A study showed that MFC with the autoclaved wastewater produced ca. 5% more power density than with raw wastewater [26]. Another pre-treatment method i.e. sonication was shown to be useful to increase the performance of the MFCs considerably. This approach was employed using raw wastewater that produced ca. 16% more power density and increased the COD removal efficiency by nearly 5%. The sonication process may improve the performance of the MFC by altering the biodegradability of the organic matter present in the wastewater or changing the molecular weight or particle size spectra of the organic matter. Moreover, wastewater stirring has also shown marginal improvement in the COD removal in the MFC [26]. However, some of these pre-treatment options are energy-intensive and may not be ideal for scale up.

Compared to industrial wastewaters, domestic wastewater is more biodegradable. Domestic wastewater can be a promising substrate for bioenergy production by MFCs. This approach can be utilized to make eco-friendly public toilets, which can generate electricity and can help to keep the surrounding environment neat and clean. For example, a single chamber air-cathode MFC (3-stage MFC/struvite extraction process system) was utilized to treat human urine with simultaneous extraction of struvite ($NH_4MgPO_4{\cdot}6H_2O$), which is an eco-friendly fertilizer. Struvite crystals are generally present in human urine; thus, these can be extracted from urine using MFCs [77]. The anode was inoculated with anaerobic sludge. Human urine, supplemented with 0.5% yeast extract and 1% tryptone, was used as the substrate. The MFC achieved a power of 14.32 W/m^3 after first stage, which reduced to 11.76 W/m^3 after the third stage [77]. Also, the MFC enhanced urea hydrolysis during the operation, which was advantageous for struvite precipitation process. In their successive study, they added sea salts in the human urine (substrate) that increased the electricity generation as well as the struvite extraction [78]. After the addition of sea salts the power output increased by 10%, while the struvite extraction enhanced from 21 to 94%. Besides, the COD removal also improved from 16% to 18% [78]. In addition, the research group of Ioannis Ieropoulos at University of the West of England, Bristol (UK) had a successful field-trial on the MFC-based public toilets in Glastonbury Music Festival. A special urinal was fabricated and the collective urine was fed in the stack of MFCs connected in parallel, as shown in Fig. 8 [79]. The MFCs were directly connected to LED lights to monitor the electricity generation. The trial was run for approximately 3 months and 2.5 – 5 L of urine was converted daily to power. For a period of 5 weeks, an average power of 75 mW was achieved each day and a maximum of 98% COD reduction was observed during the trial [79]. In addition to human urine, human feces have been also used in MFCs to generate electricity. For example, a two chamber MFC was fed with human feces wastewater for electricity generation and its treatment. The wastewater was firstly fermented prior to use in MFCs to enhance the power generation. The results showed that the MFC achieved a maximum power density of 70.8 mW/m^2 and the total COD reduction was 78% after an operation of 190 h [79].

In MFCs, the wastewater treatment efficiency can be further improved by operating the fuel cells for longer periods. For example, an MFC (air-cathode) was operated for four cycles; each cycle lasted for approximately 35 days. The results suggested that the COD removal after the first cycle was ca. 95% which increased to more than 98% after the end of four cycles (after 140 days of MFC operation) [27]. This can be attributed to the longer duration available for the microorganisms to degrade the complex substrates completely into simpler substances. However, the coulombic efficiency achieved in the demonstration was ca. 7%, indicating that most of the substrates did not convert to electricity, which could be due to the following reasons: (i) oxygen diffusion, (ii) production of fermented products, (iii) oxidization of other electron acceptors, and (iv) biomass production [27]. The integration of MFCs with other wastewater treatment technologies can extract more energy, thereby further improving the pollutant removal efficiency. Generally, the bacteria in MFCs effectively degrade the simpler or low-strength wastewaters whereas bioreactors such as anaerobic digester (AD) or UASB treat high-strength wastewaters [2]. Therefore, the wastewaters with complex composition (e.g. POME) can be subjected to the fermentation in UASB that can provide more suitable or simpler substrates for electricity generation in MFCs. Moreover, the residual organics present in the effluent of UASB can be further removed in the MFCs.

Generally, the MFCs with smaller volumes (10-100 ml) are used in the laboratory with synthetic wastewaters. However, a significant number of efforts have been made to scale-up the MFC technology. For example, Zhu et al., constructed a 2-L MFC with staggered and inline electrode system using graphite rods [80], demonstrating faster start-up and higher power output as compared to the MFC with inline electrode array. Evidently, the former MFC produced a maximum power density of 23.8 W/m^3 and the latter MFC generated a maximum power density of 19.1 W/m^3 [80]. This higher power density can be accredited to the improved mass transfer in staggered electrode array. Besides, the MFC also achieved a 84% COD reduction [80]. In another study, the MFC was further scaled-up to 20-L to treat brewery wastewater [81]. No catalyst and ion exchange membrane was used in this study. This MFC was operated for one year and a stable 75% COD removal performance was observed during the first five months [81]. Moreover, a maximum of ~94% of COD reduction was achieved at a flow rate of 1 ml/ min (hydraulic retention time=313) when the MFC was connected to an external resistor of 10 Ω [81]. In a subsequent demonstration, a MFC with 90-L capacity (stacked with five modules) was fabricated by Dong et al. [82]. This was operated in an energy self-sufficient mode for approximately 180 days to treat brewery wastewater (diluted and real wastewater) [82]. A schematic diagram of the 90-L MFC is shown in Fig. 9. The results suggested that the MFC obtained a maximum COD reduction of ~87% and 85% with diluted and real wastewater, respectively. Besides, the MFC with real wastewater obtained higher energy production (0.097 kWh/m^3) as compared with diluted wastewater (0.056 kWh/m^3) [82]. Therefore, it can be concluded that the scale-up of MFC technology has shown substantial improvements for wastewater treatment as well as for bioenergy production, which may pave the way for commercialization of MFCs in the near future.

5. MFCs FOR BIOREMEDIATION OF SPECIFIC CONTAMINANTS

The exoelectrogens produce electrons from their metabolism in the anode chamber of an MFC, which need to be reduced at the cathode chamber. Therefore, an electron acceptor is provided at the cathode to overcome the potential losses. In addition, a catalyst can also be used to increase the reduction reaction rate. Usually, the electron acceptors that exhibit a high redox potential, faster kinetics, a low cost and easy availability are significant and of great interest in MFC applications. For example, oxygen is one of the promising and widely used electron acceptors in the MFCs. In MFC system, various organic and inorganic toxic elements or compounds can be utilized as the electron acceptor in the cathode chamber for its removal or reduction to less toxic form and simultaneously for the electric current generation. For examples, metal ions, perchlorate, nitrobenzene, azo dyes, nitrate (NO_3^-) etc. have been used as electron acceptors in different MFCs to explore the bioremediation potential of this technology. Some examples of MFC performance for bioremediation application are given in Table 4.

The high concentration of toxic heavy metals (e.g. cadmium, mercury, lead, arsenic, chromium etc.) in industrial effluents is harmful to the cellular metabolism of the flora and the fauna living on our planet. Therefore, the wastewaters that contain high concentration of toxic heavy metals need to be reduced into nontoxic form before they are discharged into the environment. MFCs have shown a great potential for the reduction of heavy metals both when used in the anode as well as the electron acceptor in the cathode chamber [72-75]. Generally, the heavy metals with a high redox potential are of great interest to act as the electron acceptor, to achieve higher power output from the cell. Before discussing the MFC potential for the removal of heavy metals let us get an idea about the processes that are responsible for heavy metal removal/reduction in MFCs.

Various heavy metals have been investigated in the anode chamber as well as in the cathode chamber of MFCs for their eco-friendly removal. For anodic removal, generally, a specific concentration of a heavy metal or toxic element is added in the anolyte (supplemented with carbon source and bacterial inoculum). On the other hand, a heavy metal with a high redox potential can be used as the electron acceptor in the cathode chamber. A few mechanisms have been demonstrated that are responsible for the removal of heavy metals or other toxic elements during MFC operation. The first mechanism is biosorption that has been widely recognized for the removal of toxic elements in the MFCs [73]. Biosorption is a combined term for the processes such as microprecipitation, complexation, chelation, coordination, and ion exchange. The biomolecules like polysaccharides, proteins and lipids contain the functional groups such as amine, sulfate, carboxylate, hydroxyl, and phosphate that help in the biosorption process to remove the heavy metals or toxic pollutants. These biomolecules may be present in the anolyte or on the bacterial cell walls, which play a major role in the removal of toxic pollutants. Moreover, some processes like biological oxidation, chemical oxidation, volatilization, anode electrode adsorption have been found responsible for the sulfide removal during the MFC operation.

A single chamber air-cathode MFC demonstrated for the removal of cadmium (Cd) and zinc (Zn) showed high removal efficiencies i.e. 90% and 97%, respectively [72]. Moreover, in a dual chamber MFC, vanadium containing wastewater was employed as the cathodic electron acceptor for its simultaneous removal. The fuel cell after 10 days' operation achieved ca. 70 % removal of V (V) with a maximum power density of ca. 970 mW/m^2 [73].

In another study, a two-chamber MFC obtained a maximum power of ca. 431 mW/m^2 with more than 99.5% removal of Hg^{2+}, which was used as an electron acceptor in the fuel cell [74]. Also ammonia–copper (II) complexes have been substantially recovered from wastewater using MFC technology. Cu $(NH_3)_4^{2+}$ complexes can be reduced to Cu or Cu_2O. In a study, 96% copper was successfully removed after 12 hours of operation of an MFC at a pH-of 9.0 [75].

Different types of dyes are used for the colouring purpose in the textile industry that results in the generation of a colossal volume of dye wastewater per year around the world. Dye wastewater contains many toxic and recalcitrant organic molecules and carcinogenic chemicals [6]. The discharge of such wastewater is threatening to the environment, animals as well as to the plants. Therefore, treatment of such hazardous wastewater is essential before its discharge to the environment. MFC technology provides an eco-friendly alternative for the treatment of dye wastewater and simultaneous bioelectricity generation. MFCs use microorganisms, therefore, the dyes can be reduced by different decolorization mechanisms involving enzymes, low molecular weight redox mediators, and chemical reduction by biogenic reductants. In the MFCs, the dye decolorization occurs in the anode chamber biologically under anaerobic conditions. For example, the azo bond of congo red dye was broken into the intermediates such as aromatic amines that can be completely degraded abiotically in cathode chamber [76].

