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Bioremediation of Pollutants and Sustainable Energy Production through Bacterial Activities in Microbial Fuel Cells: An Overview

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Electrical energy generation can be achieved in microbial fuel cells (MFCs) through the catalytic action of microorganisms which can oxidize organic matter and convert it into a biofilm. In MFCs, the exoelectrogens play a crucial role. MFCs is eco-friendly promising technology that produces electricity from various organic substrates. It is a novel and environmentally friendly approach for bioremediation and sustainable electricity production. The fact that heavy metals contributing adversely to the environmental pollution thus the microbial fuel cell technology has a solution for this as well, performing the removal and recovery of heavy metals by using both single and double-chambered MFCs. Many studies show that the new strains of microbes can produce power densities individually as high as strains from mixed communities. However, the implementation of this technology is just limited to the laboratory scale because of a few challenges like low efficiencies, low production rates. This review article focuses an introduction about the role and mechanism of different microorganisms towards energy production, biofilm formation, high power producing microbes inside the microorganisms, the electron transfer mechanism to the electrodes and *vice-versa* and the removal of heavy metals.

Keywords: Biofilm, Microbial fuel cell, Exoelectrogens, Heavy metals, Electricity generation.

INTRODUCTION

In recent years, the microbial fuel cells (MFCs) are the most exciting electrochemical fuel cells in today's research area, commonly it is made up of two parts which are called anode and cathode as shown in Fig. 1. The purpose is generating electricity from organic matter, which exists abandonly on the earth's surface or in the organic wastes, however, most of the bacteria are used as biological catalysts [1]. The bacteria species served as biocatalysts which can oxidize both organic and inorganic matters into carbon dioxide and produce electrons on anode requiring anoxic conditions for generating electric current while transporting these electrons from cells to the surface (anode) [2]. Also, bacteria produce electron shuttles when transferring these electrons to the anode *e.g.* phenazines, flavins, *etc*. The electron mediators can be used to transfer the electrons which are naturally found in the extracellular environment [3,4]. The microbial nanowires are known as conductive pili filament, which can be used to transfer the electrons. In the MFCs studies, for electricity production, chemical mediators like neutral red are used for carrying the electrons from cell to electrode [5]. Further, the electrons transferred at anode combined with oxygen and the water molecules are produced in the cathode chamber [6]. During the process of energy generation, the pollutant reduces to an insoluble form. The MFCs also plays a significant role in wastewater treatment.

Today, heavy metals pollution is creating an alarming situation to the environment because of their toxicity. The heavy metals are essentially concern due to their high toxicity and density effect even at low concentration. Heavy metals cause a major hazard to the marine organism and human health [7]. Heavy metals are produced in the environment by chemical processing industries, metal finishing, dyeing sources and electroplating. They are non-biodegradable, high density and

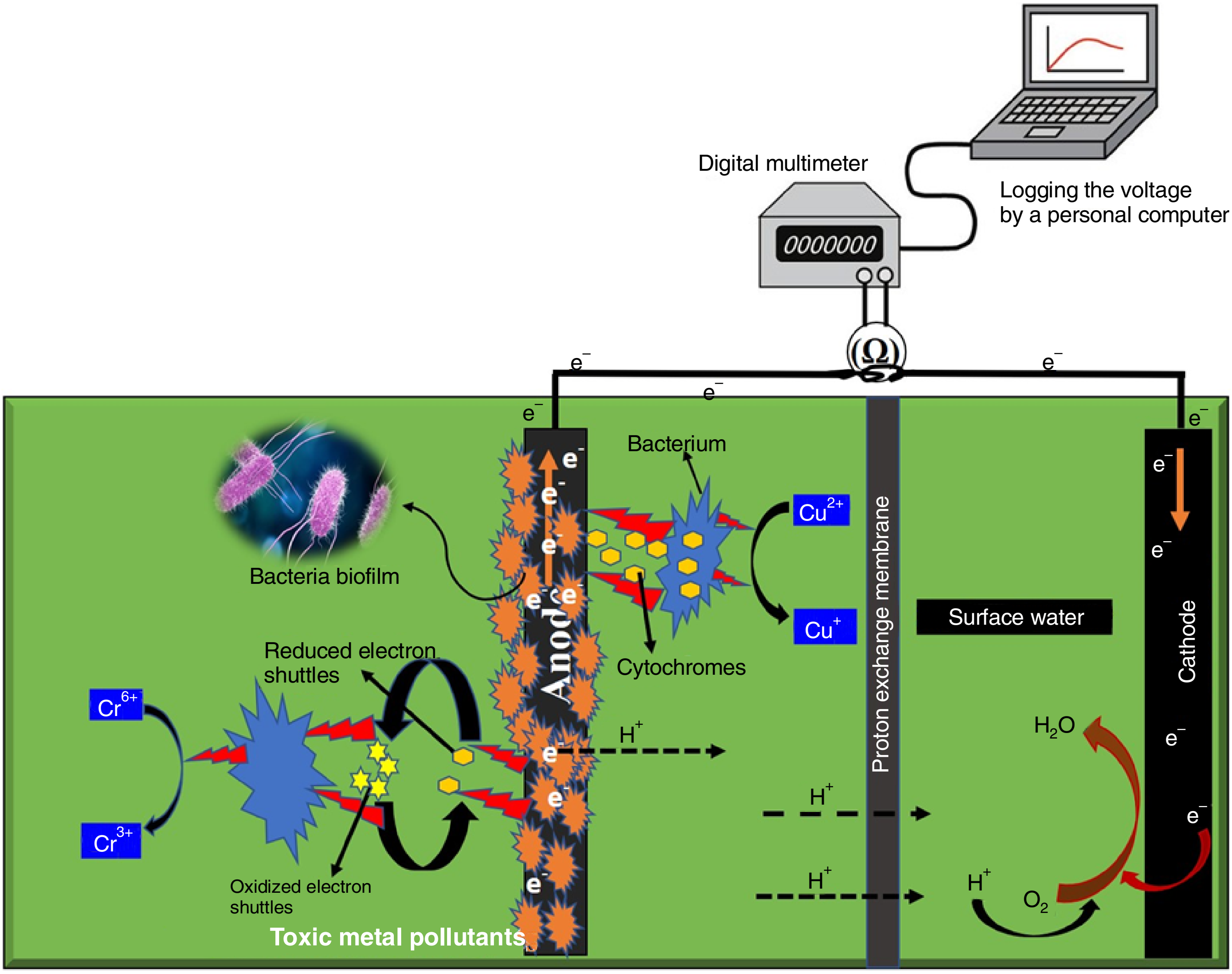

Fig. 1. Basic setup and principal of microbial fuel cells (MFCs)

