Microbial fuel cells a state-of-the-art technology for wastewater treatment and bioelectricity generation

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Abstract

Wastewater treatment and electricity generation have been the major concerns for the last few years. The scarcity of fossil fuels has led to the development of unconventional energy resources that are pollution-free. Microbial fuel cell (MFC) is an environmental and eco-friendly technology that harvests energy through the oxidation of organic substrates and transform into the electric current with the aid of microorganisms as catalysts. This review presents power output and colour removal values by designing various configurations of MFCs and highlights the importance of materials for the fabrication of anode and cathode electrodes playing vital roles in the formation of biofilm and redox reactions taking place in both chambers. The electron transfer mechanism from microbes towards the electrode surface and the generation of electric current are also highlighted. The effect of various parameters affecting the cell performance such as type and amount of substrate, pH and temperature maintained within the chambers have also been

discussed. Although this technology presents many advantages, it still needs to be used in combination with other processes to enhance power output.

Keywords: Environmental sustainability; Wastewater treatment; Microbial fuel cell; Configurations; Electron transfer mechanism; Electrode fabrication and Bioenergy.

1 Introduction

Nonrenewable energy resources have been excessively utilized to meet energy demand [1]. The scarcity of fossil fuels and their influence on climate change has put forward the requirement to search for some alternative energy resources. Many disadvantages associated with conventional treatment methods such as the generation of huge quantities of sludge that need further treatment for their disposal lead to several financial issues [2, 3]. Therefore, there is an intense need of using such energy sources that lead to a pollution-free environment [4, 5]. Because of the presence of a number of impurities and toxic components, wastewater treatment is an energy-consuming process whose approximately half of energy is being utilized in aeration processes [3, 6]. Water is an essential commodity for the survival of life on earth and it has been polluted due to anthropogenic activities, azo dyes and carcinogenic elements discharged through industries that affect human health and the ecosystem on a large scale.

Crucial and irrevocable health and environmental concerns are by highly substituted stable and recalcitrant aromatic rings of azo dyes even at ppm levels in the effluent [7] . In the coming years, the problem of water scarcity will be faced by all countries that induce the use of wastewater as valuable energy and water resource [8]. Of all the energy sources for next generation, microbial fuel cell (MFC) is getting worldwide attention The expanse of consideration in MFC to associate wastewater treatment and biological power production is a substantial approach for sustainable

bio-electrochemical system [7, 9]on account of its ability to treat wastewater and generate energy in the form of electric current simultaneously. These are the fuel cells that harvest chemical energy from organic matter present in wastewater and transform them into electrical energy with the aid of microbes [10]. In contrast to the conventional fuel cells, expensive metal catalysts are not utilized in MFCs.

Hence, this technology signifies a new approach using inexpensive ion exchange membranes and binders to generate electric current [11]. Heavy metal pollution due to the untreated wastewater emerging from industries has become one of the major environmental issues. This matter is of utmost importance because of the recalcitrance, toxicity and persistence of these heavy metals. In the past few years, several physical, chemical and biological treatment methods have been applied for heavy metal removal from wastewater including adsorption, chemical precipitation, flocculation and technologies based on electrochemical treatment [12]. The production of toxic chemicals, by-products and more sludge production is the limitation of these methods. Immense attention has been paid to induce such a novel approach for heavy metal recovery that is both eco-friendly as well as economical [13]. Using different electron acceptors in the cathodic chamber, the reduction of metals occurs and at last, can be recovered from the surface of cathode [14]. Numerous industries including textile and pharmaceutical drain out wastewater without any preliminary treatment process causing some diseases including ischaemic heart disease, chronic obstructive pulmonary disease, lung cancer and many environmental problems because of the presence of carcinogenic elements such as chromium, lead and arsenic and dyes which need to be degraded.

Among all industries, the most polluting industry is the textile industry where dyes are used for the colouration of fabrics and the waste materials consist of heavy quantities of azo dyes polluting the whole environment on a large scale Azo dyes contribute the largest production volume, approximately 70%, to dye industry [15]. Low light penetration, high chemical oxygen demand, increased chromaticity and grievous environmental pollution are the characteristics of azo dyes contaminated wastewater [16]. Regarding the extensive use and their chemical structures, dyes have been categorized into five major branches which are; basic, acidic, reactive, direct and disperse dyes [17]. The most frequently and predominant organic dye present in industrial wastewater is azo dye which is chemically a compound having a nitrogen-nitrogen double bond (–N=N–) and because of its complicated framework, it is difficult to be reduced. However, these dyes are easily decolourized by acquiring electrons being released from organic compounds oxidation process [18, 19]. The chromophoric group is azo group i.e. colour generating constituents imparting a specific colour to the dye. Along with this group, the phenomenon of resonance is shown by delocalized electrons of p-orbitals in the benzene ring.

The presence of different chemical substituents with the azo bond would alter the properties of the dye. Microbial fuel cell (MFC) represents a promising bio-electrochemical technology that makes use of microbial catalytic activity to extract chemical energy stored in organic matter and convert into electrical energy [20-24]. Microbial fuel cell (MFC) has proved to be an excellent emerging approach because of its cost-effectiveness and sustainability to harvest electrical energy from wastewater [25]. In general, MFC is comprised of an anode and a cathode chamber (Fig. 1) in between which a proton exchange membrane is inserted to avoid the diffusion of electrolytes between two compartments [26].

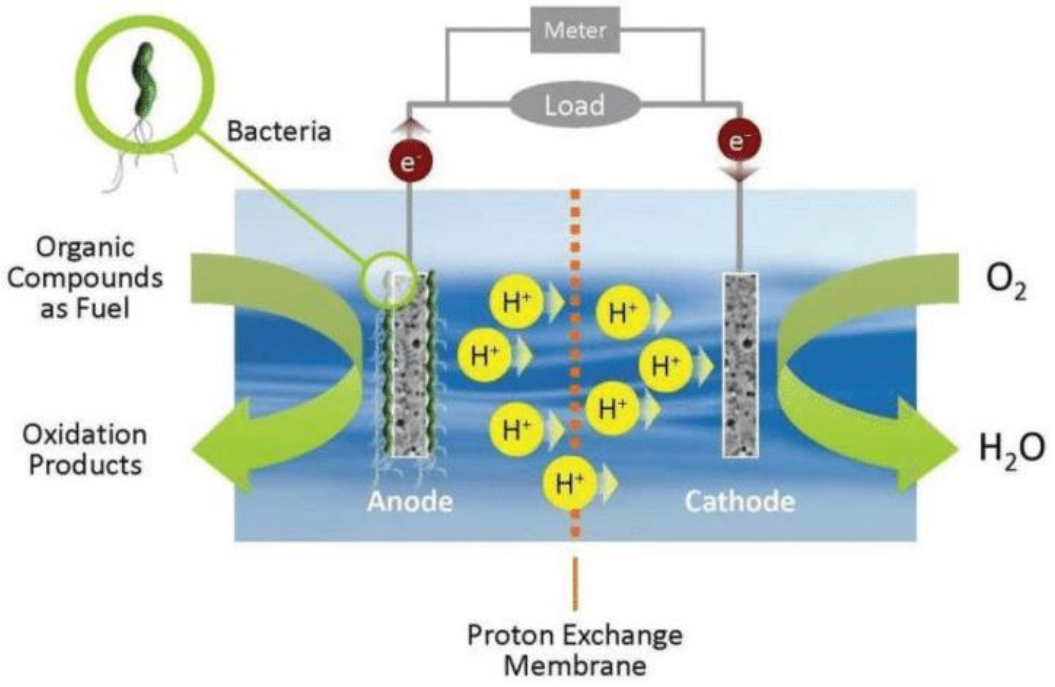

Fig. 1. Schematic representation of typical Microbial Fuel Cell [27].