An sMFC with bioanode and biocathode was demonstrated to decolorize an azo dye congo red, after the operation of the fuel cell for approximately one day. More than 98% congo red decolourization was achieved in that study [76]. Transfer of electrons from anode microorganisms and protons through PEM leads to the degradation of azo bond (–N=N–) in the cathode. Reduction of azo bond results in the formation of colourless and biodegradable aromatic amines [76]. Dechlorinating microorganisms can be used in MFCs for the bioremediation of pentachloroethene (PCE) and trichloroethene (TCE) to reduce them into non-toxic end product ethene. Strycharz *et al.* successfully used *Geobacter lovleyi* and graphite electrodes (as the electron donor) for reductive dechlorination of PCE [83]. A consortium of anaerobic and aerobic bacteria in the cathodic chamber of dual chamber MFC demonstrated efficient degradation of pentachlorophenol (PCP). In the study, degradation rate for PCP was investigated at different pH values and variant temperatures. The most effective degradation rates achieved at a constant temperature of 50° C and pH 6 were 0.52 mg/L-h and 0.36 mg/L-h, respectively [83]. In addition, *Geobacter species* have shown the tendency to reduce aqueous, soluble U (VI) into an insoluble form as U (IV). Multiple lines of evidence suggest that *G. sulfurreducens* entails the outer-surface *c*-type cytochromes for U (VI) reduction but do not require pili for the same purpose [84]. Further investigation revealed that *G. sulfurreducens* strain lacking the *pilA* gene reduced U (VI) to the parallel extent to wild type strain. Similarly, *c*-type cytochromes are also indispensable for *S. oneidensis* to reduce U (VI). Gene deletion studies demonstrated the importance of outer membrane, decaheme cytochrome MtrC in the electron transport to U (VI), as the strains deficient in mtrC and/or omcA were unable to reduce U (VI) [85]. Moreover, MFCs utilizing anaerobic biocathodes have shown the ability to reduce highly toxic Cr (VI) to much less toxic Cr (III) and subsequent precipitation to Cr $(OH)_3$ with simultaneous electricity generation [86]. The MFC with set biocathode potentials reduced Cr (VI) with increased reduction rate of 19.7 mg/L-d. Further, use of *Shewanella oneidensis* MR-1 (produced riboflavin, an electron shuttle mediator to transfer electrons) as a biocatalyst in the cathode under aerated conditions in the presence of lactate showed increased reduction rate for Cr (VI) [87]. An MFC fed with sulfide and glucose and predominated by *Firmicutes* obtained sulfide removal efficiencies of up-to 85% and a power output of 572.4 mW/m^2 at acurrent density of 1094.0 mA/m^2 [88].

Recently, analysis of 16S rRNA revealed that a strain showing similarity to *Klebsiella sp.* is capable of bioremediation of cyanide-containing wastewater in MFC. That study achieved more than 99.5% removal of cyanide and ca. 88% COD removal rate [89]. The investigations described in this section reflect that the MFC technology is a promising alternative for the bioremediation of hazardous contaminants.

6. MFCs AS BIOSENSORS

The online water-monitoring system is indispensable to maintain the proper usage of wastewaters from industries or municipal to conserve the aquatic environment as well as the public health. The MFC has been proven a successful biosensor to detect the organic compounds and contaminants in the wastewaters [90-92]. The conventional biosensors usually require a transducer whereas MFC in itself acts as a transducer, therefore MFC can prove to be a cost-effective biosensor. In the MFC-based biosensor, the exoelectrogens in the anode chamber serve as a signal generator or biological recognition element whereas electrodes and PEM (if used) acts as the transducer. The main advantage of the MFC-biosensor is its long-term stability. This is because the exoelectrogenic biofilms extend the lifespan of sensing element and curtail the replacement of sensing elements.

The basic principle of MFC-based biosensor is presented in Fig. 10. Generally, a toxin (or a sample to be detected) is provided at the anode chamber and its effect on the voltage output is measured. A sudden change in the voltage i.e. either fall or rise in the voltage is taken as the signal for toxin detection. For example, if a toxic element (i.e. chromium) is injected in the anode chamber, a sudden or slow fall in the voltage can be expected because it inhibits growth and activity of the exoelectrogens and, consequently, decreases the voltage [93]. On the other hand, if a carbon source (i.e. acetate) is injected in the anode chamber, a rise in the voltage is anticipated because it accelerates the growth and activity of the exoelectrogens and, therefore, increases the voltage [93]. The results from this study are depicted in Fig. 11, which demonstrates different MFCs as the biosensors using low and high concentrations of different types of contaminants. Typically, the demonstration used three samples i.e. chromium (acute toxin), iron (non-toxic metal) and acetate (organic substrate) at different concentrations (chromium-1 mg/L and 8 mg/L, iron-1 mg/L and 48 mg/L, acetate-200 mg/L) in separate MFCs. The injection of acute toxic and non-toxic metal suddenly decreased the voltage marginally at low concentrations and severely at high concentrations. On the other hand, the addition of carbon substrate increased the voltage [93].

The MFC sensors can be operated in two modes. The first is flow-through and the second is flow-by electrodes. In the first mode, the water sample moves through the porous electrode, while in the second mode, the water sample flows parallel to the electrode surface [94]. The operation of MFC sensor in a flow-through mode can improve the diffusion of ions and the electrolytes, thereby increasing the sensitivity of the MFC-based toxicity sensors. Moreover, a study reported that flow-through anode in an MFC sensor also enhanced the diffusion of protons through anodic biofilm, improving the biocatalysis of the substrates by the exoelectrogens [95]. Evidently, the sensitivity of an MFC-based toxicity sensor was increased approximately 40 times by using a flow-through anode as compared to the flow-by anode [96].

According to the Michaelis-Menten equation, the biocatalytic activity of exoelectrogens in the anode chamber depends on the concentration of dissolved organic matter and it keeps increasing until the concentration of the organic matter reaches a saturation point [97]. MFC sensors are usually operated in turn-off mode for toxicity monitoring, and the metabolic activity of exoelectrogens can be supressed by adding a certain concentration of a toxic pollutant in the anolyte, resulting a certain change in the electric output [94, 96]. The biological toxicity of the target toxic pollutants is generally measured by correlating the concentration of the toxin to the electric signal output. Therefore, current change (ΔI) and inhibition ratio (IR) can be evaluated. Further, ΔI can be utilized to obtain the sensitivity of the MFC-based toxic sensor by normalizing the ΔI to the concentration of the toxic agent. On the other hand, IR represents the amplitude of the electric signal output and can be used to evaluate the toxicity of pollutants [98]. However, it is still unclear what maximum concentration of the toxic agent is required to obtain a signal output for toxicity-monitoring.

In the conventional MFC-based sensors, the sensitivity of toxic agents depends on the bioanode in the system or we can say bioanode acts as a sensing element in the MFC sensor to monitor the water toxicity. But recently, Yong et al. designed an MFC sensor with biocathode as the sensing element. The results revealed that the MFC sensor with biocathode showed better sensitivity than the MFC sensor with bioanode [99]. Such MFC sensors could be advantageous in comparison to bioanode because they do not need organic matter supplementation for baseline signal output and can reduce the negative effects of combined shock of toxicity and organic matter. Moreover, the signal output of an MFC sensor is greatly dependent and influenced by the performance of the anode and the cathode. Therefore, the modifications can be done in both the chambers to reduce the response time and increase the detection capacity. For example, the anode potential of the MFC sensor significantly affects the biosensor sensitivity and, therefore, can be optimized using a potentiostat. The anode potential usually determines the energy level of the electrons that get transferred from the surface of exoelectrogens to the anode surface and, hence, affects the electron transfer rate and the electric output signal [94]. A study revealed that the MFC sensor operated at a constant anode potential (-1.5 V) showed the highest sensitivity and an unbiased measurement of toxicity as compared to the MFC sensor without applying anode potential

[96]. Similarly, the cathode of MFC sensor can be altered to improve its water-monitoring. The performance of cathode (stability and catalysis) can affect the amplitude and the accuracy of output signal under non-toxic conditions as well as toxic conditions. In a study, a cathode-based MFC sensor array was designed like a bioanode MFC sensor array to detect Cu^{2+} and acidic toxicity. An immediate voltage drop was observed when the MFC was injected with Cu^{2+} (2-6mg/L) and the pH was decreased from 6 to 4 [100]. Results are given in Fig. 12.

The application of an MFC-based BOD sensor with municipal or industrial wastewater could be more challenging in real-world applications because the wastewaters contain easily degradable organic matters as well as toxic pollutants. During the operation of an MFC-based BOD sensor, sudden changes in BOD and toxicity could simultaneously occur [101]. In a MFC-based sensor, the current density decreases with respect to the toxicity of the toxic agents, while the current density increases with rise in BOD [101]. Therefore, the sudden variation in BOD might wane the responses of MFC sensor for toxicity. Evidently, a study demonstrated that a combined shock of BOD and toxicity affected the signal output when using the MFC sensor for the detection of Cr (IV) [102]. In other words, it can be stated that signal interference is caused by the combined shock of BOD and toxicity when MFC sensor is used for water-monitoring. Recently, Yong et al. studied the effect of organic matter concentration (in anode) on toxicity monitoring to avoid the signal interference by the combined shock of BOD and toxicity [103]. The study revealed that the background organic matter concentration should be fixed at a high level of oversaturation for maximizing the signal output when the 'ΔI' is selected relative to the concentration of a toxic agent. On the other hand, IR should be fixed to a lower value near to the detection limit to maximize the signal output [103]. The results of this study are shown in Fig. 13.

The passage of oxygen into the anode chamber affects the metabolic activity of anaerobic microorganisms in MFC-based biosensors, thereby, affecting the biosensor sensitivity. Therefore, it is important to solve this limitation to improve the performance of these kinds of biosensors. The oxygen diffusion can be diminished by placing an ion exchange membrane between the cathode and the anode that is less permeable to oxygen. Generally, nafion is used as the proton exchange membrane in MFCs, but it shows high oxygen permeability [94]. Recently, a sulfonated ketone ether membrane was applied in a MFC-based biosensor replacing nafion. The MFC with the new membrane showed better sensitivity results as compared to nafion [104]. The better performance was attributed to the lower oxygen permeability of the membrane [104]. The other challenges include its long response time and detection reliability to replace the commercialized real water-monitoring systems. However, the longer response time for detection of contaminants can be minimized by modifying the MFC sensor structure. For example, in a study, the response time was significantly reduced from 36 min to 5 min by decreasing the volume of anode from 25 ml to 5 ml in the MFC [104]. On the other hand, the detection reliability can be further ameliorated by connecting various MFCs in parallel. Such MFC array has been reported for effective water quality monitoring [94].