high solubility power in surface/ground water [8,9]. It can be served as a major source for dangerous health issues for a human being if consumed more than allowed concentration. There are different factors associated with water contamination. Mostly, ground and surface water sources are susceptible due to having different anthropogenic activities like industrial activities, agriculture, mining and many other activities are included [10-13]. These activities are responsible to enhance the heavy metal concentration in water sources due to bad disposal of waste material and wastewater management. If a high concentration of heavy metal enters the human body it may cause serious health issues *e.g.* high quantity of chromium is responsible for serious kidney issues, skin ulceration, skin irritation, nerve tissue syndrome, cardiovascular system and liver damage problems [14]. Similarly, cadmium can cause lung disease, renal dysfunction syndrome and bone defects. Further, nickel and copper can cause different diseases like kidney failure, anemia, stomach pain, liver problems, intestinal irritation and heart diseases. Lead and mercury are most dangerous for human well-being, they are responsible for different diseases such as kidneys, respiratory system, heart, skin, brain damage and Huter Russel syndrome [15-17]. Arsenic is not essentially a metal, but it can be called a semi-metal because arsenic's properties are transitional between non-metals and metals. Arsenic can cause serious medical issues if it is present in the body at an elevated level. The leading source of toxic arsenic is from ground natural water that certainly holds elevated concentrations. In 2007, a case studied by the scientific community noticed that 137 million people are affected by drinking arsenic pollutant water [18]. Therefore, the treatment of heavy metals is highly significant. Scientists have found various ways to remove heavy metals from wastewater. There are many chemical, biological and physical treatment approaches and hence these are being in practice too. Electrochemical treatment technologies, coagulation-flocculation, adsorption, membrane filtration and chemical precipitation are the following methods that are being practiced these days [19-21]. Many researchers are keen to develop a novel approach that can be economical and eco-friendly for heavy metal treatments [2,5]. The low cost and sustainability of MFCs have been an emerging approach for producing energy from wastewater treatment. The process of organic matter oxidation at the anode is joined to the reduction of oxygen at the cathode [22]. At cathode, any compound with higher redox potential than oxygen can be reduced. An element like dye molecules, persulfate, permanganate, ferricyanide, nitrate, along with heavy metals are proved to be sufficient electron acceptors in MFCs [23]. The fact behind the reduction of these compounds is a thermodynamically favourable condition making the electrons flow from anode to the cathode with no external power consumption. The MFCs represents a modern and exciting solution to cost-effectively producing electricity [24,25]. For more than 100 years, the idea of energy generation through bacterial respiration has been considered [26]. Furthermore, the present article reviews the mechanism of energy generation and metal remediation *via* MFCs. Different types of bacterial species are involved in MFCs, which are discussed in the present article.

Energy generation and metal remediation mechanism through microorganism in MFCs: Many bacterial species are capable of transferring electrons through MFCs which has a broad spectrum of applications. In MFCs, in total five groups of firmicutes have shown energy production which includes yeast, fungi, *Microalgae, Proteobacteria phyla* and acid bacteria, iron-reducing *Geobacter* sp., *Aeromonas hydrophila*, *Shewanella* sp., *Rhodoferax ferrireducens*, *Enterococcus gallinarum*, *Clostridium butyricum*, are the typical bacterial species capable to interchange electrons with electrodes [27-29]. Among these bacterial species some of them like *Geobacter species* show electronic characteristics such as conductive polymers, biofilms can play the role of supercapacitors. The bacterial respiration generates the electrons which could be transferred to the electrodes and electric current could be measured. Bacterial species produce a biofilm around the anode and electrons are transferred to the cathode. Carbohydrates as organic substrates are usually used in MFCs during bacterial metabolism and bioelectrogenesis [30]. Entering glycolysis, acetyl coenzyme is produced from molecules of carbohydrate which enters the tricarboxylic acid cycle (citric acid cycle and Kreb's cycle). During Kreb's cycle it generates one reduced flavin adenosine dinucleotide (FADH2) with CO_2 as a byproduct and three molecules of reduced nicotinamide adenine dinucleotide (NADH). This mechanism usually takes place in cytoplasm functioning as electron transporters, the generation of NADH and $FADH_2$ and later to the electron transport chain (ETC) they pass their electrons. through subsequent protein channels, these electrons pass (NADH dehydrogenase, ubiquinone, coenzyme Q and cytochromes) of the ETC and ultimately to the electron acceptor [1,31]. By pumping from the anode, they are transferred to the cathode electrode. Fig. 2 shows that approximately 34 adenosine triphosphate (ATP) molecules and H_2O from the carrier molecules are produced by this whole cycle.

Another side, the reduction of metal during the energy generation is also very significant. Electrotrophs are the microbes capable to accept electrons from the electrode through reduction for remediation of heavy metals. This discovery of electrotrophs has opened a new area in the research. Till today, several microbial consortia showed electrotrophs properties [32]. Gregory *et al.* [33] first explored the opposite direction of the flow of electrons (from electrodes to microbes) and further studied by Thrash & Coates [34]. The bacteria have diverse types of redox-active molecules that serve as electrons shuttles. They can receive the electrons from electrodes and by promoting the fermentation and reduction of inorganic substrates by delivering to bacteria. The species of bacteria which show such capability are *Lactobacillus farciminis*, *Dechlorospirillum anomalous*, *Staphylococcus carnosus*, *Clostridium jungdahlii*, *Shigella flexneri*, *Acinetobacter calcoaceticus*, *Kingella denitrificans*, *Enterococcus faecalis* and *Streptococcus mutans* [32,35]. Due to the low electrode potential driven by bacteria, the protons remain reduced to hydrogen gas. Hydrogen gas is insoluble and the application of this technique is limited by its explosive nature. The hydrogen gas requires an expensive metal catalyst or energy involvement at the electrode surface to counteract the protons reduction. Therefore, the best alternative to