Electrons and protons are generated in the anode chamber through oxidation of organic substances owing to the catalytic activity of bacteria. Electrons then move towards the cathode electrode through external circuit connections while protons move through proton exchange membrane [28-30]. In the cathodic compartment, a reduced compound or water is produced due to the reaction of electrons and protons with oxygen or any other compound serving as a terminal electron acceptor. Electric current is generated on account of the utilization of electrons moved from anode to cathode through external connection. Different kinds of wastewaters having variable compositions are utilized as fuels and treated in microbial fuel cells such as industrial, domestic and agricultural wastewaters [31-37].

The performance of MFC is affected by bacterial adhesion, electron transfer and electrochemical efficiency which rely upon the selection of adequate electrode material. The efficacy of MFC can

be improved by an increase in the surface area of electrodes to offer more reaction sites, reduction in porosity to enhance electrical conductivity, the use of 2D-nanomaterials can expand the domain of electro-microbiology [38, 39], high electrical conductivity and low interfacial impedance to facilitate the flow of electrons in external circuit with least resistance [40] [41], fouling and life of electrodes can be controlled by selection of materials resistant to acids and bases and for commercial purposes, the material should be low cost. Moreover, the supercilious biocompatible electrode material improves bacterial adherence and life of MFC Electrode materials for microbial fuel cells: nanomaterial approach, Mater Renew Sustain Energy[42] To scale up the efficiency of MFC, a number of studies are reported with different electrode materials; activated Carbon [43] carbon cloth dopped with nitrogen gas [39], carbon-based plain carbon paper [44] granular graphite [45], metal and metal oxides [46] [47] and composites [48].

The performance of MFCs has been examined by changing the configurations, substrate material, conditions of operation and indicators. The variability in each of these key parameters significantly affects the efficiency of treatment and generation of electric power. Many ongoing researches on microbial fuel cells have shifted their attention from batch lab-scale configurations towards continuous-flow scalable setups of MFCs. This necessitates a complete thoughtful of the separate and mutual effects of operating conditions and configuration of the reactor. Scaling up can be done by piling up multiple cells, more than one electrode in the same cell or by increasing the size of the cell [49]. MFC seems to be a promising approach from both financial and environmental aspects [50, 51]. The ultimate goal of this present review is to conclude various key parameters, different designs and electrode materials with little modifications best suited to the performance of cells in terms of power generation and treatment efficiency.

The purpose of a model is to replicate the performance of a system employing a set of laws and equations, to study the density and steady/dynamic nature of the MFC poses boundaries. Dynamic and steady-state 1-D, 2-D and 3-D models for simulation of MFC have been studied and intricacy of the model depends upon dimensions of coherence and established assumptions [52]. [38] studied one-dimensional modelling and simulation by Solid Edge. Electrochemical behaviour of MFC can be studied by various numerical expressions/laws like Ohm's law, Nernst-Monod expression to describe the rate of electron-donor (ED) oxidation [53], Butler–Volmer expressions [54] finite volume method/finite difference method/ parallel computation/ multiple step times and computational fluid dynamics method, Nernst-Planck equation [55].

Electrode materials play an inevitable role in the power output and cost of MFCs, to convert chemical energy into electricity by employing bacteria to oxidize organic/inorganic matter. In this paper, the recent progress of anode/cathode materials for MFCs has been scientifically swotted, subsequently detailed insights into the characteristics, some prevailing problems, advancements, assessment parameters of the electrode materials and their effectiveness in terms of wastewater treatment. It is observed that the difference in materials of electrodes resulted in a significant difference in the output and electrode modifications came out to be a promising substitute to upscale the efficacy of MFCs. From the perspicacity of recent developments, the exploration of electrode materials will be very imperative and vendible as an affordable price and exceptional performance will significantly outspread the application of MFCs.

However ongoing studies at electrode modification have substantially upgraded the performance of MFCs, at present the working mechanism in most of the studies, still demands more workforce, which is not very clear. For this promising wastewater treatment technology, durability and long-working life of electrode materials are unavoidable aspects. Unfortunately, most of the present studies remained heedful to the performance/output power, not copiously addressing the stability of the electrode materials, which would eventually lack to offer a valuable standard for their long-term provision in industrial application. This review is novel in respective of an assemblage of recent advances in electrode fabrication strategies analyzing MFCs performance with reference to electricity generation and it highlights the crucial need for extensive studies on the working mechanism of electrodes other than improving their operational life to improve the durability of MFCs.

2 Microbial fuel cells (MFCs)

Wastewater treatment has become a major global concern these days on account of a tremendous increase in industrialization and anthropogenic activities causing various health issues. **Error! Reference source not found.** represents the schematic overview of anaerobic treatment that majorly produce biogas, effluents and sludge. The industrial waste containing hazardous chemicals and dyes is discharged into water bodies without being given any preliminary treatments. When sustainable energy resources come under discussion, we talk about technologies that are pollution-free and non-combustible [56]. The inadequacy of water supply is predominantly contributed by wastewater generated from domestic as well as industrial sources [57]. This led to the treatment of wastewater before ejection in the environment by applying various expensive chemicals [58, 59]. Therefore, we need such sustainable energy sources as alternatives inducing a less hazardous environment.

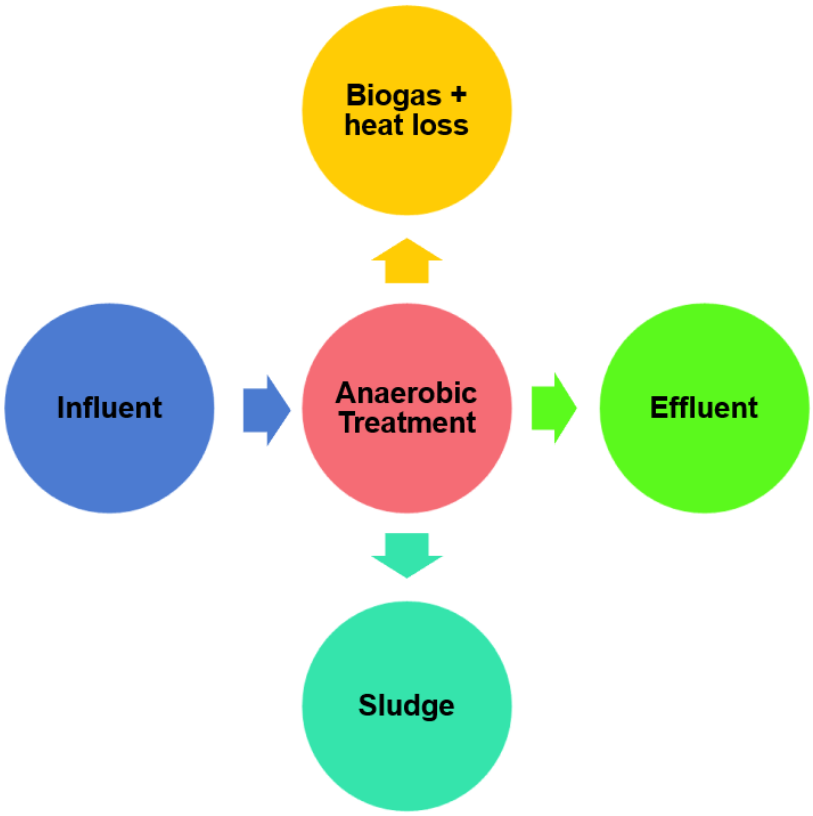

Fig. 2. A Scheme of anaerobic wastewater treatment.