A few MFC-based biosensors have been commercialized. One such product is named Biomonitoring system (HATOX-2000), which has been invented by a Korean company and can be utilized for online monitoring of water toxicity. More detailed information of this product can be accessed from elsewhere (www.ecotrade.org).

7. MICROBIAL ELECTROLYSIS CELLS FOR HYDROGEN PRODUCTION

An MFC produces electricity from organic waste while a microbial electrolysis cell (MEC) produces hydrogen gas. The working principle of an MEC is similar to an MFC as the electrons generated by the exoelectrogens in the anode combines with protons at the cathode to produce hydrogen gas as the final product. But unlike MFC, electricity is provided in the MEC to produce hydrogen. Theoretically a voltage of 0.2 to 0.8 V is required to reduce the protons to form hydrogen. Such low voltage is easily achievable in the MFC. Therefore, an MFC can be used to supply the voltage to the MEC for hydrogen production. The electrode material used in the MFCs can be employed in the MECs as well. Moreover, the exoelectrogens are also required to produce hydrogen gas in MECs. In MECs, similar to MFCs, a cathode catalyst such as platinum is used to overcome the overpotentials to drive hydrogen production. Unlike MFCs, the MECs require strictly anaerobic conditions for hydrogen production. However, the higher concentration of hydrogen gas promotes the growth of methane-producing microorganisms. Subsequently, the hydrogen gas is contaminated by methane and the resultant hydrogen output is decreased. Different types of organic sources and wastewater can be applied in MEC for hydrogen production. Notably, MEC has shown higher hydrogen yields than that obtained with fermentation. For example, the maximum theoretical yield of 7 mol-H_2/mol-glycerol by oxidation is achievable. The hydrogen yields reported in some studies using fermentation vary from 0.05-1.05 mol-H_2/mol-glycerol [106, 107], but a hydrogen yield of 3.9 mol-H_2 /mol-glycerol has been achieved using MEC [108]. In addition, a hydrogen yield of 7.2 mol-H_2 /mol-glucose was also obtained in the study against the maximum theoretical yield of 12 mol-H_2/mol- glucose [108].

There are some obstacles that limit the application of MECs at the large scale. For example, a single MFC generally produces an OCV of approximately 0.8 V and a resultant working voltage of ~0.5 V can be achieved in an MFC [109]. This decrease in voltage could be due to higher internal resistance in the MFC system, energy utilization by bacteria, and electrode overpotentials [109]. Therefore, three or five MFCs can be connected in series to increase the resultant voltage output. But the voltage reversal can reduce the voltage output over the long-term [109]. This problem was resolved by Hatzell et al. by using a capacitor in the circuit to prevent the voltage reversal. In this study, the MFCs were connected in a parallel configuration to charge the capacitors. Then the capacitors were connected in series to discharge the voltage to the MECs. Such a system increased the hydrogen production rate approximately 2.3 times as compared to coupled systems without capacitors [110]. Another major limitation in MECs is the consumption of hydrogen by methanogens to produce methane, which consequently reduces the hydrogen generation. Many approaches have been used to inhibit the methanogens in MECs. For example, the cathode can be exposed to oxygen or ultraviolet radiation to inhibit the methanogens. In a demonstration, the exposure of cathode to air decreased the methane concentration from 3.4% to less than 1% [111]. On the other hand, the exposure of ultraviolet (UV) radiation in the MEC maintained high concentrations of hydrogen (91%), while without UV irradiation, methane concentrations increased significantly [112]. Recently, the use of antibiotics has shown the potential to inhibit the methanogens [113]. In a study, Catal et al. used different concentrations of four antibiotics (neomycin sulfate, 2-bromoethane sulfonate, 2-chloroethane sulfonate, and 8-aza-hypoxanthine) to measure the inhibition of methanogenesis on a mixed culture community to improve the hydrogen production. The results showed that the increasing concentrations of the antibiotics decreased the concentration of methane effectively that resulted in a comparatively higher hydrogen production [114]. The third major problem that hinders the use of MEC at pilot scale is the necessity of a catalyst at the cathode. Usually, platinum is used as the cathode catalyst in MECs that is very expensive. Moreover, it can be easily poisoned by sulfide present in the water. Therefore, its replacement with a catalyst which is cost-effective and has similar catalytic properties is required to launch the technology at a large scale. Some catalysts have been already experimented in MECs to replace platinum. For example, Yang et al. recently used polyaniline/multi-walled carbon nanotube as the cathode catalyst in a single chamber MEC. The results suggested that a maximum hydrogen production rate of 1.04 m^3/m^3/day was achieved with the catalyst, which was comparable to the performance with platinum [114]. The same catalyst was further used in a different study with biocathodes that achieved a maximum hydrogen production rate of 0.67 m^3/m^3/day [115]. Moreover, nano-Mg $(OH)_2$/graphene composites at different concentrations were demonstrated as the cathodic catalyst in MEC to improve hydrogen production. The cathodic hydrogen recovery and hydrogen production rate obtained with the catalyst were ca. 84% and 0.63 m^3/m^3/day, which were higher as compared to the Pt/C cathode [116].

8. CONCLUSIONS AND CHALLENGES

The MFCs provide a suitable, eco-friendly alternative to produce energy and to treat wastewater simultaneously. Several wastewaters ranging from low-strength to high-strength have been utilized in MFCs for their treatment and electricity generation simultaneously. However, the power outputs achieved in the MFCs are low and can be enhanced by the following approaches; 1) a suitable design that results in low internal resistance; 2) using nanoparticles that increase the electron transfer mechanisms; 3) use of genetically engineered microorganisms; 4) addition of pre-treated inoculum or control inoculum; 5) decreasing the start-up time of the MFC. For example, graphene/ Fe_3O_4 nanocomposites coated carbon paper as the anode electrode decreased the start-up time and achieved a maximum current density of 1800 mA/cm^2, which was ~6 times higher than the bare anode [56]. The electricity generated from MFCs can be further used to power electric instruments or machines. As noted earlier, MFCs have been successfully applied to operate the "Gastrobots" for bioenergy production and environmental monitoring.

Further efficient treatment of wastewater can be achieved by operating the fuel cells at mesophilic temperatures. Moreover, the MFCs integrated with other anaerobic fermentation technologies such as with UASB, have shown enhanced COD removal efficiency. Significant efforts have been made to scale-up the MFC technology. For example, a MFC with 90-L capacity obtained a maximum COD reduction of ~87% with brewery wastewater [82].

MFCs have shown a great potential for the reduction of heavy metals or toxic pollutants when used in the anode as well as in the cathode chamber as the electron acceptor. The heavy metals with a high redox potential are of great interest to act as the electron acceptor, to achieve higher power output from the cell. The biomolecules that may be present in the anolyte or on the bacterial cell walls contain the functional groups, which play a major role in the removal of toxic pollutants. MFCs have achieved heavy metal removal of even upto 99.5% (Hg^{2+}) and 97% (Zn). The MFCs can also be applied as a BOD or COD sensor to detect the availability of a toxic pollutant in the wastewater. The voltage drop/rise is taken as the signal for the detection of the toxin or the sample. The change in voltage is usually proportional to the concentration of the toxin. The low sensitivity and detection reliability are the main challenges in MFC-based biosensors. The sensitivity of an MFC-based toxicity sensor can be improved by operating them in a flow-through mode. A study showed that the sensitivity of the biosensor increased approximately 40 times by using a flow-through anode as compared to the flow-by anode [96]. In addition, an MFC can be amended to an MEC to produce another biofuel i.e., hydrogen energy, while the MFC may be a substantial alternative to supply the required voltage. One of the major limitations in MECs is the consumption of hydrogen by methanogens to produce methane, which consequently reduces the hydrogen generation. However, the use of antibiotics and exposure of ultraviolet radiations have shown the potential to inhibit the methanogens [113]. The results showed that the increasing concentrations of the antibiotics decreased the concentration of methane effectively, resulting a higher hydrogen production [114].

The MFC technology has been used for various applications, however, there are some challenges that need to be addressed to make the technology economically viable. The first prime hurdle is a feasible design for upscaling the MFC. The previous designs exhibit some drawbacks such as high internal resistance, electrode spacing, exchange of anolyte and catholyte across the PEM etc. when we think to scale up them for long-term operations. However, some designs have already been introduced but have not been explored at the industrial scale. The second challenge is to provide cost-effective electrode materials and PEM (if used) for MFCs. For scale up, the available electrode materials such as carbon paper and carbon cloth would be very expensive. Another obstacle is the choice of an electron acceptor at the cathode. Oxygen is abundantly available and is the preeminent choice for the electron acceptor. But continuous sparging of oxygen at the cathode can also affect the activity of anaerobic microbial community at the anode during long-term operations since oxygen can diffuse through the PEM to the anode. Platinum is most commonly used for oxygen reduction reaction, but it is very expensive, and a cheaper alternative is required. For example, at the small scale (MFC of 250 ml capacity), commercially available 0.5 mg/cm^2 20% platinum on carbon paper of 20 cm^2 costs ~250 US$ (Fuel Cell Earth, USA). If we want to scale up the MFC reactor, we need larger electrode and obviously, a large amount of platinum. This makes the use of platinum uneconomical at the large scale. Moreover, platinum turns poisonous when it reacts with certain elements/chemicals in the water such as sulphide, making the use of platinum impractical for wastewater treatment application. Therefore, the replacement of platinum is the must in scaling-up the MFCs.

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Highlights

- The state-of-the-art information on major applications of MFCs and strategies to improve them is provided in this article.
- The basic principles of all the applications are thoroughly discussed.
- The obstacles that limit the technology to use in real world applications are reported.
- Many approaches such as electrode modification, genetic engineering etc. can be utilized to improve the MFC performances.