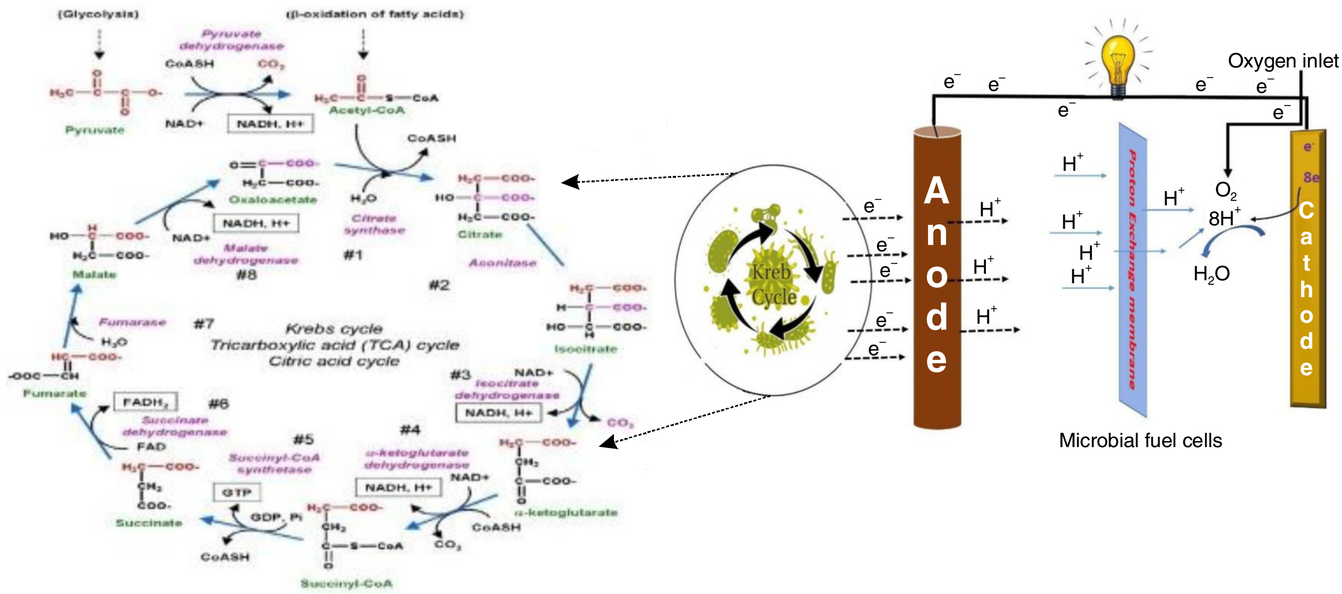

Fig. 2. Graphic demonstration of bacterial metabolism for the transfer of electrons from bacteria to the anode (reproduced from reference [35] with Elsevier Permission Copyright 2020)

solve this constraint and create a high electron transfer rate is powering bacteria with electricity. The only planktonic cells proliferate and electrode-attached cells are not activated by hydrogen gas and normal redox-active molecules. The bacteria cell-electrode remain attached and disconnected from the products, making it possible for the cells attached to the electrode to be forged with electrons [36]. Thrash & Coates [34] first suggested the influence of microbes after considering the *Geobacter* species as electron acceptors from the electrodes. As electron acceptors several species of *Geobacter* decrease uranium(VI), nitrate, fumarate, chlorinated solvents. Many experiments have shown the species of *Geobacter* sp. can accept electrons directly from electrodes [26,27,29]. In *G. Sulfurreducens*, the movement of electrons to electrodes by sulphur reduction was different from the biofilms gene expression which accepted the electrons from the electrode.

The cytochrome gene deletion required for the production of electricity did not affect the movement of electrons to the electrode and the omcZ and pili genes deletion importance for the electricity generation had no significance on operating of the bacterial species activities [37]. Also, electrons enter *G. sulfurreduces* MR-1 for fumarate reduction. Riboflavin developed from this bacterium in the presence of lactate as an electron shuttle mediator and reduces Cr(VI) by the similar Mtr pathway through, which electrons migrate out of the cells [38]. Cytochrome c (Cyc2) was found in the outer membrane used by *Acinetobacter ferroxidase* for O_2 reduction. Inside bacteria (*A. calcoaceticus* and *S. putrefaciens)* the transfer of electrons to pyrroloquinoline quinone releases electron shuttles. Cyt579 and Cyt572 are excreted by *Acidophilic leptospirillum* group II bacteria for oxidation and reduction of Fe(II) as electron shuttles [39]. In electron-receiving cells, the processes of power storage are unstated; across the membrane, only the proton gradient was generated when protons were used for the reduction of electron acceptors. *Geobacter* sp. suggesting that current saving is a better option for current production than for current consumption as current-storage biofilms are considerably thinner than the up-to-date biofilms [40]. The electrons were obtained from the electrode by *Geobacter sulfurreducens* and soluble U(VI) was reduced into the insoluble U(IV) form. The insoluble U(IV) form, which is adsorbed on the electrode surface. By lifting the electrodes from the MFCs, immobilized uranium was quickly removed [39]. The *G. sulfurreducens* have reduced another toxic, soluble form of Cr(VI) from the less toxic to insoluble Cr(III). To pass the electron to these bacteria, onto the anode chamber the reduction of Cr(VI) depends entirely on the oxidation of acetate and the reduction of Cr(VI) in the cathode chamber. By powering bacteria, other organic compounds have also been reduced from the electron-accepting to electron donor. Other than *Shewanella*, *Geobacter* and many bacterial species act as electron acceptors, for instance, *Methanobacterium palustre* can accept electrons and reduced to phenol from dehalogenated 2-chlorophenol [41]. After the 16S rRNA pyrosequencing, Guan *et al*. [42] isolated the *Macellibacteroides*, *Lactococcus* and *Enterobacter* sp. with a high power density of 543.4 mW/m^2 these species can reducing vanadium(V) up to 93.6%. Zhao *et al*. [43] isolated *Spirochaetes*, *Firmicutes*, *Chlorobi*, *Armatimonadetes*, *Chloroflexi* and *Gammaproteobacteria* from concentration 75 to 150 mg/L. With a power output of 419. 11 mW/m^2 after 12 h of operation these bacterial species can remove vanadium(V). However, it is still unknown how electrons are accepted by electrode by molecular mechanisms and can be significant for further research direction. The metal reduction mechanism is also systematically shown in Fig. 1.

Mechanisms of electron transference from bacteria cell to the electrodes: In generating bioenergy through MFC technology it is usually critical for transferring electrons to electrode from the respiration chain of exoelectrogens. Transferring of electrons by microorganisms, process does not count in a natural phenomenon. This mechanism is also not identified yet [44]. Therefore, the electron transfer by exoelectrogens to electrodes several pathways have been suggested. The mechanism, in general, is distributed into two kinds: (i) direct electron transfer (electrodes and cell surface have direct contact); and (ii) indirect electron transfer (in which the contact through electron mediators) (Fig. 3).