In the coming years, one cannot be dependent merely upon fossil fuel energy due to a drastic increase in the energy crisis, hence we need renewable energy resources. To cope up with the problems relevant to wastewater drained out by textile industries, a new biological approach and an emerging technology under consideration is a single-chambered microbial fuel cell (SCMFC) which treats wastewater and simultaneously converts chemical energy through oxidation into electricity due to catalytic activity of microbial community [60]. MFC has gained much attraction due to its environmental profits. This technology discovers the metabolic reactions of different microbial species such as *Shewanella sp.*, *Geobacter sp.*, yeast and consortium of species that help in catalysis of redox reactions [61]. In MFC, electrons are emitted through oxidation of organics

192 at the anode via electrogenic microorganisms, which then combine with diffused protons at
193 cathode and electricity is generated through reduction reaction [62].
194
195 MFC is a biological approach that not only raises power generation standards but also addresses
196 fuel economy by the utilization of domestic and industrial wastewater. This system generates
197 power along with wastewater treatment leading to a sustainable future that can use non-fossil
198 resources for power generation [63]. In general, power is generated owing to the metabolism of
199 bacteria that produce electrons by consuming the substrate present as contaminants in wastewater
200 inside MFC and these electrons then complete the reaction by moving through an external circuit
201 towards the cathode [64]. As the electrons are only produced from bacteria, so the thickness of
202 active biofilm growth on the anode is responsible for the extent of generated electric power [65].
203 The cell performance is affected by the significant contribution of many factors including the
204 oxygen received by cathode compartment, substrate oxidation in an anodic compartment,
205 movement of electrons towards anode surface and extent of proton diffusivity through the cation
206 or anion exchange membrane [66].
207
208 Advantages linked to the use of MFCs are the lower yield of sludge to be disposed of and no need
209 for aeration as compared to other conservational techniques. The process of electricity generation
210 through MFCs is clean and further does not need any kind of purification process, as in the case
211 of hydrogen and methane produced under anaerobic conditions [67]. In future, an increase in
212 wastewater emitting from industries and houses which is estimated to be 7% per year would need
213 more energy resources for the treatment. Vast research has been carried out to produce electricity
214 simultaneously with the treatment of wastewater using MFCs generating current densities within

0.06 – 1000 mA/cm^2 range [31, 68]. Recently, the MFC system was used not only to generate the power density but also to effectively remove the chemical oxygen demand (COD). The two different MFC reactors were analyzed and the results showed a maximum power output and COD removal with R_{cc} MFC reactor at the concentration of 2900 mg/L (Fig. 3). The greater efficiency of R_{cc} MFC reactor was attributed to the high external load in closed circuit, greater metabolic activity and maximum breakdown of the substrate [69].

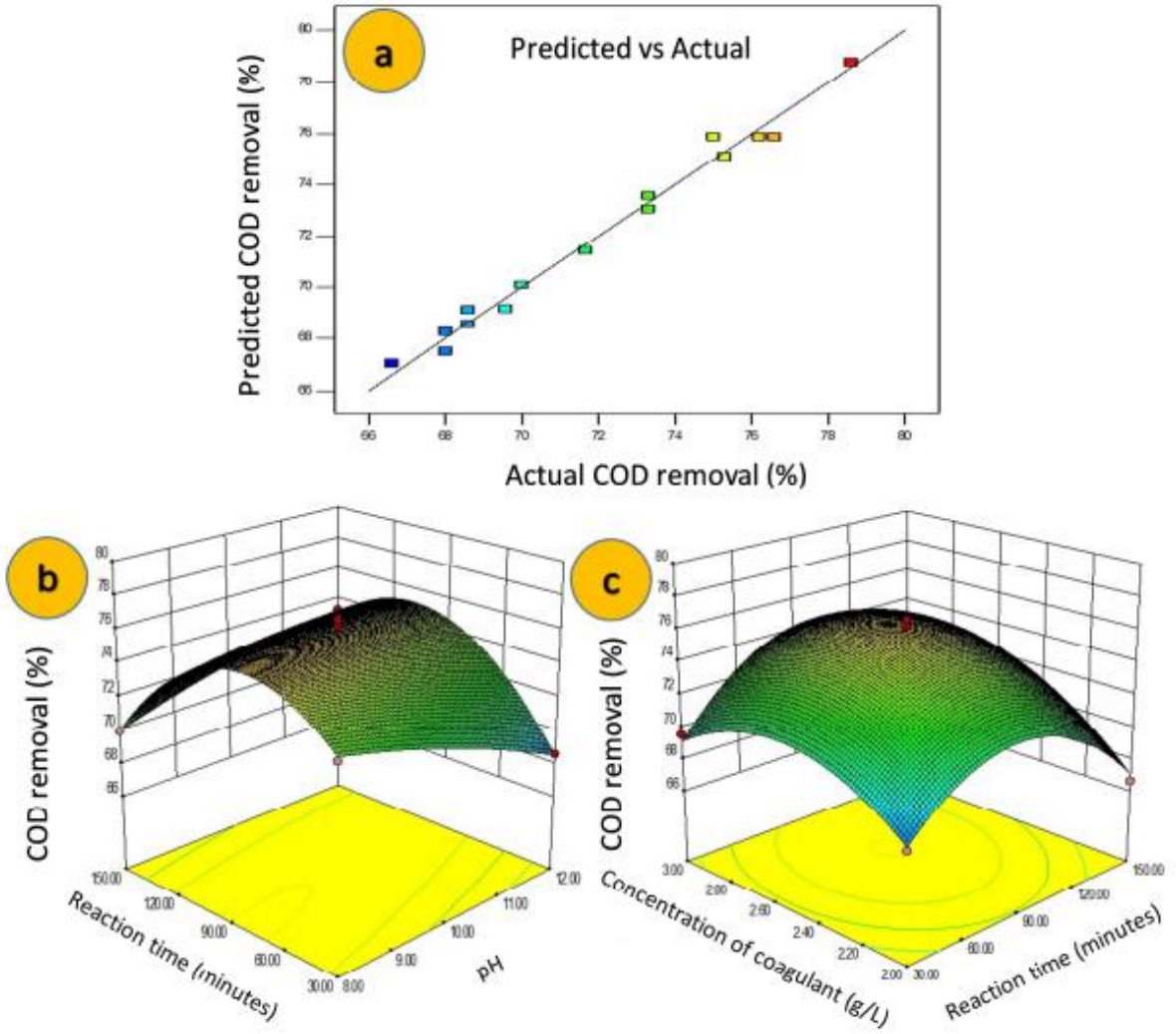

Fig. 3. (a) Efficiency correlation of actual and predicted COD removal, (b) pH and reaction time response surface plot for COD removal, (c) combined effect plot between concentration and reaction time on COD removal [69].