List of tables

Table 1. Performance of microbial fuel cells for bioelectricity generation using pure cultures

Inoculum	Type of MFC	Substrate	Electrode materials	Current density/Power density	References
Klebsiella pneumonia	Single-chamber MFC	Glucose	Carbon cloth	199 mA/m^2	25
Desulfovibrio desulfuricans	Double-chamber MFC	Wastewater	Graphite felt	233 mA/m^2	26
Escherichia coli	Double-chamber MFC	Glucose	[1]PAN/ TiO_2 composite-anode Carbon cloth–cathode	3390 mA/m^2	27
Saccharomyces cerevisiae	Single-chamber MFC	Synthetic wastewater	Graphite plates	282 mA/m^2	28
Thermincola ferriatica	Double-chamber MFC	Acetate	Graphite carbon fibres	12000 mA/m^2	14
Lysinbacillus sphaericus	Double-chamber MFC	Glucose	Graphite felt	85 mW/m^2	30
Citrobacter sp.	Single-chamber MFC	Acetate	Carbon cloth	205 mA/m^2	31
Ochrobactrum sp.	Double-chamber MFC	Xylose	Carbon fibres brush	2625 mW/m^3	32
Shewanella putrefaciens	Single-chamber MFC	Lactate	Carbon cloth	4920 mW/m^3	33
Scenedesmum	Double-chamber MFC	Acetate	Carbon fiber brush-anode Carbon cloth-cathode	1926 mW/m^2	34
Shewanella oneidensis	Mini-MFC	Lactate	Graphite-felt	3000 mW/m^2	35

Cyanobacteria	Single-chamber MFC	Domestic wastewater	Graphite felt-anode, Carbon cloth-cathode	114 mW/m^2	36
Chlorella vulgaris	Double-chamber MFC	Wastewater	Carbon felt-anode Carbon cloth-cathode	2485 mW/m^3	37
Rhodopseudomonas palustris	Single-chamber MFC	Wastewater	Carbon paper-anode Carbon cloth-cathode	2720 mW/m^2	38
Coriolus versicolor	Double-chamber MFC	[2]ABTS	Carbon fibres	320 mW/m^3	39
Geobacter metallireducens	Double-chamber MFC	Domestic wastewater	Carbon paper	40 mW/m^2	40
Geobacter sulfurreducens	Double-chamber MFC	Acetate	Carbon fibres	1.9 mW/m^2	17

Note-: [1]PAN= Polyaniline

[2] ABTS = 2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

Units of surface power density are given in milliwatts per square meter; volume power density in watts per cubic meter; and current density in milliampere per square meter.

Table 2. Performance of microbial fuel cells for bioelectricity generation using mixed cultures

Source of inoculum	Type of MFC	Substrate	Electrode material	Current density/Power density / Voltage	Reference
Dairy manure wastewater	Single-chamber MFC	Dairy manure wastewater	Graphite fiber brush	190 mW/m^2	42
Potato wastewater	Single-chamber MFC	Potato wastewater	Graphite fiber brush	217 mW/m^2	42
Activated sludge	Double-chamber MFC	Acetate, glucose	Carbon paper	410 mV	43
Primary wastewater	Double-chamber MFC	Acetate	Graphite rods	152 mA/m^2	44
Activated sludge	Single-chamber MFC	Acetate, glucose	Carbon cloth	1084 mW/m^2	45
Activated sludge	Double-chamber MFC	[1]POME	Polyacrylonitrile carbon felt	107 mW/m^2	46
Activated sludge	Single-chamber MFC	Glucose	Carbon cloth	68 mW/m^2	47
Activated sludge	Single-chamber MFC	Acetate	Graphite coated with graphene -anode, carbon cloth-cathode	670 mW/m^2	48
Primary wastewater	Single-chamber MFC	Acetic acid	Graphite fiber brushes-anode Carbon cloth-cathode	835 mW/m^2	49
Primary wastewater	Single-chamber MFC	Ethanol	Graphite fiber brushes-anode Carbon cloth-cathode	820 mW/m^2	49
Primary wastewater	Single-chamber MFC	Lactic acid	Graphite fiber brushes-anode Carbon cloth-cathode	739 mW/m^2	49
Primary wastewater	Single-chamber MFC	Succinic acid	Graphite fiber brushes-anode Carbon cloth-cathode	444 mW/m^2	49

Anaerobic sludge	Double-chamber MFC	Slaughterhouse wastewater	Carbon cloth-anode Titanium mesh-cathode	578 mW/m^2	51
Anaerobic reactor effluent	Double-chamber MFC	Acetate	Carbon cloth-anode Granular active carbon-cathode	1200 mW/m^3	52
Soil	Double-chamber MFC	Cellulose	Carbon paper	188 mW/m^2	53

Note: [1]POME =Palm Oil Mill Effluent

Units of surface power density are given in milliwatts per square meter, volume power density in watts per cubic meter, and units of voltage in millivolts.

Table 3. Performance of microbial fuel cells for wastewater treatment

Wastewater	Type of MFC	Electrode material	% COD reduction	Reference
Swine wastewater	Single-chamber MFC	Toray carbon paper as anode carbon cloth as cathode	92	54
Starch processing wastewater	Single-chamber MFC	Carbon paper	98	55
Real urban wastewater	Double-chamber MFC	Graphite electrodes	70	60
Olive mill wastewaters	Single-chamber MFC	Carbon cloth as electrodes	65	61
Protein-rich wastewater	Double-chamber MFC	Graphite rods as electrodes	80	4
Paper recycling wastewater	Single-chamber MFC	Graphite fibers-brush	76	11
Cassava mill wastewater	Double-chamber MFC	Graphite plates electrode	86	62
Food processing wastewater	Double-chamber MFC	Carbon paper electrodes	95	68
Domestic wastewater	Double-chamber MFC	Plain graphite electrodes	88	69
Chocolate industry wastewater	Double-chamber MFC	Graphite rods as electrodes	75	70
Biodiesel wastes	Single-chamber MFC	Carbon brush electrodes	90	71
Beer brewery wastewater	Single-chamber MFC	Carbon fibers	43	72
Brewery wastewater	Single-chamber MFC	Carbon cloth as electrodes	98	73
Potato Processing wastewater	Tubular MFC	Graphite particles as anode Graphite felt as cathode	91	74

Palm oil mill effluent	[1]UML-MFCs	Graphite granules, Carbon fiber felt	90	75
Animal carcass wastewater	Up-flow tubular MFC	Graphite felt as anode Carbon cloth as cathode	51	76
Food waste leachate	Double-chamber MFC	Carbon felt	85	83
Chemical wastewater	Double-chamber MFC	Graphite plates	63	84

Note: [1]UML-MFCs = Up-flow membrane-less microbial fuel cell

Table 4. Performance of microbial fuel cells for bioremediation

Heavy metals/ Wastewater	Type of MFC	Electrode material	% Removal	Power density	Reference
Chromium (VI)	Double-chamber MFC	Graphite granules-cathode Graphite brush-anode	94	6.4 W/m^3	85
Chromium (VI)	Double-chamber MFC	Carbon fiber felt	76	970 mW/m^2	86
Sulfide	Double-chamber MFC	Carbon fiber felt	85	572.4 mW/m^2	87
Cadmium	Single-chamber MFC	Carbon cloth	90	3600 mW/m^2	88
Zinc	Single-chamber MFC	Carbon cloth	97	3600 mW/m^2	88
Vanadium	Double-chamber MFC	Carbon fiber felt	68	970 mW/m^2	89
Ammonia–copper (II)	Double-chamber MFC	Graphite felt-anode Graphite plate-cathode	96	140 mW/m^2	90
Mercury (Hg^{2+})	Double-chamber MFC	Graphite felt-anode Carbon felt- cathode	99.5	433 mW/m^2	91
Azo dye Congo red	Single-chamber MFC	Carbon brush	98.3	-	92
Cyanide	Double-chamber MFC	Carbon cloth	88.3	-	93
Copper (Cu^{2+})	Double-chamber MFC	Graphite felt electrodes	99.5	319 mW/m^2	106
Chromium (VI)	Single-chamber MFC	Carbon brush-anode Carbon cloth-cathode	99	419 mW/m^2	107
Nitrate	Single-chamber MFC	Graphite rods	30	3900 mW/m^3	108
Nitrite	Single-chamber MFC	Graphite rods	37	3600 mW/m^3	108

Note: Units of surface power density are given in milliwatts per square meter; volume power density in watts per cubic meter.