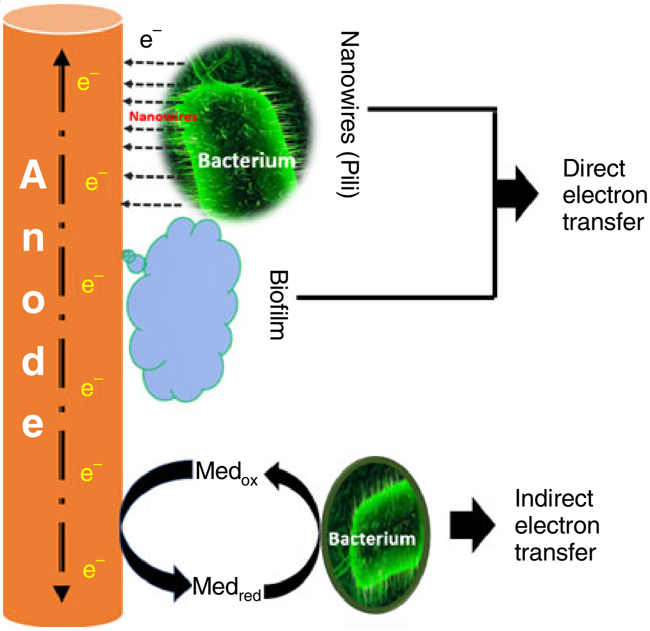

Fig. 3. Mechanism of electron transference of exoelectrogens to the anode

It is necessary for direct electron transfer through the outer membrane of the cell electrons should reach there and for physical interaction between the cell and anode is needed. The electrically conductive nanowires (flagella and pili) at anode surface the electrogene forms biofilms [45]. Through, the direct interaction, the electron moves *via* the cytochrome outer membrane and nanowires. Additionally, the *trans*-membrane electron transportation *via* proteins or limited diffusional electron mediators is also used. An electrode as the electron acceptor is not in direct cell contact. The nanowires are linked to cytochromes attached to the membrane and allow exoelectrogens to use it. In direct electron transfer, electron transport proteins play an essential role in transferring electrons to the outer membrane and finally to the anode from the cytoplasm. The effective current generation in MFCs is the first alternative for direct electron transfer. The drawback of direct electron transfer is that proteins usually bury within proteins the active sites of electron transport proteins, resulting in a low rate of electron transfer [46]. To date, only a variety of species of electrochemically active bacteria, such as *Geobacter* and *Shewanella* are known to carry electrons away from the cell with bacterial nanowires [47]. The transfer of electrons is carried out with the help of lower soluble molecular-based mediators. For indirect electron transfer, removing the need for close interaction between the acceptor of the electron and the cells. The electron mediators have been able to penetrate bacterial cells, electrons collected from the results of the exoelectrogens metabolic reactions which further supplied the electrons to the anode electrode.

The existence of electron mediators was first considered necessary for the activity of the MFC. They can be produced through the exoelectrogens or applied to the anode chamber externally. Several classes of bacteria have been recognized for self-mediator syntheses, such as pyocyanin, phenazine, *etc*. The mediators and the redox proteins will have a major effect on electron transfer efficiency because of the potential difference [28,48-51]. To increase the performance of electron transference, a variety of chemical composites such as anthracenedione, methylene blue, neutral red, humic acid, thionine and riboflavin have been tested [52,53]. The involvement of exogenous mediators, however, is not recommended since they often contribute to comparatively low current densities as well as becoming costly and dangerous to microorganisms, allowing low efficiency over long periods, rendering it impossible to commercialize the technique. Also, the addition of exogenous mediators daily is technologically unworkable and environmentally uncertain. Therefore, if the bacterial species can be effectively used as a catalyst without any mediators. It is theoretically practicable that, as well as being environmentally healthy, there is no need to eventually incorporate electron mediators.

Biofilm of electrode: The biofilm is generally known as a microbial city. The polymeric matrix containing carbohydrates, proteins, lipids, *etc.* It is usually due to the bacteria who are involved in its surface and it is the most preferable environment for them to live and they form a biofilm. Efficient energy can generate through the production of electroactive biofilms. The biofilm formation can be monitored *via* various pathways, which is more likely depend on the microbes used in MFCs with the operating conditions and electrode material along with the substrates of the MFCs. Biofilm formation can be affected by the electrode's physical and morphological properties in some of the recent studies [54].

In recent studies, it is suggested that on electrode the bacteria are inadequate to make biofilms the same way in MFCS, cannot generate significant current densities. On the other hand, the anode bacteria are skilled to produce higher current densities on thick biofilms rivalry to a bacterium which can form thin biofilms. Gram-positive *thermincola ferriacetica* can produce a persistent current density 7-8 A/m^2 while forming thick biofilms (~38 μm) [55]. In contrast with *Thermincola potens* can produce relatively lower current densities while forming monolayer biofilms [54]. Following are some of the ways, which can trip the mechanism of biofilm formation like in MFC on anode or cathode the microbe's attachment to the surface and by the transport towards the surface, maturation of biofilm and microcolonies formation [56]. The formation of biofilm is through the bacterial production of nucleic acids, carbohydrates (polysaccharides), adhesins and proteins that interrelate and cover the bacteria [57]. During the metabolism of the electroactive, biofilms can respirate through terminal electrons onto electrode surfaces. For example, *Shewanella* sp. in the biofilm formation flavins which are the redox-active mechanisms can moderate the exocellular electron transport [58]. With the moving motion of flagella, it transports bacteria to the surface during the biofilm formation in *P. aeruginosa.* mushroom-shaped biofilm formed by the maturation method which needs a cell-signaling with that type IV pili can form microcolony and cellular aggregation [59]. There is a method called quorum sensing, which allows the communication and coordination of group behaviour in the bacterial population. Quorum sensing manages the outflow of biofilm-related qualities for auxiliary advancement of biofilm in *P. aeruginosa* and the different microorganisms. The blended culture of biofilms creates higher power density than pure culture. The blended culture or diverse culture injected into MFC delivered around 20% higher power efficiency than the pure culture [60]. Nonetheless, the function of non-exoelectrogens and generating power isn't known. Bacterial cells are in close interaction with the anode electrode surface and electrons move directly to the anode, without any c-type cytochromes. The utilization of bacterial species at the cathode to catalyze oxygen which decreases the enthusiasm for cathodic biofilm. In an adversary to anode biofilms, it has been seen that age influence gradually diminishes with increment in the width of cathode biofilms.