2.1. Electron transfer mechanism in MFCs

Bacterial strains generate electrons via various bio-electrochemical processes (**Error! Reference source not found.**), and a specific mechanism is developed for extracellular electron transfer (EET). These systems involve the use of electroactive species that catalyze redox reactions at cathode and anode having the unique capability of transforming chemical energy of wastewater pollutants into bioelectricity or some other valuable products or hydrogen in microbial electrolysis cells (MEC). Pure anaerobic strains called exoelectrogens transport electrons from the oxidation product to the electrode with the aid of projected nanowires, external mediators or specific proteins. Many strains have been investigated for their potential to produce more power in MFCs. The bacteria can transfer electrons to the electrodes by three principal mechanisms. Fermentative bacteria, in an indirect MFC, yield end products like hydrogen, carbon dioxide, alcohols, or ammonia. Under anaerobic conditions, these reduced products are formed when the electron produced through substrate breakdown is used to reduce some intermediate products like proton or alcohol.

Therefore, these fermentative bacteria are not able to donate the electrons to anode, so to make use of these bacteria, external mediators are which can shuttle between cell membrane and anode. The bacterial strains which need mediators for electron transfer include *Streptococcus lactis, Proteus mirabilis* and *Klebsiella pneumonia* while the bacterial strains that do not require any mediator include *Aeromonas hydrophila*, *Geobacter metallireducens, Rhodoferax ferrireducens* and *Geobacter sulfurreducens*. Recent studies revealed that some species can perform in the absence of any mediator, but if they are added, their efficiency can be improved such as *Shewanella putrefaciens* [70]. Another extracellular electron transfer mechanism includes the use of redox

mediators for shuttling of electrons. A mediator should exhibit a few salient features like the ability to develop a physical attachment with the electrode surface, electrochemically active, should have low oxidation potential, should not absorb on the surface of bacteria or electrode.

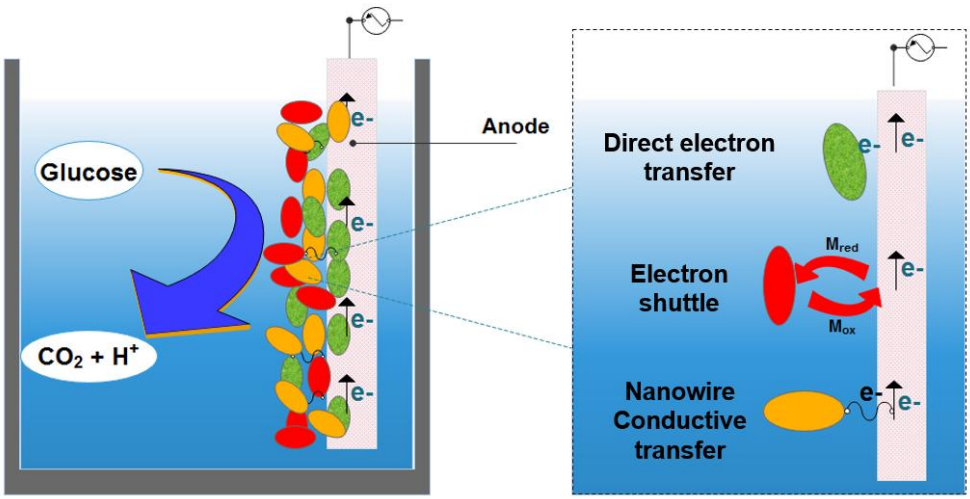

Fig. 4. Electron transfer mechanism in a microbial fuel cell.

The oxidation potential of the mediator should be closer to the redox potential of the primary substrate. The mediator can be membrane-bound or soluble in the medium [71]. Some commonly used natural/synthetic electron mediators ferrying between inside cell membrane of bacteria and anode are Phenazine ethosulphate, 2-hydroxy-1,4-naphthoquinone, gallocyanine, benzylviologen, 2,6-dichlorophenolindophenol, antraquinone-2- disulfonate, and thionine [72]. Generally, it is known that electroactive bacterial strain *Geobacter sulfurreducens* directly transfer electrons with projected appendages named as nanowires. The direct transference of electrons, produced through respiration from the electroactive bacteria to the anode is the third and the most significant mechanism. it was first projected that microbes can transfer electrons to an electrode surface when strains of *Shewanella putrefaciens* during metabolism of lactate generated electricity [73]. The

metal-reducing bacterium *Shewanella putrefaciens* were found to have cytochromes (electron carriers) in its external membrane and were capable of generating anodic current in the absence of terminal electron acceptors. The selection of mechanism mainly depends on the microbial potential for EET and the type of environmental pollutant to be removed [74].

3 Configurations of microbial fuel cell

Generally, a microbial fuel cell is comprised of an anodic compartment, cathodic compartment, proton exchange membrane or salt bridge and assembly of electrodes. The classification of microbial fuel cells was done based on the mode of operation and design [75].

3.1 Dual-chambered

A dual-chambered MFC (**Error! Reference source not found.** (B), as the name implies, consists of an anode and cathode that are separated by placing a salt bridge. This is basically H type design of MFC (**Error! Reference source not found.** (A)). Microbes, media solution and electrodes are present in the anode chamber while fresh water and electrode are contained within the cathode chamber having a supply of oxygen from the surrounding. In Fig. 5(A), the circular orientations around anode compartment are representing microbial growth and biofilm production. The major limitation associated with the less efficiency of this configuration is the increase of internal resistance due to increased distance between the electrodes. Commonly used electrodes are made of graphite, copper, stainless steel mesh, carbon paper and carbon cloth [76].