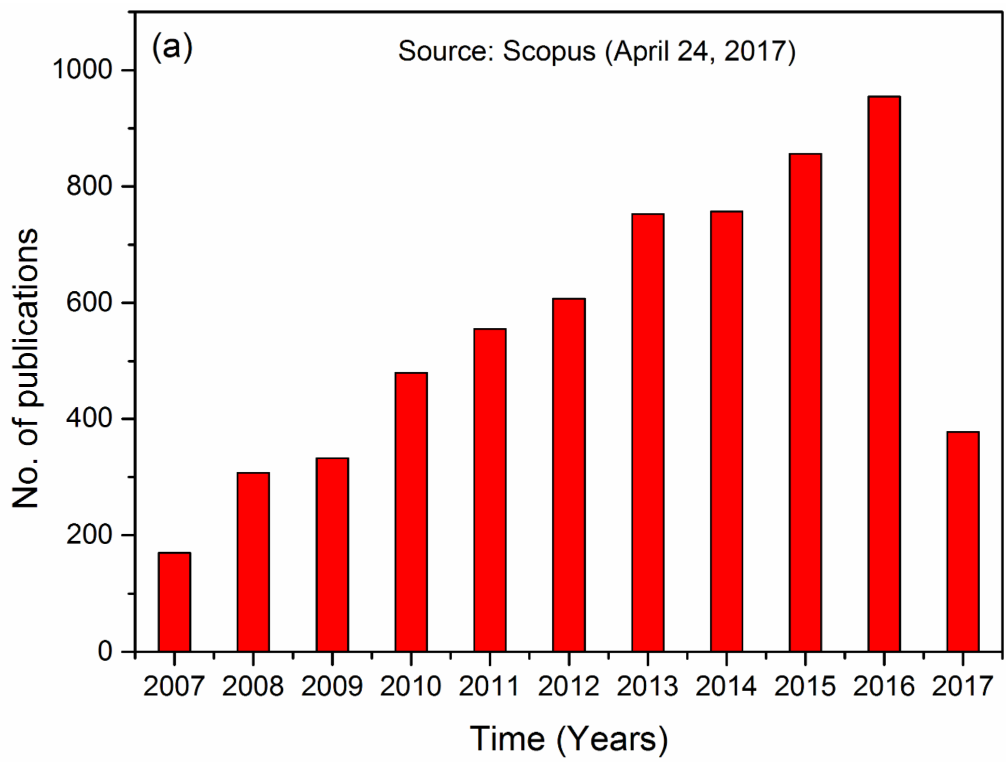
List of figures

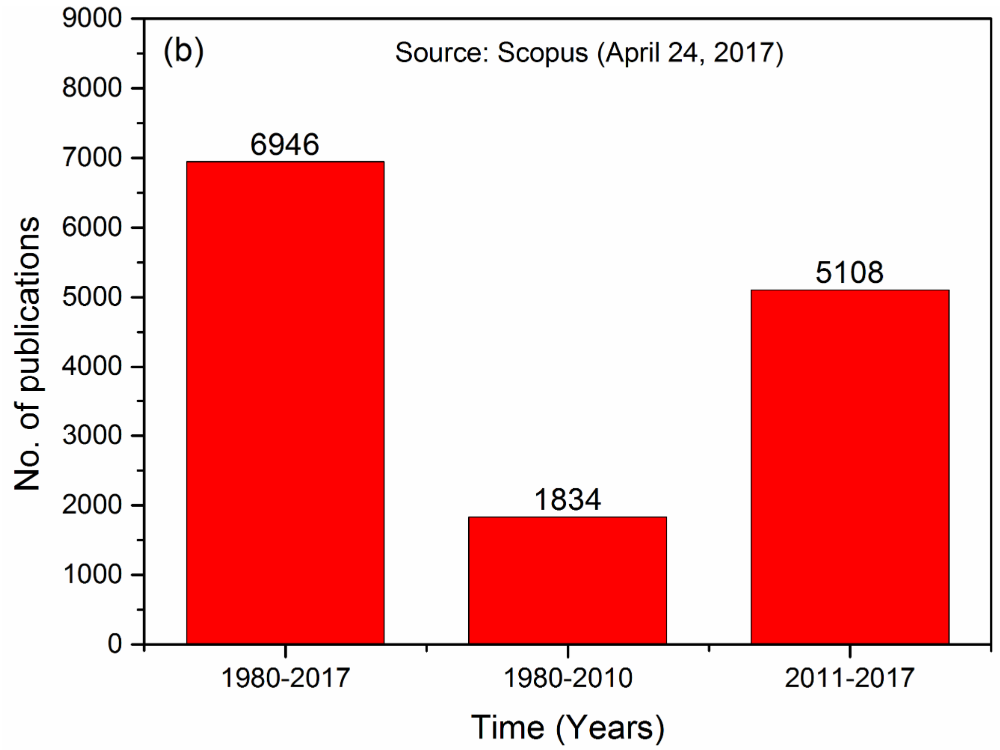
11. Voltage responses of MFC-based biosensors to different samples. The figure shows the performance of four MFCs used to sense the addition of three samples (with different concentrations) in the anode chamber, resulting five shocks (a) to (e). **(a)** The MFC was injected with iron (non-toxic metal) of concentration 48 mg/L after 150 minutes of operation. The injection suddenly decreased the voltage from 121 mV to 67 mV. **(b)** The MFC was injected with chromium (acute toxin) of concentration 1 mg/L after 74 minutes of operation. After 134 minutes of the first fall (shock), the voltage decreased from the steady point (89 mV) to 81 mV. **(c)** After 74 minutes of operation, there was a steep fall in the voltage from 109 mV to 91 mV. **(d)** In another MFC**,** iron of concentration 1 mg/L was injected in the anode chamber after 30 minutes of operation. This low concentration decreased the voltage slightly from 121 mV to 118 mV, though higher concentration sharply decreased the voltage as mentioned earlier in (a). **(e)** The effect of carbon substrate was also sensed in the MFC, addition of 200 mg/L sodium acetate showed instant rise in the voltage from 102 mV to 114 mV after 2 minutes, which further increased to 122 mV after 4 minutes.

12. **(a)** The MFC array used for Cu^{2+} toxicity monitoring, **(b)** acidic toxicity monitoring [100].

13. The signal interference of an MFC sensor by the combined shock of biochemical oxygen demand (BOD) and toxicity in a continuous flow-through mode: (**a**) the MFC sensor operated with background acetate of 0.3 mM; (**b**) the MFC sensor operated with background acetate of 5 mM [103].

Figure 1.


(a)
Source: Scopus (April 24, 2017)
No. of publications
0
200
400
600
800
1000
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
Time (Years)


(b)
Source: Scopus (April 24, 2017)
No. of publications
0
1000
2000
3000
4000
5000
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7000
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9000
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1834
5108
1980-2017
1980-2010
2011-2017
Time (Years)

(c)

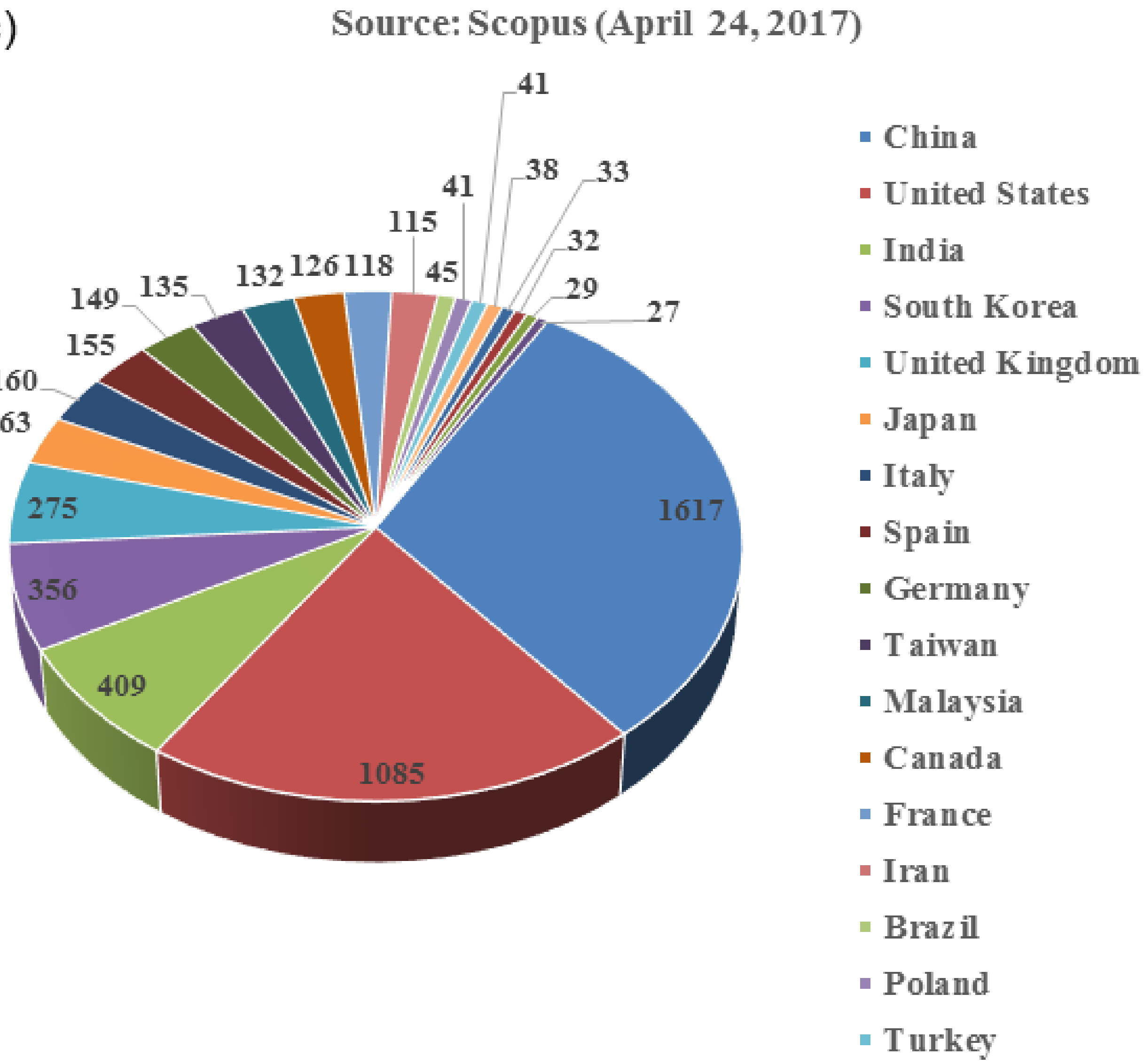
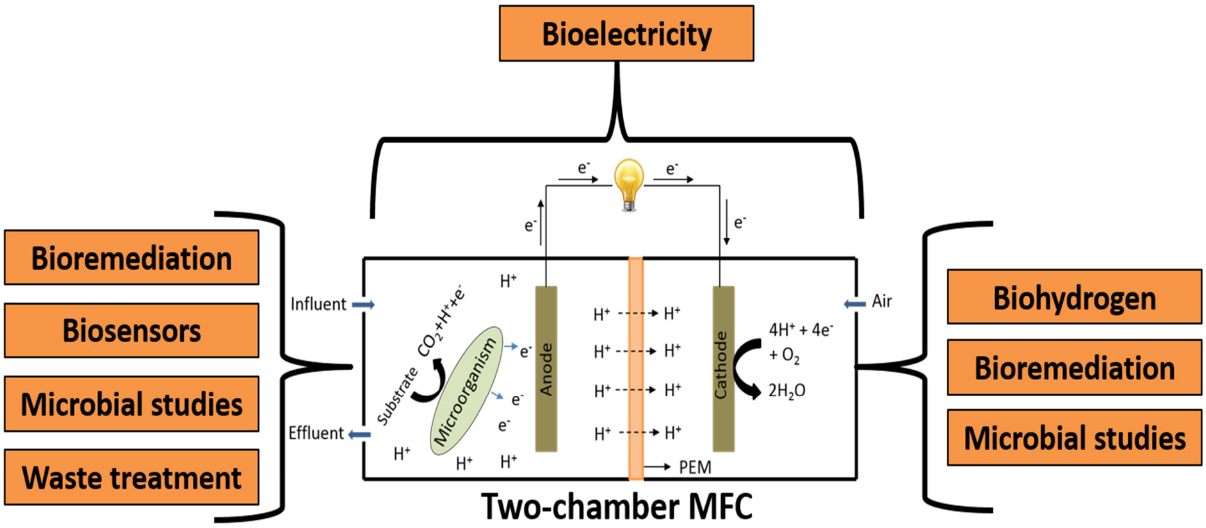

Source: Scopus (April 24, 2017)
1617
1085
409
356
275
163
160
155
149
135
132
126
118
115
45
41
41
38
33
32
29
27
China
United States
India
South Korea
United Kingdom
Japan
Italy
Spain
Germany
Taiwan
Malaysia
Canada
France
Iran
Brazil
Poland
Turkey

Figure 2.



Bioelectricity
Bioremediation
Biosensors
Microbial studies
Waste treatment
Influent
Effluent
Substrate
$CO_2+H^++e^-$
Microorganism
Anode
Cathode
e^-
H^+
$4H^+ + 4e^- + O_2$
$2H_2O$
PEM
Air
Biohydrogen
Bioremediation
Microbial studies
Two-chamber MFC

Figure 3.