Bacterial role in MFCs: For electricity generation, bioremediation and for many more fundamental applications many microbes have been tested. In the MFC technique, the substrate is used from different sources like wastewaters (protein-rich wastewater, chocolate industry water, paper recycling wastewater, brewery wastewater, swine wastewater) and many nutrients (lactate, sucrose, xylose, glucose, acetate, ethanol and starch) [61]. However, only a few of the selected microorganisms can produce electricity in MFCs. In several types of MFCs, the exoelectrogens from different groups of organisms like algae, yeast, cyanobacteria, Gram-positive/negative bacteria and fungi have been used in the anodic chamber [62]. Different microorganisms producing sufficient generation of electricity can oxidize the multifaceted organic matter into their components (Table-1). However, for its energy production, specific exoelectrogens may oxidize specific substrates. Moreover, each exoelectrogens has diverse paths and genes, proteins or enzymes for its oxidation or degradation, depending on the type of substrate [63,64]. The selection of an effective bacterial consortium and the preferred substrate therefore determines the performance of the MFCs. For instance, when worked for 3 months and MFCs nourished with aerobic-anaerobic slush inoculant and glucose to generate and convert the electricity [65]. Organic substrates that contain lipids, proteins and carbohydrates as electron donors are used in MFCs for redox anode reactions to generate energy. Through, the glycolysis and other related processes, these multifaceted organic molecules supplementary undertake acetyl Co-A dispensation processes, which then contributed in the citric acid cycle. Three reduced NADH are produced from three nicotinamide adenine dinucleotide (NAD^+) one flavin adenosine dinucleotide (FAD) reduces to FADH2 and CO_2 is released as a by-product in the single cycle of citric acid [66]. Metabolic pathways such as (glycolysis and Krebs cycle) occur in both prokaryotes (bacteria) and eukaryotes (yeast) in their cytoplasm. NADH and FADH2 serve as electron carriers that transfer their electrons to the ETC for making energy carrier molecules called ATP. Respiratory reactions occur in bacteria in their cell membrane (constituting the outer cell membrane, inner cell membrane and periplasm) that contains the machinery [67]. All the proteins or enzymes necessary for electron transfer (MFC base) resides on the inner mitochondrial membrane in yeast. Usually, ETC contains four intermediary proteins, cytochromes, NADH dehydrogenase and ubiquinone, coenzyme Q (however these intermediate proteins may differ by species). The electrons are transmitted to the final electron acceptor *via* these proteins and the protons (reduced) are pumped out of the cell in the anode is then transmitted *via* PEM to the cathode [68]. Chemical mediators were used to catalyzing electron transfer from within the bacterial cell to the anode surface before the idea that bacteria could promote electron transfer. While reacting with ETC components they get reduced transferring their electrons to the anode when release out from the cell. Having the potential anode metabolism of the bacteria usually switch from oxidative phosphorylation (metabolism) to fermentative metabolism [69]. Bacteria choose fermentation metabolism in the unavailability of electron acceptors, but when the electrons are accumulated on electron acceptors bacteria modified to oxidative metabolism in the existence of electron acceptors on the low anode potential. Only one-third of electrons used for electricity generation and other electrons used in the fermentation products and can oxidized by anaerobic bacteria such as *Geobacter* sp. in MFC for current generation in fermentation processes. Many bacteria (*Clostridium* sp., *Enterococcus* sp.) have been anaerobically inoculated in MFCs to generate fermentation products, beyond electricity generation [70]. The bacteria *Geobacter* sp. *Clostridium* sp. is the most effective exoelectrogens present in MFC. It's the most effective source of hydrogen in the MFCs showing higher power densities than pure cultures biofilms of mixed consortia because of the networks of metabolisms between the bacteria it can be conceived in biofilms, but it needs to be thoroughly elucidated and experimentally validated [71]. In deciding bacterial metabolism, the capacity of the anode plays an important role. Negative anode potential delivers the electrons which can affects the bacteria by more reduced complexes. As a result, less energy is absorbed by the bacteria and the energy recovery in MFC is greater and hence the power production is greater. Clearly, at negative anode potentials, the microbial population of sulfate-reducing bacteria developed greater power density, 45 mA m^{-2} at -0.6 V than 15 mA m^{-2} at -0.2 V [72]. Setting the cathode potential has also been shown to boost MFC efficiency. A study showed that the reduction of MFC for Cr(VI) with fixed cathodic potential at -300 V increased the maximum power density from 4.1 W/m^3 (control, no fixed potential) to 6.4 W/m^3 and the start-up time was reduced relative to control to 19 days from 26 days [73].

Anodic bacterial species: In MFC technology, the most studied and adequate exoelectrogens belongs to the bacteria family of *Geobacteraceae*. With approximately 100% electron recovery to produce energy, *G. sulfurreducens*, δ-*proteobacteria*, can reduce acetate. In an MFC with metal electrodes, acetate as electron donor and fumarate as electron acceptor, the species has effectively developed the present density of 3147 mA/m^2 [74]. While using wastewater as inoculum in MFC, *G. metalloproteins* (pure culture) can generate only 40 mW/m^2 power output using them as electron acceptors, *Shewanella* spp., γ-*proteobacteria* reduces manganese and iron [75]. Generating power density of 3000 mW/m^2 in a miniature MFCs lactate *Shewanella oneidensis* DSP10 as being anolyte it uses lactate and as catholyte, it used ferricyanide which is highly appreciable [76]. Generating the highest power density of 4.92 W/m^3 in a single-chamber MFCs it uses $CaCl_2$ as anolyte [77]. With a power density of 2720 mW/m^2 in the same MFCs, the bacteria's such as a photosynthetic purple non-sulfur bacterium, α-*proteobacteria Rhodopseudomonas palustris*, usually utilizes thiophosphate, volatile acids, yeast extract comparing with mixed cultures it is considered to be very high. *Thermincola ferriacetica*, which is a thermophilic Gram-positive, metal-reducing bacterium displays the maximum power density 12 A/m^2 from acetate. Producing the power density of 4310 mW/m^2 γ-*proteobacteria Pseudomonas aeruginosa* uses graphite electrodes as the electron acceptor and glucose as an electron donor [70]. In MFC, *Desulfovibrio desulfuricans* which is a sulphate-reducing bacterium generating the most power density of 233 mA/m^2 from surface-treated graphite felt electrodes displaying 50 % higher than untreated electrode [78]. Gram-negative bacteria *E. coli* offered 1300 mW/m^2 at 3390 mA/m^2 energy efficiency *via* MFCs.

In MFC, *Saccharomyces cerevisiae,* with no use of artificial mediators (open-air cathode) using non catalyzed graphite fed with synthetic wastewater as electrode produces 282.83 mA/m^2 power density [79]. Using ferricyanide as catholyte and Pt as electrode many yeast *Hansenula anomala* produced 2.9 W/m^3 current density [80]. Producing the outstanding 720 mW/m^2 current output in MFCs, *Candida melibiosica* with surface nickel nanostructures on altered carbon fed electrode was used [81]. Few microorganisms not widely used in MFCs have also demonstrated the potential to generate electricity and a few novel exoelectrogens have recently been discovered. A new exoelectrogens has been revealed by 16S rRNA gene sequence analysis; *Geobacter anodireducens* has demonstrated 98% resemblance to *Geobacter sulfurreducens*, but cannot decrease (fumarate) as the electron acceptor [82]. The *Ochrobactrum* sp. another novel strain 575, recently isolated from xylose MFC's anodic chamber, produced a maximum power density of 2625 mW/m^3. Also, the findings showed that the digestion of xylose in *Ochrobactrum* sp. was distinct from other electroactive bacterial strains, which rely on the respiratory chain of succinate oxidation rather than the conventional respiratory chain of NADH