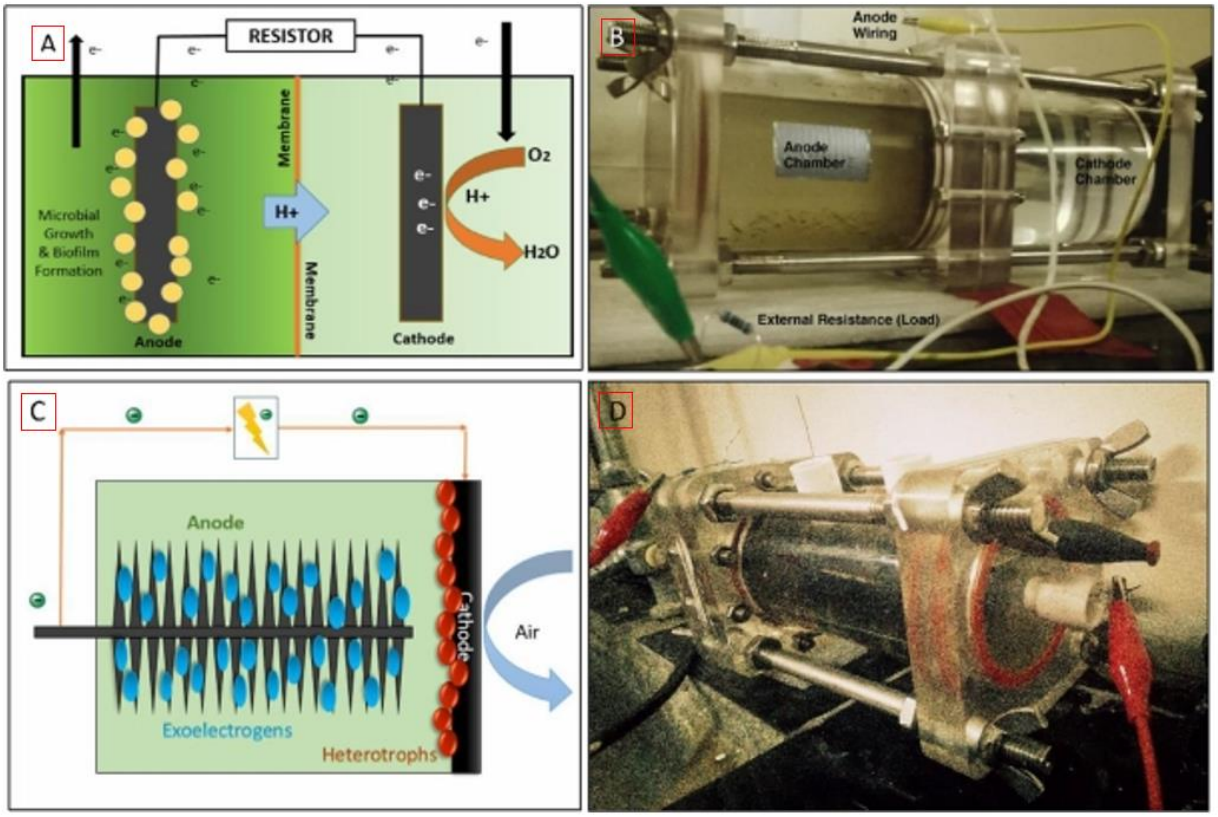

Fig. 5. (A) Working of dual-chambered cell, (B) Dual-chambered, (C) Working of single-chambered cell and (D) Single-chambered cell.

3.2 Single-chambered

The second configuration of MFC is single-chambered (**Error! Reference source not found.** (D)) that is composed of an anode chamber only and the cathode is faced towards the atmosphere (**Error! Reference source not found.** (C)). Active aeration is not compulsory in this configuration. This is a simple design that can be functioned in two modes i.e., batch or continuous. The design is cost-effective and because of its simplicity, scaling up the process is very easy. This cell configuration is efficient and preferred due to lower internal resistance in comparison to dual-chambered MFCs. The main advantage associated with the use of single-chambered MFC is cost-effectiveness along with the generation of more power output [76].

3.3 Up-flow MFC

The up-flow configuration is made in such a way that wastewater is entered from the bottom and effluent is pumped out from the top side. The mode of operation in this configuration is continuous [77].

3.4 Stacked MFC

As the name indicates, stacked MFC is comprised of many microbial fuel cells connected in either series or parallel combinations to obtain high power output. Different studies conducted on stacked MFCs revealed parallel combination to be more efficient than series combination [2].

3.5 Paper MFC

The material used for the fabrication of electrodes in this type of MFC was paper having the benefits of being cost-effective and chemical-free. The design is simply comprised of an anode and cathode [78].

4 Electrode fabrication

Many factors affecting the cell performance include the type of substrate, pH, temperature, electron transfer mechanism opted by microbes, the configuration of the cell, membrane and the material of electrode [79]. The fabrication of electrodes is the major factor having a dominant effect on the working of MFCs. Many researchers have now shifted their attention towards materials of the electrode as the performance is directly dependent on the electrode kinetics [80]. The selection of anode material has a significant impact on the performance of microbial fuel cells in terms of treatment efficiency, adhesion of microbes to the electrode and the transference of electrons from microbial appendages to the electrode surface [81]. Therefore, the materials for the construction of anode should possess some specific properties that not only improve the adhesion

capacity of electroactive bacteria on the anode and the movement of electrons towards the electrode surface but also the collection of current. A number of anode materials including carbon and metal-based with different modifications have been assessed to enhance the performance of the cell [82].

The most commonly used materials for anode fabrication were carbon-based because of its several characteristics such as high conductivity, chemical stability and biocompatibility. Numerous carbon-based materials have been utilized as anode electrodes including graphite rods [83], carbon felt [84], carbon cloth [85], carbon mesh [86], carbon brush [87], carbon veil [88]. In contrast to that, bacteria cannot grow on the surface of metals due to their antimicrobial activity but this characteristic is not present in electrogens having the capability to generate a highly active biofilm on the electrode surface [89]. One of the mechanisms for the fabrication of hybrid G-CNT biofilm is shown in Fig. 6. According to this process, carbon nanotubes can easily bind with the graphene oxide sheet and by incubation of the microbes, sheets turn into biofilms. These biofilms with enhanced microbe loading then successfully transfer electron direct to the electrodes via OM c-Cysts [90]. Instead of biofilms, now metal-based materials can also be used in MFC. The main advantage of using metal-based electrode materials is high conductivity, robustness and inexpensiveness as compared to carbon-based materials [8, 91].

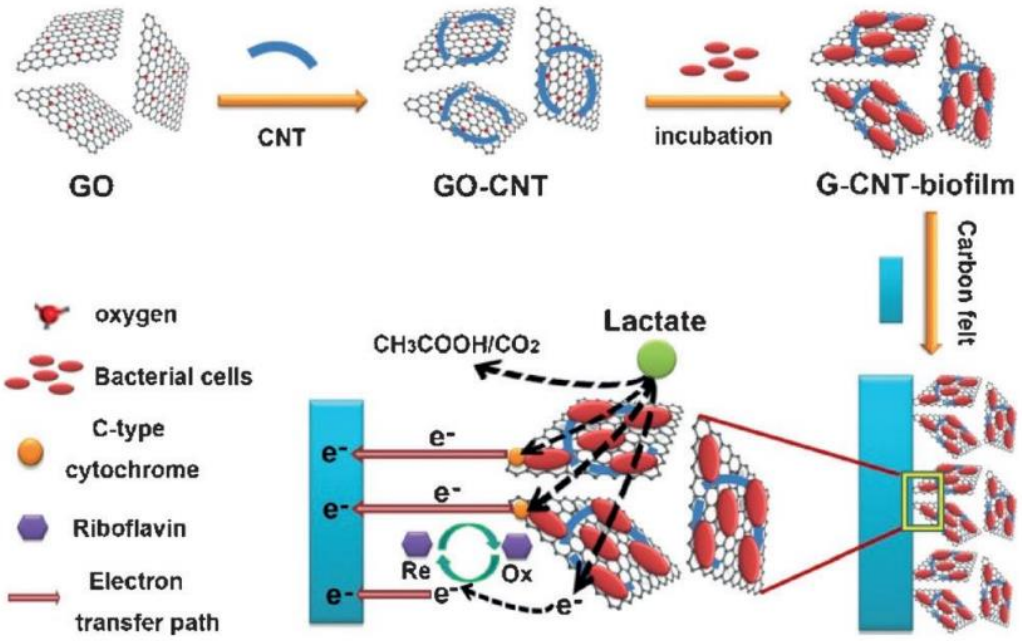

Fig. 6. Fabrication and electron transfer process of hybrid G-CNT biofilm [92].