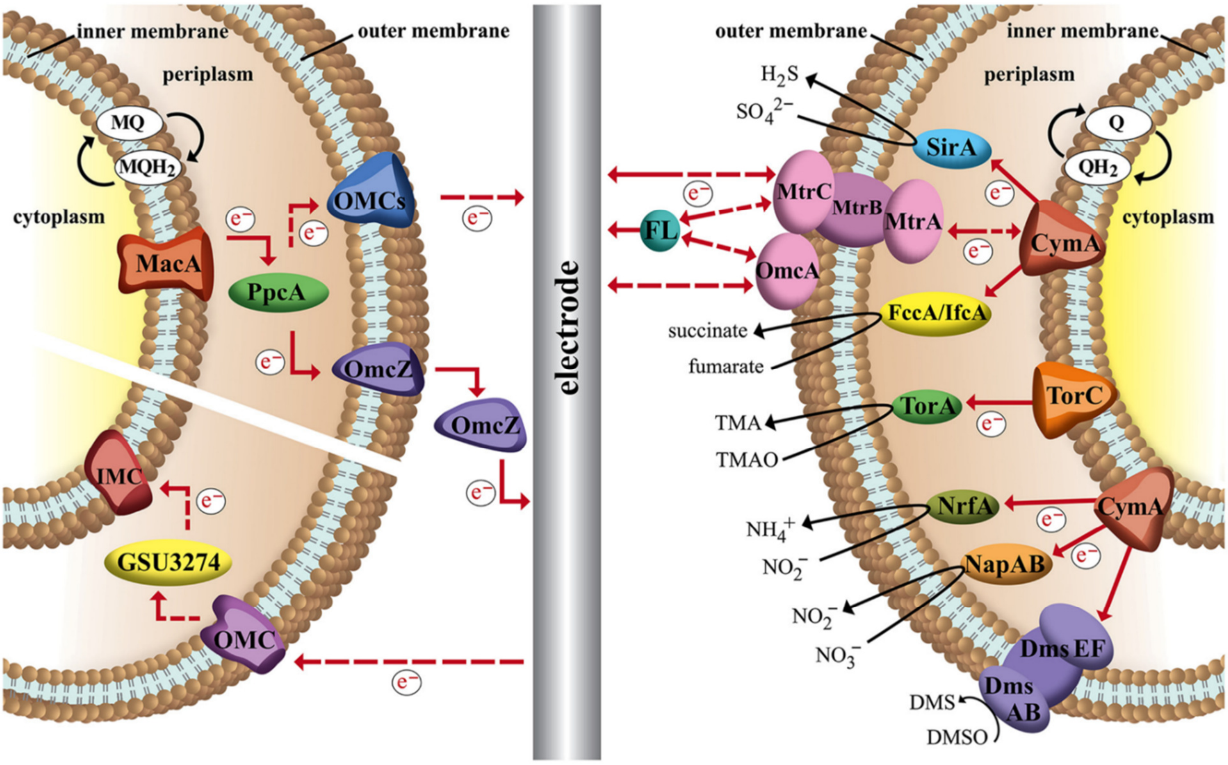

inner membrane
outer membrane
periplasm
MQ
MQH2
cytoplasm
MacA
PpcA
OMCs
OmcZ
OmcZ
IMC
GSU3274
OMC
e⁻
electrode
outer membrane
inner membrane
periplasm
H2S
SO4 2−
SirA
Q
QH2
cytoplasm
MtrC
MtrB
MtrA
FL
OmcA
CymA
FccA/IfcA
succinate
fumarate
TorA
TorC
TMA
TMAO
NrfA
CymA
NH4+
NO2−
NapAB
NO2−
NO3−
Dms EF
Dms AB
DMS
DMSO

Figure 4.

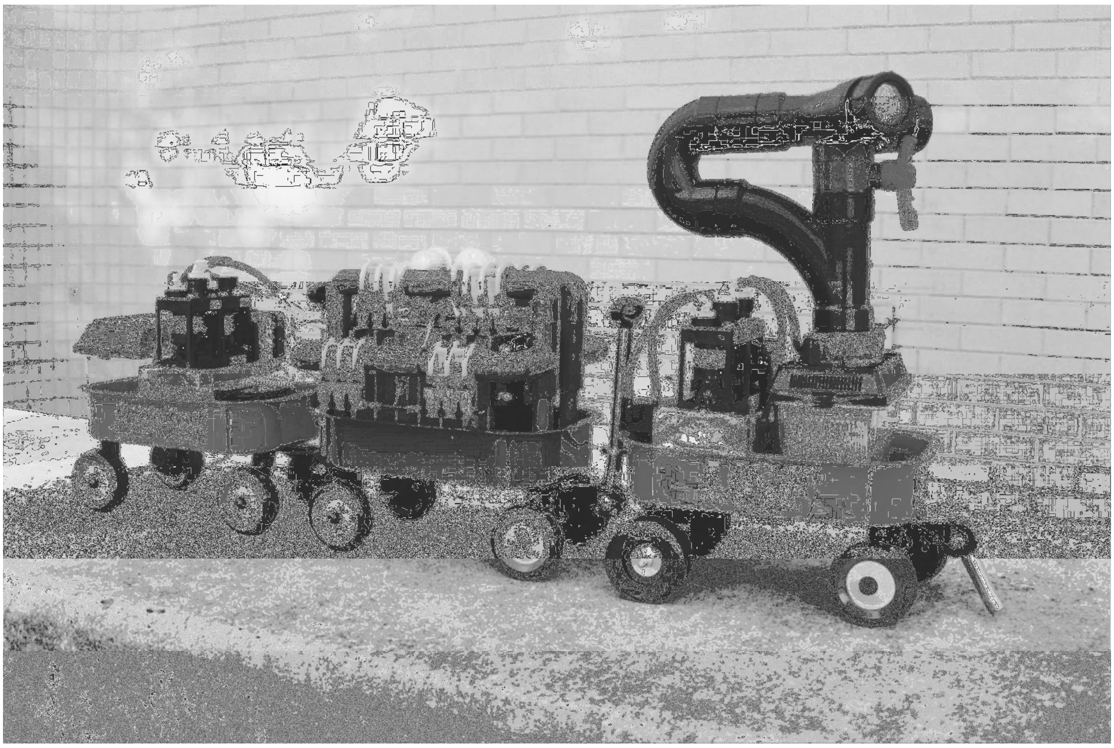


Figure 5.

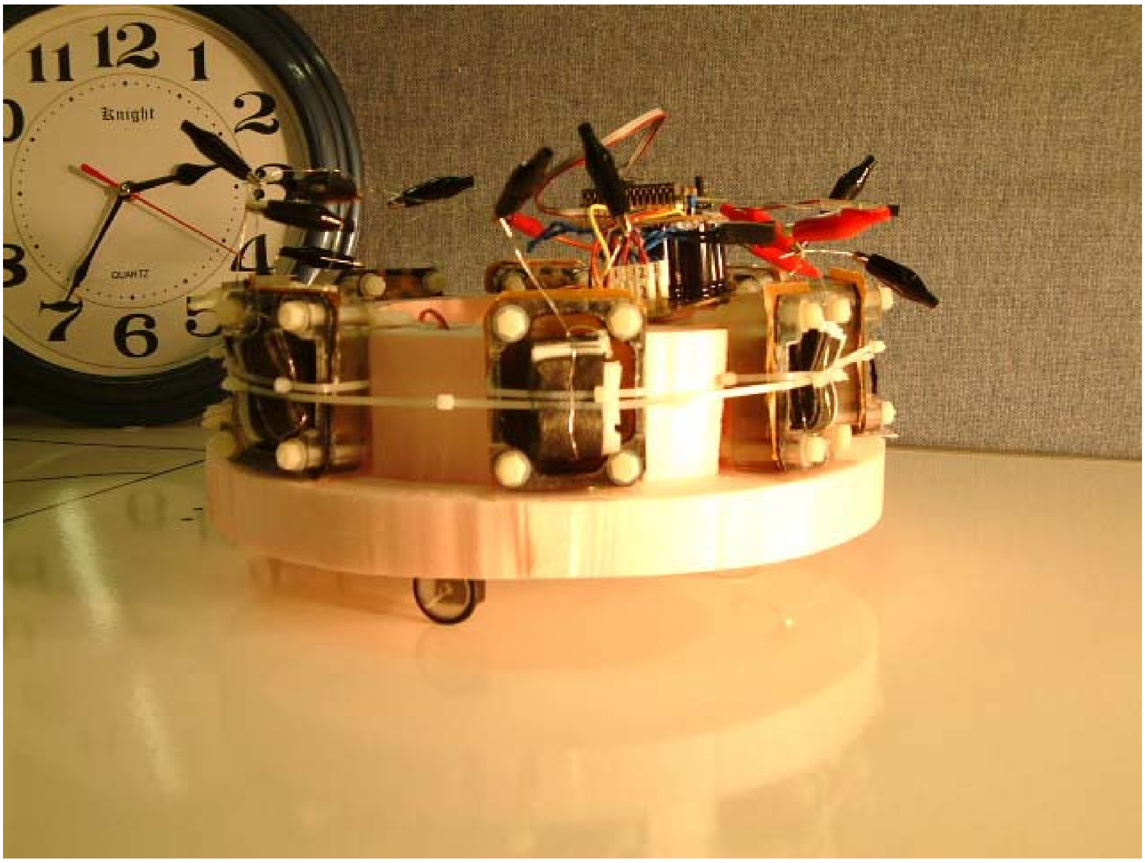


Figure 6.