TABLE-1
SUMMERY OF HEAVY METALS REMEDIATION EFFICIENCY AND ENERGY GENERATION *via* MFCs

Incubation source	Target metals	Electrodes		Operating time (h)	Temp. (°C)	pH	Initial conc.	Power density	Removal efficiency (%)	Ref.
Shewanella oneidensis MR-1	Cr	Graphite felt	Graphite rod	192	30	7	200 mg/L	32.5 mW/m^2	67	[36]
Mixed microbial culture	Cr	Graphite plates	Graphite plates	26	25	2	26 mg/L	-	97	[110]
Anaerobic sludge	V	Carbon fiber felt	Carbon fiber felt	240	30	6	100 mg/L	970.2 ± 60.5 mW/m^2	67.9 ± 3.1	[111]
Actinobacteria, β-*Proteobacteria,*	Cr	Graphite felts	Graphite felts	144 and 192	35	-	5 mg/L 25 mg/L	-	5 mg/L with 93, 25 mg/L with 61	[112]
Shewanella genus	Au	Pt-graphite	Pt-graphite	-	25	2	200 ppm Au with 1,000 ppm standard solutions of HAuCl4	-	60	[113]
Indigenous bacteria from Cr(VI)-	Cr	Graphite plate	Graphite granular	173	22-26	2	39.2 mg/L	6.9 W/m^3	-	[114]
Mixed microbial culture	Cu	Graphite felt	Graphite felt	-	25	2-5	10-200 mg/L	0.319 W/m^2	> 99	[110]
Anaerobic sludge	Cr	Carbon fiber felt	Carbon fiber felt	240	30	6	100 mg/L	970.2 ± 60.5 mW/m^2	75.4 ± 1.9	[111]
Sludge mixture	Ag	Carbon brush	Carbon cloth	8	25	7	50-200 ppm	4.25 W/m^2	99.91	[115]
Shewanella decolorationis S12, K. pneumonia	Cr	Carbon felt	Carbon felt	3.5	30	2	10 mg/L	52.1 mW/m^2	99.9	[116]
Mixed microbial culture	Cr	Graphite plates	Graphite plates	150	25	2-6	200 mg/L	0.150 W/m^2	100	[110]
Oil sands tailings affected water	Hg	Carbon cloth	Carbon cloth with Pt coating	800	21 ± 0.	1	581 ± 26 Se, 226.4 ± 4.7 Ba, 152.8 ± 16.8 Mo, 35.8 ± 13 Pb	392 ± 15 mW/m^2	97.8 Se, 96.8Ba, 77. Mo, 32.5 Pb	[117]
Mixed microbial culture	Cu	Graphite felt	Graphite felt	480	27	2	600 mg/L		92	[110]
Anaerobic granular sludge	V	Carbon fiber felt	Carbon fiber felt	72	35	1	25 mL	578.3 mW/m^2	87.9	[118]
Anaerobic sludge	Cr	Unpolished graphite plate	Natural rutile-coated polished graphite	26	22	2	26 mg/L	-	97	[119]
Mixed microbial culture	Cu	Graphite plate	Graphite plate	264	25	4.7	200 mg/L	339 mW/m^3	> 96	[110]
Sewage sludge	Cd, Zn	Carbon cloth (no wet proofing)	carbon cloth (30% wet proofing)	-	32	6.8	200 mM Cd; 400 mM Zn	3.6 W/m^2	Cd 90, Zn 97	[120]
Domestic wastewater	Cr	Graphite plates	Graphite plates	150	25	2	100 mg/L	150 mW/m^2	100	[121]
Anaerobic sludge	Cu	Graphite felt	Graphite plate	144	35	-	500 mg/L	314 mW/m^3	70	[122]
Mixed microbial culture	Cr	Carbon cloth	Carbon cloth	48	25	2	100 mg/L	0.767 W/m^2	99	[110]
Dysgonomonas and Klebsiella	V	Carbon fiber felt	Carbon fiber felt	168	22 ± 2	-	200 mg/L	529 ± 12 mW/m^2	60.7	[123]
Dysgonomonas and Klebsiella	Ag	Carbon cloth	Graphite	21	-	6.2	-	317 mW/m^2	99.9	[124]
Algae biomass	Cr	Activated charcoal	Activated charcoal	96	-	2	200mL	207 mW/m^2	98	[125]
Tetrachloroaurate wastewater	Au	Carbon brush	Carbon cloth	12	25	2.8	2,000 ppm	6.58 W/m2	99.89	[126]
Anaerobic digestion sludge	Cr	Carbon felt	Carbon felt	16	-	2	50 mg/L,	343 mW/m^2	98	[127]
Mixed microbial culture	Cu	Graphite plate	Graphite foil	144	-	3	1 mg/L	0.80 W/m^2	99.8	[110]