The type of cathode used is a major hindrance significantly affecting the performance of the cell. To manufacture a cathode that is cost-effective and generates maximum electric power and columbic efficiency is the most challenging part in this technology to be applied successfully specifically for wastewater treatment [66]. Along with robustness, a good cathode must possess some specific properties such as catalytic, high conductivity and mechanical stability [80]. Fig. 7 explains the fabrication process of rGO-AC cathode. The rGO-AC equipped cathode utilized in the single-chamber MFC device is not only potentially affordable but also gives the maximum power density of 2.25 W/m^2 [93]. Most of the materials used for the fabrication of anodes in MFCs have also been utilized for the construction of cathodes. The most widely used catalyst for the cathode is platinum. Cathodes have been classified into two categories i.e., biotic and abiotic cathodes while biocathodes can be further either aerobic or anaerobic [94].

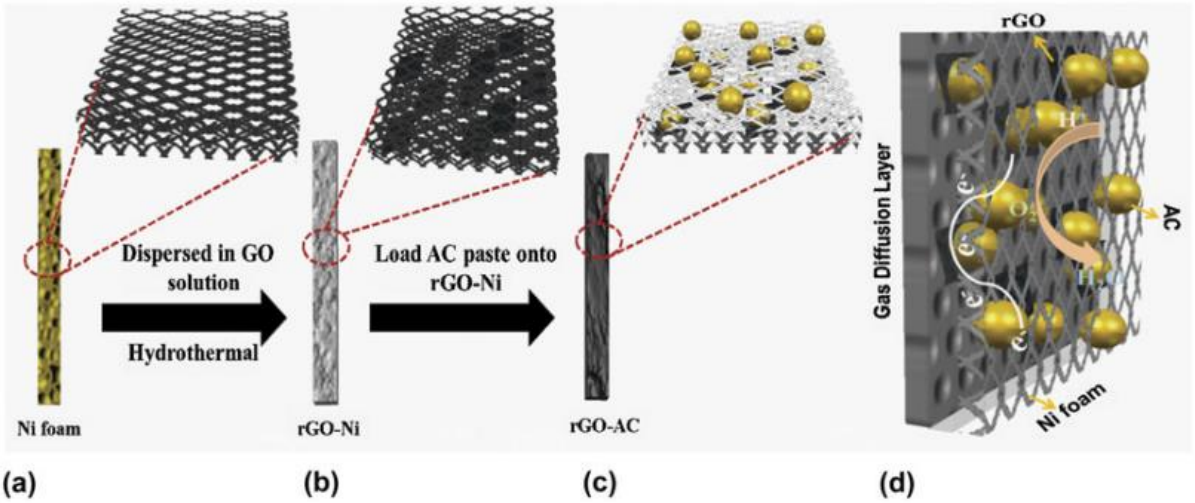

Fig. 7. (a-c) Schematic representation of the fabrication process of rGO-AC. (b) Final diagram of fabricated rGO-AC cathode [93].

4.1 Terminal electron acceptor in cathode

Electrons are released from the degradation of organic matter which eventually travels towards the cathode to be finally oxidized by the aid of electron acceptors [95]. Much attention has been paid to the substrate employed in the anodic chamber while studies are less focused on the terminal electron acceptors in the cathode chamber in spite of their significant impact on the cell performance. A good electron acceptor must have high reduction potential and fast kinetics along with its availability, cost-effectiveness and stability [96]. Up to now, oxygen has been regarded as the most useful and frequently utilized electron acceptor on account of its characteristics of high oxidation potential, availability and the production of clean water after the reduction process [97]. But many studies have reported this oxygen supply to the cathode as a power-consuming process [10, 98]. To cope up with the need for some other electron acceptors having varied redox potential and to make cathodic reactions more effective, studies are being conducted to assess the feasibility of altered electron acceptors [14]. The most commonly used electron acceptors in addition to oxygen are ferricyanide [99-101] and permanganate [102, 103].

You et al. [102] concluded that power density with permanganate was higher (116 mW/m^2) in comparison to ferricyanide (16 mW/m^2). Microbial fuel cells (MFCs) have been studied frequently by many scientists and different modifications have been done to commercialize it on a large scale to produce more power with efficient fuel consumption. The biofilm used at the anode performs a dual function of pollutants degradation, generation of power and producing 50–90% less disposable solid residue [15]. Based on the kind of microbes used in MFC, the biological approach for azo dye degradation can be aerobic, anaerobic or a mixture of both conditions [104, 105]. Some researchers [106] recently evaluate the aerobic and anaerobic degradation by adopting iMFC and dMFC systems (Fig. 8). Both of them successfully process biodegradation and revealed comparative results. Table 1 summarizes maximum power output and colour removal efficiencies using different configurations of MFCs for different azo dyes.

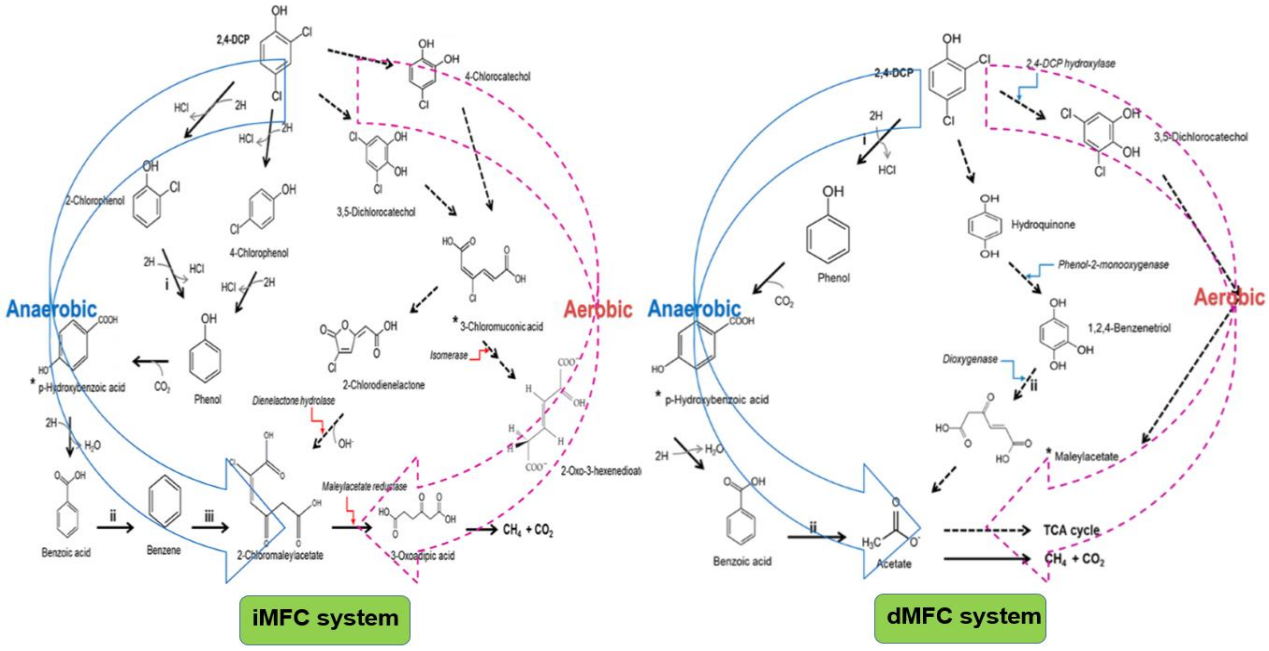

Fig. 8. Proposed aerobic and anaerobic biodegradation pathway of 2,4- DCP. Proposed reactions in roman number represent reductive dehalogenation, decarboxylation and aromatic ring cleavage [106].