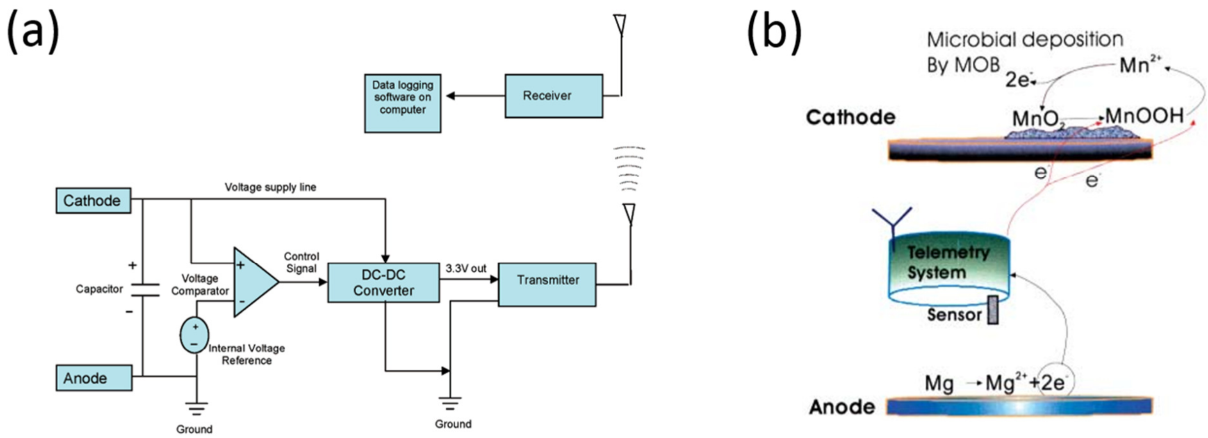
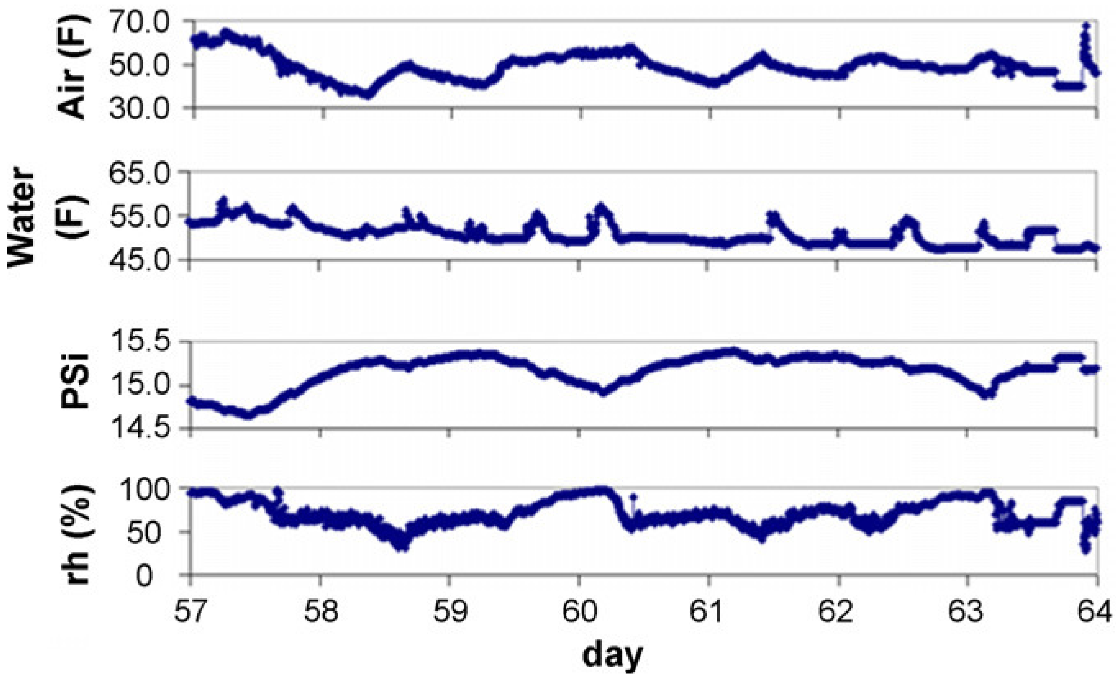

(a)
Data logging software on computer
Receiver
Cathode
Voltage supply line
Capacitor
Voltage Comparator
Control Signal
DC-DC Converter
3.3V out
Transmitter
Internal Voltage Reference
Anode
Ground
Ground
(b)
Microbial deposition By MOB
2e
Mn2+
Cathode
MnO2
MnOOH
e
e
Telemetry System
Sensor
Mg → Mg2+ +2e
Anode

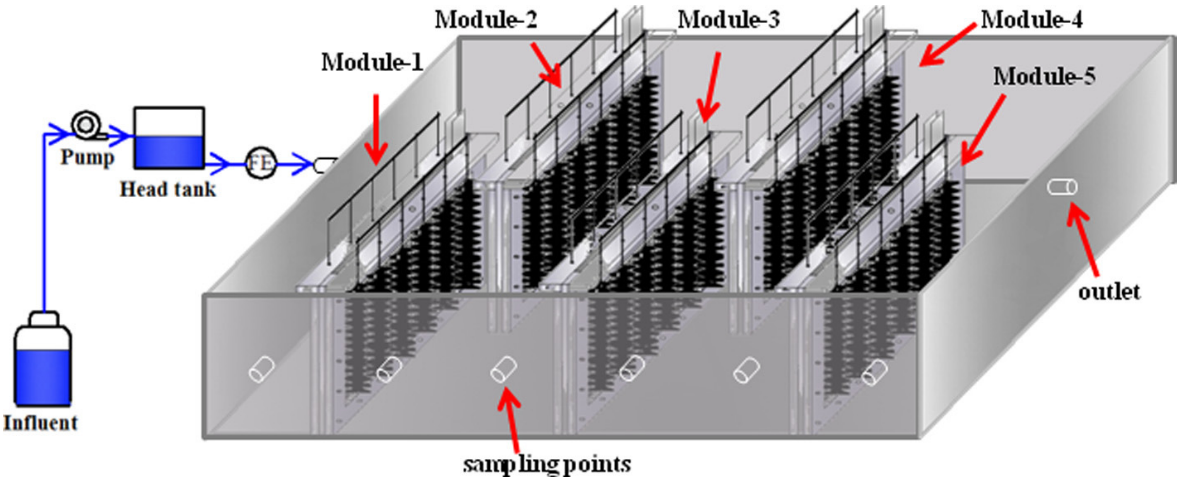
Figure 7.



Air (F)
70.0
50.0
30.0
Water (F)
65.0
55.0
45.0
PSi
15.5
15.0
14.5
rh (%)
100
50
0
57
58
59
60
61
62
63
64
day

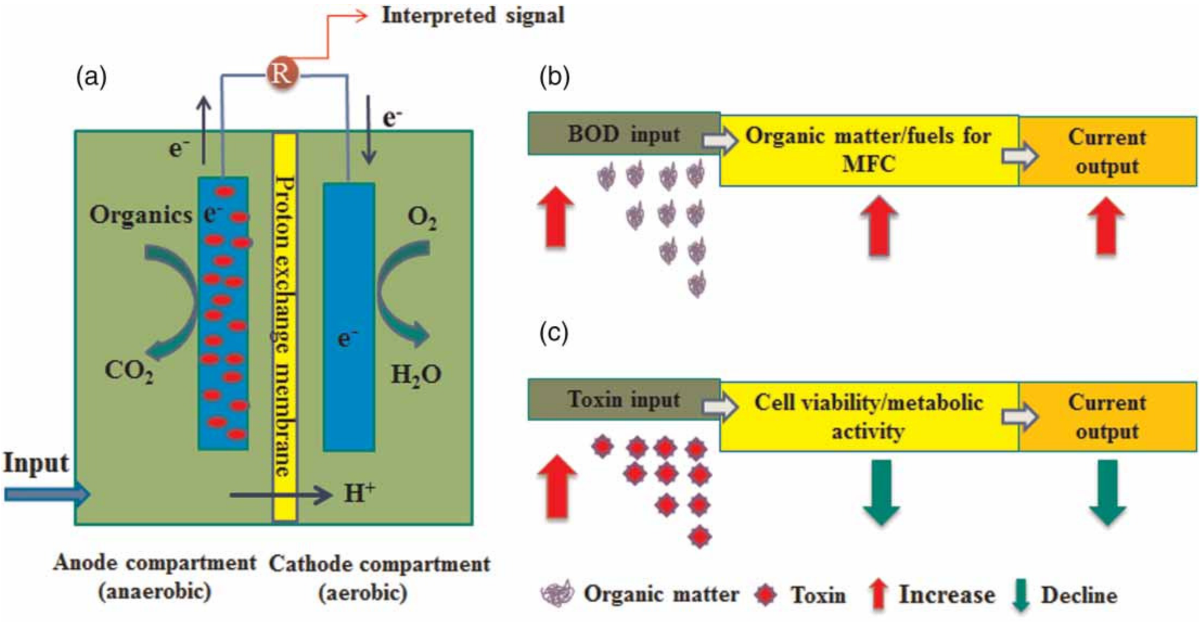
Figure 8.

Figure 9.



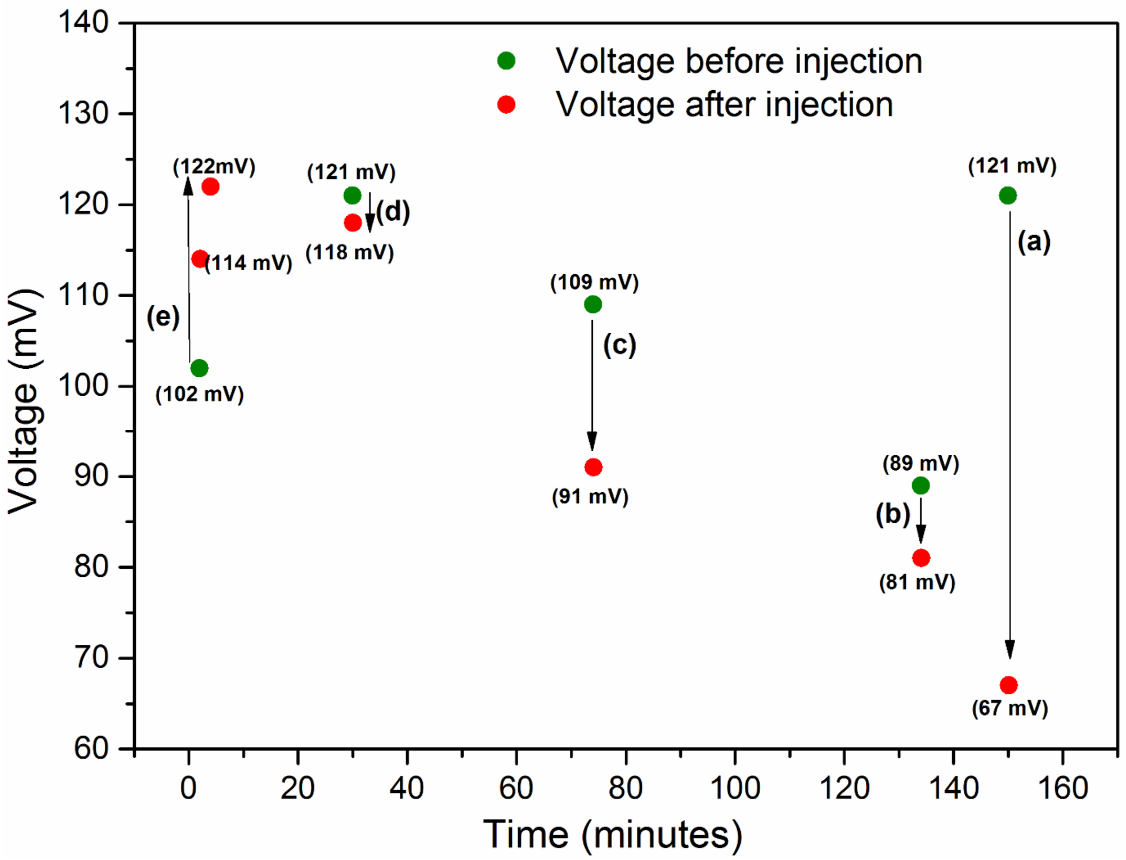
Module-1
Module-2
Module-3
Module-4
Module-5
Pump
Head tank
FE
Influent
outlet
sampling points

Figure 10.