Anaerobic cultures mixed with Cr(VI)	Cr	Graphite plate	Graphite plate	1080	22-24	-	80 mg/L	55.5mW/m^2	60	[128]
Mixed microbial culture	Ag	graphite plate	Graphite felt	26	-	2	200 mg/L	0.109 W/m^2	95	[110]
Klebsiella sp. MC-1	Cr	Carbon cloth	Carbon cloth	75	25	5	50 mg/L	412 mW/m^2	99.1	[129]
Anaerobic sludge bed	Pt	Graphite plate	Graphite plate	24	25	7	16.88 mg/L.	844.0 mW/m^2	90	[130]
Anaerobic sludge with Copper	Cu	Graphite felt	Graphite plate	144	35	-	500 mg/L	314 mW/m^3	70	[122]
Anaerobic sludge	V	Carbon fiber felt	Carbon fiber felt	72	25	7	500 mg/L	0.572 W/m^2	25	[110]
Anaerobic sludge	Cu	Graphite plate	Graphite felt	20	35	3.5	200 mg/L	314 mW/m^3	> 99	[131]
Anaerobic sludge	Hg	Graphite felt	Carbon paper	10	30	2	100 mg/L	433.1 mW/m^2	98.22-99.54	[132]
Mixed microbial culture	Cd	Graphite felt	Graphite felt	168	25	7.1	50mg/mL	700-750 mW/m^2	60	[133]
Sediment sample	Cr, Cu	Graphite felt	Graphite felt	2160	37	2	250 mg/L	400-450 mW/m^2	96	[134]
Lithium cobalt oxide Solution	Co	Graphite felt	Graphite felt	-	35	1	200 mg/L	298 ± 31 mW/m^3	62.5 ± 1.8	[135]
Mixed microbial culture	Se	Carbon cloth	Carbon cloth	48	25	7	75 mg/L	2.90 W/m^2	99	[110]
Geobacter and *Pseudomonas*	Cu	Carbon brush	Reduced Graphene oxide	-	25	6	12 mg/L	0.95 W/m^2	98	[136]
Anaerobic sludge	Cr	Graphite brushes	Graphite granules	-	25	7	10 mg/L	6.4 W/m^3	94	[137]
Mixed microbial culture	Hg	Graphite felt	Graphite felt	360	25	6.8	25mg/mL	800 mW/m^2	55	[133]
Anaerobic sludge	TI	Carbon felt	Plain carbon paper	72	22 ± 2	-	100 µg/L	457.8 ± 15.2 mWm^{-2}	67	[138]
Activated sludge	Cu	Graphite felt	Graphite plate	672	35	-	100 mg/L	140 mW/m^2	96	[137]
Mixed microbial culture	Ag	Carbon brush	Carbon cloth	8	-	7	200 mg/L	4.25 W/m^2	99	[110]
Dilute synthetic sample	Cd, Zn	Carbon felt	Carbon felt	1344	25	3	7.4 mg/L Cd^{2+} 19.5 mg/L Zn^{2+}	-	Cd 82, Zn 89	[133]
Anaerobic sludge	Cu	Graphite plate	Graphite plate	20	35	4.7	6412.5±26.7mg Cu^{2+}/L	339mW/m^3	> 99	[131]
Anerobic pure culture	V	Carbon fiber felt	Carbon fiber felt	-	30	-	-	970 mW/m^2	68	[137]
Anerobic pure culture	Cr, Cd	Carbon rod	Graphite felt	24	30	5.8-6.0	Cr (VI) 385 µM, Cd (II) 179 µM.	14.2 mW/m^2	73 for Cr, 61 for Cd.	[139]
Primary clarifier effluent	Cr	Graphite brushes	Graphite granules	2	22 ± 3	7	20 mg/L	970 mW/m^2	76	[137]
Activated sludge	Zn	Carbon cloth	Carbon cloth	-	25	-	-	3600 mW/m^2	97	[137]
Mixed microbial culture	Au	Carbon brush	Carbon cloth	-	-	2	200 mg/L	6.58 W/m^2	99.8	[110]
Contaminated soil	Cd	Graphite granules	Carbon felt	3432	25	6.8	100 mg/L	7.5 mW/m^2	31	[140]
Mixed microbial culture	Pb	Graphite granules	Carbon felt	2592	25	6.9	900 mg/L	3.6 mW/m^2	44.1	[134]
Anaerobic sludge	Ag	Carbon cloth	Graphite felt	21	26	9.2	1000 mg/L	0.3 W/m^2	99	[110]
Mixed microbial culture	Cr	Carbon brush	Carbon cloth	-	30	7	100 mg/L	419 mW/m^2	99	[137]
Dilute synthetic sample	Cu, Pb	Carbon felt	Carbon felt	864	25	3	1.1 mg/L Cu^{2+}, 2.5 mg/L Pb^{2+}	17.1 to 25.2 mW/m^2	Pb 92, Cu 99	[141]
Mixed microbial culture	Co	Graphite felt	Graphite felt	48	30	1-3	1000 mg/L	-	99.15	[110]
Anaerobic sludge	Ni	Graphite felt	Graphite plate	24	30±1	7.1	26.4 mg/L	0.68 - 0.7 mW/m^2	95	[142]
Anaerobic sludge bed.	Cu	Carbon brush	Carbon cloth	5	35 ± 1	6	12.5 m/Lg	0.2W/m^3	98.3	[143]
Mixed microbial culture	Ni	Graphite felt	Graphite felt	720	25	7	32.9 g/180mL	150-200 m W/m^2	-	[133]

oxidation. In a cubic air-chamber MFC, *Klebsiella pneumonia*, Gram-negative, non-motile, lactose fermenting bacteria produced 199.2 mA/m^2 current density and 426.2 mV maximum voltage output [83]. A Gram-positive *Lysinibacillus spherics* bacterium in MFCs with graphite used as an electrode provided a maximum current density of approx. 270 mA/m^2 and 85 mW/m^2 power density [84]. In comparison, *Citrobacter* sp. SX-1 can use different basic substrates in MFCs such as lactose, glucose, glycerol, sucrose and acetate, but has provided citrate with the maximum current density of 205 mA/m^2 [85]. In addition to bacteria microalgae and yeast, have also been used either as a bioanode or a substrate supporting the anode for the typical application in MFCs technology. In the anode, *Scenedesmus*, green algae as a substrate in powder form and *Chlorella vulgaris* as a biocathode in MFCs provided a combined power density of 1926 mW/m^2 [86]. In another research, Arthrosporic maxima were used for the metabolism and development of as a substrate as well as a carbon source. *Palustris* demonstrated a volumetric power density of 10.4 mW/m^3 in micro-MFCs, which was the highest in contrast with other substrates used in the analysis [87]. Also, in the MFC, blue-green algae (cyanobacteria) developed a maximum power density of 114 mW/m^2 at a current density of 0.55 mA/m^2 [88]. The degradation of algae produces transitional compounds such as acetate and lactate in microalgae aided MFCs that can be more utilized through exoelectrogenic agents such as *G. sulfurreducens*.

Cathodic bacterial species: In MFC *Geobacter* spp. also act as biocathodes for accepting electrons from cathodic electrodes. The studies show that *Geobacte sulfurreducens* as the sole electron donor with electrode it can reduce fumarate to succinate producing the current density of 20.5 A/m^2 in a reactor with stainless steel electrodes, while *G. metallireducens* it can reduce nitrate to nitrite [89]. In the air-cathode MFC, *Shewanella oneidensis* MR-1 acts as biocatalyst and lactate as electron donor which showed improvements in the reduction rate of Cr(VI) with a maximum current density of 32.5 mA/m^2 [38]. The study shows the expression of riboflavin in the transport of electrons by using external membrane-bound cytochromes. The *Shewanella putrefaciens* and *Acinetobacter calcoaceticus* demonstrated the ability to reduce oxygen in water at an elevated rate [90]. *Acidithiobacillus ferrooxidans* which is an acidophile microorganism in MFC, can feed as a biocathode up to 5 A/m^2 of current densities and at low pH, it can obtain oxygen reduction. On acetate oxidation catalysis, the electrochemical reduction of oxygen produces a maximum current density of 145 mA [88]. On the carbon electrode, *Micrococcus luteus* and other Gram-positive (*Staphylococcus* spp., *Lactobacillus farciminis*) and Gram-negative bacteria (*Pseudomonas fluorescens*, *Escherichia coli*, *Acinetobacter* sp.) can catalyze the electrochemical reduction of oxygen shown by cyclic voltammetry. At various set potentials the seawater coated on stainless steel electrode forms aerobic biofilms showed effective catalyzation of oxygen reduction and gained current densities up to 460 mA/m^2 [91]. The maximum power density of 1926 mW/m^2 was produced by an acetate fed MFC using *Chlorella vulgaris* as a biocathode. CO_2 provided by the anode was used by *C. vulgaris* as a carbon source for its formation. The analysis also revealed that *C. vulgaris* without the availability of anodic CO_2, did not expand in acetate-fed MFCs. During the immobilization of *C.vulgaris* the MFCs became highly effective in the cathode chamber, resulting in a power density of 2485.35 mW/m^3 at a current density of 7.9 A/m^3, while the MFC with suspended *C. vulgaris strain* of white-rot fungus, *Coriolus versicolor* (secretes laccase to reduce oxygen at the cathode), inoculated in the cathode chamber of an MFCs to catalyze the cathodic reaction, developed a combined power density of 320 mW/m^3 [92].