381 **Table 1.** A comparison of maximum power output and colour removal efficiencies of various microbial fuel cells.

MFC type	Dye/s	Concentration (mg/L)	Anode – Cathode	Power output	Colour removal (%)	References
H-shaped MFC	Methyl orange, congo red, Reactive blue 172, reactive yellow 145 and reactive red 2		Graphite based Metal salt - GECE	Methyl orange – 4100×10^{-6} W/m^2 MO – 4676×10^{-6} W/m^2 RB – 2593×10^{-6} W/m^2	94 90	[107]
Planted CW-MFC	Reactive brilliant red X-3B (ABRX3)		Granular Activated Carbon	0.3 W/m^3	91	[108]
Pilot-scale anaerobic baffled reactor with biocatalyzed electrolysis system	Alizarin yellow R (AYR)	200	Granular graphite	-	96	[109]
Single-chambered	Thionine-based textile dye	40	Porous carbon cloth (anode) and PTFE diffusion layer (cathode)	83×10^{-3} W/m^2	-	[110]
Constructed wetland – MFC	Reactive brilliant red X-3B (ABRX3)	300	Activated carbon and stainless steel mesh	0.9 W/m^3	96	[111]
Single-chambered up-flow	Acid orange 7 (AO7)	-	Carbon felt and carbon plate	-	81	[77]
Single-chambered	Orange I, II and methyl orange	-	Carbon felt	-		[112]
Air-cathode single-chambered MFC	Active brilliant red X-3B (ABRX3)	100	Porous carbon papers & Pt-coated air cathode with PTFE layer	373×10^{-3} W/m^2	90	[113]

Air-cathode single-chambered MFC	Active brilliant red X-3B (ABRX3)	300 - 1500	Porous carbon papers & Pt-coated air cathode with PTFE layer	274×10^{-3} W/m^2	90	[114]
Anaerobic-aerobic sequential reactor and MFC coupled system	Congo red	100	Plain carbon felt (anode) and graphite-granules (cathode)		93	[115]
Single-chambered biocathode-MFC	Azo dye		Granular activated carbon	8 W/m^3	75	[116]
Biocathode-MFC	Congo red	300	Porous carbon papers	9×10^{-3} W/m^2		[117]
Dual-chambered	Orange G	-	Graphite rods	91×10^{-3} W/m^2	- 97	[118]
Dual-chambered	Reactive blue (RB221)	-	Modified graphite electrode	21×10^{-3} W/m^2	74	[119]
Single-chambered air cathode	Congo red	-	Graphite felt (anode) and Pt-coated carbon paper (cathode)	53×10^{-3} W/m^2	90	[120]
Biocathode with dual-chambered MFC and air-cathode with single-chambered	Congo red	-	Non-wet proofed porous carbon paper and Pt- coated wet-proofed PCP	324×10^{-3} W/m^2	96	[121]
Dual-chambered MFC (saline catholyte)	-	-	Graphite fiber brushes & Pt-coated wet-proofed CC	491×10^{-3} W/m^2	-	[122]
Air-cathode buffer-free MFC			Graphite felt & activated carbon air-cathode	512×10^{-3} W/m^2	-	[123]
Continuous-flow MFC	X-3B	200	Air-cathode	Decreased slowly with increased operational time	-	[124]
Dual-chambered	Reactive blue 19 (RB19)	50	Carbon felts	-	89	[16]

Single-chambered	-		Cu_2O nanoparticles/graphene oxide and Pt/C as cathode catalysts	-	-	[62]
Two rectangular perspex frames	Acid Orange 7	0.06	Granular graphite	0.31 W/m^3	-	[125]
Air-cathode single-chambered	Reactive blue 160 (RBu160)	450 - 560	Activated and hydrophobic carbon cloth	197×10^{-3} W/m^2	-	[126]
Dual- chambered	Methyl orange	10-20	Rutile-coated graphite	-	73	[127]
Air-cathode single-chambered	Congo red	300	Porous carbon papers	$192\times10^{-3}W/m^2$	90	[128]
Dual-chambered	Congo red	300	Carbon paper electrode	214 mV	-	[129]
Dual-chambered	Congo red	300	Porous carbon papers	0.25 V	95	[130]
Dual-chambered	4-Nitrophenol	139,00	Carbon felt	143×10^{-3} W/m^2	-	[131]
Cylindrical single-chambered MFC	Acid Orange 7	70-210	Carbon fabric	-	-	[132]
Dual-chambered	1,2-Dichloroethane	102	Graphite granules	-	43	[133]

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Some microbial communities *Geobacter sulfurreducens* and *Beta Proteobacteria* were also used in mediator-free planted CW-MFC to examine electricity production and dye degradation [108]. A *Pseudomonas*-catalyzed microbial fuel cell was designed to examine the degradation of various dyes including reactive blue 172 (RB), methyl orange, Congo red, reactive red 2 and reactive yellow 145, which have capability to form electron-shuttling substances [107]. Alizarin yellow R-bearing wastewater treated in anaerobic baffled reactor coupled with bio catalyzed electrolysis system (ABR-BES) containing sludge from wastewater plant exhibited colour removal up to 96% and generated current output of 24 A/m^3 [109]. Rather than using a single bacterium for the removal of dyes from wastewater, a more pragmatic approach to make use of sludge emerged with promising results. Anaerobic sludge collected from municipal wastewater for decolourization of commercially used reactive and azo dyes (brilliant red, acid orange 7, congo red) MFC coupled constructed wetlands (CW-MFC) showed significant colour removal efficiency (more than 80%) [55, 90, 99].