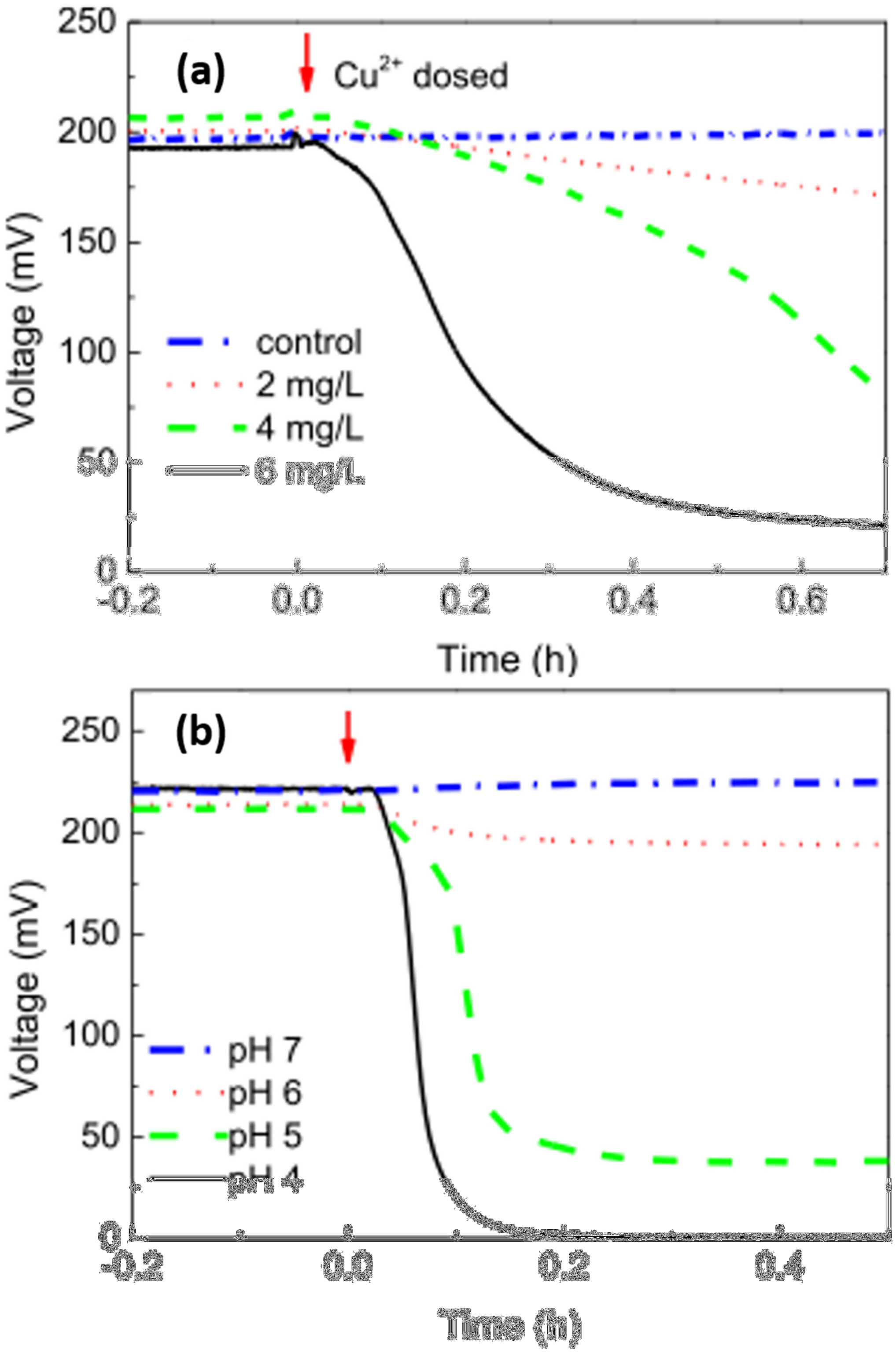
Interpreted signal
(a)
R
e^-
e^-
Organics
e^-
Proton exchange membrane
O_2
CO_2
e^-
H_2O
Input
H^+
Anode compartment (anaerobic)
Cathode compartment (aerobic)
(b)
BOD input
Organic matter/fuels for MFC
Current output
(c)
Toxin input
Cell viability/metabolic activity
Current output
Organic matter
Toxin
Increase
Decline

Figure 11.



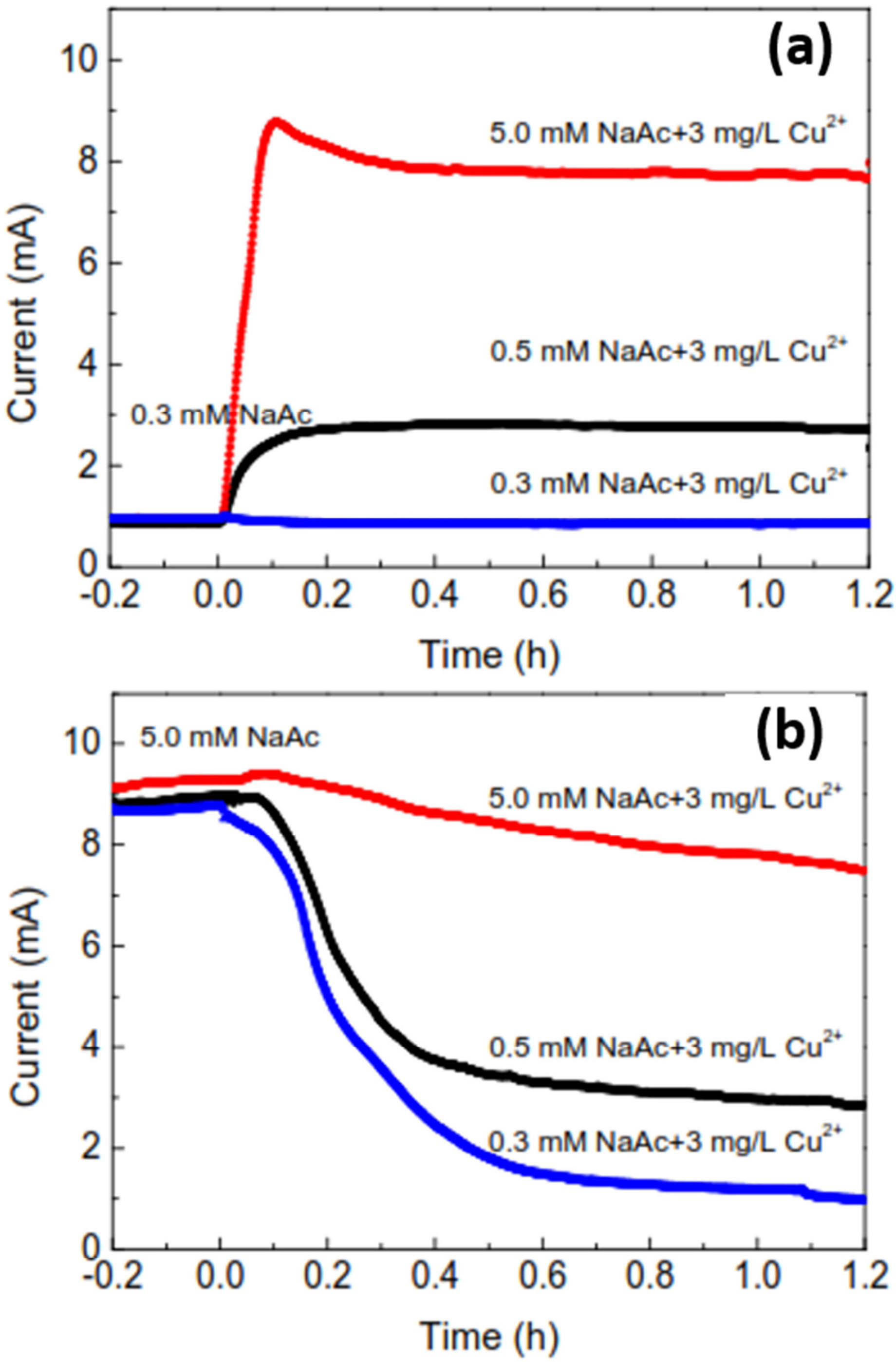
Voltage before injection
Voltage after injection
(122mV)
(114 mV)
(102 mV)
(e)
(121 mV)
(118 mV)
(d)
(109 mV)
(c)
(91 mV)
(89 mV)
(b)
(81 mV)
(121 mV)
(a)
(67 mV)
Voltage (mV)
Time (minutes)
140
130
120
110
100
90
80
70
60
0
20
40
60
80
100
120
140
160

Figure 12.



(a)
Cu2+ dosed
Voltage (mV)
250
200
150
100
50
0
-0.2
0.0
0.2
0.4
0.6
Time (h)
control
2 mg/L
4 mg/L
6 mg/L
(b)
Voltage (mV)
250
200
150
100
50
0
-0.2
0.0
0.2
0.4
Time (h)
pH 7
pH 6
pH 5
pH 4

Figure 13.


(a)
5.0 mM NaAc+3 mg/L Cu^{2+}
0.5 mM NaAc+3 mg/L Cu^{2+}
0.3 mM NaAc
0.3 mM NaAc+3 mg/L Cu^{2+}
Current (mA)
Time (h)
(b)
5.0 mM NaAc
5.0 mM NaAc+3 mg/L Cu^{2+}
0.5 mM NaAc+3 mg/L Cu^{2+}
0.3 mM NaAc+3 mg/L Cu^{2+}
Current (mA)
Time (h)