Removal of heavy metals by MFCs

Chromium: It is present on earth crust in two oxidation states *viz.* Cr(III) and Cr(VI). Between them, the Cr(VI) state is considered as harmful in the sense of contaminants from tanning and metal industries and become the toxic and carcinogenic source for the ecosystem. Due to its toxicity, it is a major concern that how we do remove it from the environment [93]. In literature, the first-time removal of chromium is done by using graphite paste electrodes for the reduction of this toxic element. That study shows appreciable results such as a power density of 1540 mW/m^2 and a reduced rate of ~2.13 g/m^3/h at a concentration of 100 mg Cr(VI) [94]. Moreover, instead of using the expensive membrane, literature shows the use of the salt bridge for more efficient reduction of Cr(VI) up to power density in the range of 92.65 W/m2 for 5 mg/L of Cr (VI) and 75.08 mW/m^2 for 80% of 10 mg/L of Cr(VI) [95]. In previous studies, it is explained the pH imbalance between anion and cation chambers in MFCs become the cause of the less stable system and also reduces the bioelectricity production [96]. For evaluating the problem, the use of bipolar membranes supplies better performance and increase the production of bioelectricity. Moreover, it also increases the efficiency of the removal of Cr(VI). With the help of PEM, pH of both chambers was reduced due to the flow of electrons along with PEM. It is not only cost-effective but also increases the reduction ranges of MFCs. In previous studies, effective CNF carbon nanofibers electrode doped with alumina/nickel nanoparticles were also used efficiently along mediator less MFCs [97]. In earlier study, Cr(VI) was removed at maximum power density of 93% cathodic columbic effect. Moreover, Hanak *et al.* [98] discussed Fe(III) as mediator in MFCs to boost up the reduction of Cr(VI). The aspect of Fe (III) exposed that it is the source of enhancement of cathodic columbic efficiency and also the increment of reduction of rate of Cr(VI). There is an evidence of the electrodes used which are made up of carbon, carbon cloth and carbon brushes for efficient removal of Cr(VI). Moreover, the most efficient functioning in these three electrodes is of carbon cloth through the electrochemical reduction of Cr(VI).

Vanadium: This element is produced in bulk which is about 38000 tons production yearly. It is known as steel addictive because of its unique properties *i.e.* steel-vibration and shock resistance. Vanadium is also considered as an effective element for various human diseases. The reduction of vanadium(V) is achieved by different types of microbes named as *Rhodoferax ferrierducens* in the anode chamber 300mg/L of $NaVO_3$ added in anode chamber to increase the current range up to 0.06 mA

[99]. Various factors such as pH, temperature and stirring speed affected the proceedings of reduction in vanadium(V). Kilicarslan *et al.* [100] introduced the beneficial procedure of two different electron acceptors in the cathodic chamber. Along with this procedure, not only the reduction of vanadium is achieved but also the reduction of Cr(VI) is also achieved. And the power output shows considerable efficacy in this study using only one electron acceptor. Moreover, the results of this study as the reduction efficiencies of V(V) and Cr(VI) were 67.9 and 75.4%, respectively, proved its high power output demand.

Copper: This element has a controversial issue globally in terms of its supply and demand. Its generation from industrial and radioactive wastes makes its extraction and recovery crucial for researchers. It is known as a toxic material for human beings as well as for the environment. Due to its toxicity, its removal is more necessary than its recovery stage. Birloaga and F. Vegliò [101] used the bipolar membranes in MFCs to improve the efficiency of copper removal up to 99.88% along with maximum power output of 0.43W/m^2. The whole setup is generated in anaerobic conditions in cathodic chambers. In literature, there is also the removal of copper in anodic chambers of MFCs along with sulfate-reducing bacteria. This metal up to 20 mg/L stimulates the biological reactions by improving the MFC performance at the low metal concentration [102]. It has been done by introducing four alterations to reduce the internal resistance. The first one is basically to reduce the anodic and cathodic distance from 3 to 0.5 cm, the second one is to reduce internal resistance by the use of an anion exchange membrane, third is the use of copper plate as a cathode in the place of graphite paper, the fourth one is the replacement of graphite paper with carbon felt as an anode to achieve more surface area [103].

Silver: It is one of the precious elements present in different types of industrial effluents. Its unique characteristics such as malleability, ductility, high conductivity, light reflexive and firm strength make it more demanding in the field of ornaments making, electronics and photography [104]. The major drawback of this element is that it has fewer natural resources or its availability. That is why, researchers recovered this element from industrial effluents for economic and environmental benefit. In literature, the mentioned methods of Ag removal from wastewater were adsorption, chemical precipitation, bioreduction and bioabsorption [105]. In one study, there is the removal of silver in the range between 99.91-98.26% for the cost-effective MFC after 8 h work. Its initial concentration ranging from 50 to 200 ppm generates a power density of 4.25 W/m^2 [106]. In another study, 95% removal of silver is achieved with acetate as an electron donor in cathodic chamber reduction [107]. The removal of silver is also done in the presence of ammonia and generate 3.2 J energy and achieved 1.6 g of pure silver on cathodic chambers. Along with 1 g COD (83%) was removed from anodic chambers [108].

Cobalt: According to literature, Co(III) is considered an efficient terminal electron acceptor in MFCs due to a high potential (1.61 V) [109]. Moreover, the addition of Cu(II) improved the efficiency of the cobalt leaching process and acid utilization up to 308% and 171%, respectively, in MFCs [103]. A summary of metal removal *via* MFCs are shown in Table-1.

Conclusion

Unfortunately, microbial fuel cells (MFCs) technology is not promoted for commercialization yet and this technique is only limited to the laboratory level. The microorganisms used in MFCs have been called the powerhouses of the MFCs. So far among the different microorganisms from exoelectrogens to electrodes, the transfer of electron mechanism is only found in *Shewanella* sp. and *Geobacter* sp. and they are capable to perform electron transfer through pili. High power densities can be generated from microorganisms that exhibit conductive pili. Formation of conductive biofilm has an important role that can produce by the bacteria through specific c-type proteins *e.g.* c-type cytochromes and pili. Removal of different types of heavy metals, MFCs technology plays a significant role in terms of electricity generation. Nevertheless, this technology became more economical with the use of biocathodes and the MFCs have emerged as the only technology for renewable energy production along with other practical applications too. For better improvement in this technology, it is highly important to select and breed high-quality exoelectrogens to improve the performance by modifying present exoelectrogens or creating novel exoelectrogens, which shows the best electrochemical activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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