In view of flexibility in regulating the pH, phosphate buffer is popularly used as a catholyte [134]. Electricity generation was enhanced by short-term adjustment of pH of anolyte in anodic compartment leading to increase of electroactive bacteria (EAB) biofilm formation from 59 to 75% triggering an acceleration of redox reactions and decrease in resistances of electrodes but it is not advantageous on a large-scale [123]. Disadvantages related to the use of glucose is methanogenesis and fermentation causing the inhibition of electricity production, as well as regular replacement of the medium, which also results in less power output [92]. In single-chambered MFC, Kalathil et al. [116] revealed a power output of 8 W/m^3 by using graphite-activated carbon (GAC) as biocathode. The associated advantages are that pH adjustments and preheating process

406 are not required. Fang et al., [108] reported 91% decolourization of dye (ABRX3) in planted MFC
407 generating a voltage of 610 mV when GAC anode/cathode was used.
408
409 The design of such a microbial fuel cell that can be commercialized on a large scale is still a
410 challenge to be accomplished. It is essential to find out the power generation with dye
411 decolourization efficiency. A single-chambered air-cathode MFC was used to decolourize active
412 brilliant red X-3B for the first time by Sun et al [114] resulted in 90% colour removal with power
413 generation of 274 mW/m^2 when glucose was used as an organic substrate. In contrast to that, an
414 attempt was made by Liu et al. [112] to decolourize orange I, orange II and methyl orange in the
415 presence of phosphate buffer in the cathodic compartment and the results indicated a complete
416 reduction of dyes with voltage generation of 282 mV utilizing glucose in the anodic chamber.
417 [113]. Li et al. [115] have discussed the step by step mechanism of azo dye degradation in an
418 anaerobic-aerobic sequential MFC reactor, describing the cleavage of azo bond in anode chamber
419 under anaerobic situation and reduction into aromatic amines and dissociation of these amines
420 under an anaerobic situation in the cathodic compartment, but its maintenance is difficult with a
421 continuous mode of action.
422
423 MFC exhibited substantial performance while exploiting enzymatic oxidation in cathode
424 compartment in terms of decolourization [119]. Another design of MFC was developed for the
425 removal of commercially used dyes by the name of constructed wetlands MFC (CW-MFC) which
426 attained extensive decolourization with efficient power output [108, 109]. The colour removal of
427 active brilliant red (ABRX3) and maximum power output reported to be 93% and 0.06 W/m^3
428 respectively in cylindrical planted constructed wetlands MFC [111]. A green approach was used

for the generation of electricity from waste and results described those pure strains were less productive than mixed cultures and MFC using Pt as catalyst exhibited 16 times more power density compared with MFC without the catalyst. But the Pt catalyst cannot be used because of its high cost. In MFCs, the microbial communities in anode and cathode chambers were quantified using various gene sequencing techniques including 16S rRNA gene sequencing, next gene sequencing and fluorescence in-situ hybridization (FISH) which makes use of specific probes to quantify the specific populations [135].

Electricity generation was evaluated by comparing the efficiencies of Cu_2O nanoparticles/graphene oxide and Pt/C as cathode catalysts in single-chambered MFC and the results depicted the highest voltage output (0.223 V) and columbic efficiency (93%) using Cu_2O nanoparticles/graphene oxide. The relative abundance (49%) of microorganisms *Geobacter sulfurreducens* in the anode with Cu_2O nanoparticles/graphene oxide was higher as compared to commercial Pt/C (32%) [62]. Electron exchange is strongly based on material conductivity, characteristics of the surface according to the type of microbes used in MFC being the composition of an electrode a deciding factor for the implementation of MFC on a large scale [15].

A plain carbon felt was used as electrode material by Li et al. [115] which resulted in colour removal of Congo red dye to be 93% along with 95% COD removal of wastewater generated a maximum voltage of 360 mV. By increasing the surface area of the anode from 18 to 36 cm^2 comprising of porous carbon papers, the colour removal of Congo red was raised to 163%, apart from that, decolourization time declined from 168 to 72 h. In addition, increasing the surface area of the anode by four times (72 cm^2) caused complete removal of dye within 26 h [136]. Graphite

granules showed conductivity, but the drawback was the formation of clogs in the system. To sort out this problem, a cuboid basket composed of titanium made by Cui et al. [109] was compactly filled with graphite granules and a titanium rod was utilized to establish a connection with an external circuit. In this configuration, the large surface area of granules provided maximum colour removal of alizarin yellow azo dye and voltage simultaneously.

5 Assessment of parameters effecting power output of MFCs

Several parameters affect the performance of MFC including the type of substrate to be added for consumption by electroactive microorganisms, temperature maintained in the cell, pH variations due to anolyte and catholyte composition and hydraulic retention time (Fig. 9) [49].

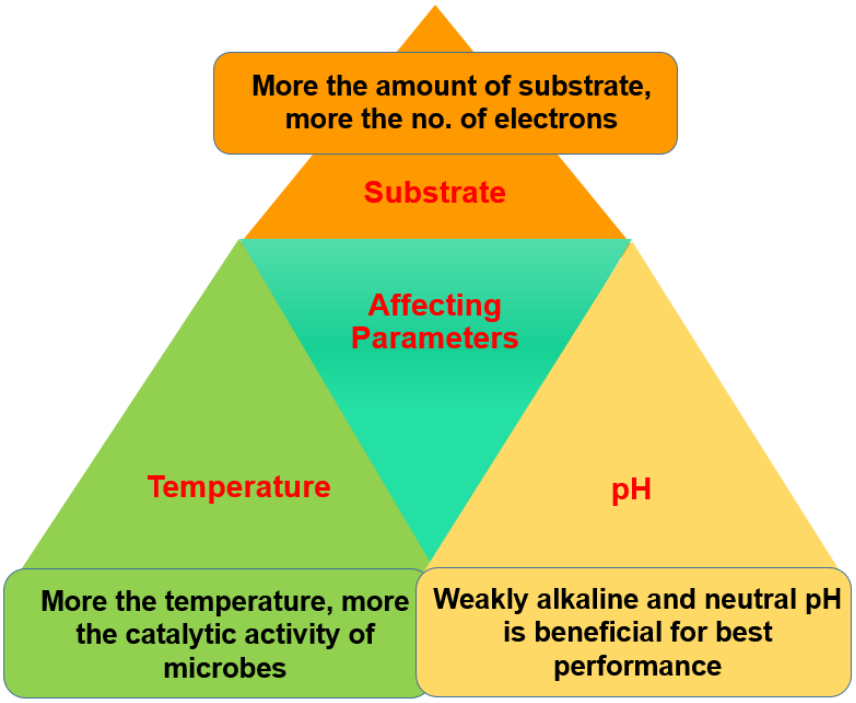

Fig. 9. Summary of parameters affecting the performance of microbial fuel cells.

5.1 Effect of substrate

The substrate origin plays a vital role in power production by MFC. Many organic materials e.g., glucose, acetate and lactate can be utilized as substrates for microbes. Some organic compounds

present in wastewater can also be consumed by microbes as energy sources. Acetate is frequently employed due to stability in its nature and showed approximately 66% higher efficiency in comparison to butyrate [137]. Sodium acetate has generated power output of 308 mW/m^2 and COD removal up to 99% [138, 139]. More the amount of substrate available for oxidation by microbes, more electrons will move through the external circuit and hence more electricity generation. All substrates exhibit different efficiencies to get oxidized in an anodic chamber from which acetate depicts the highest conversion efficiency of 72% [75].

5.2 Effect of temperature

Cell thermodynamics, reaction kinetics and microbial community are significantly affected by the temperature maintained inside the MFC. The temperature between 20–30 ºC has been adopted in most of the studies because of the maximum microbial activity in this range. [86]. The performance of the cell was reduced at lower temperatures. The power output was increased by 10% in an experiment conducted at a temperature ranging from 17–22 ºC [140]. An exponential relationship instead of a linear was observed between temperature and power density. Increased temperature is favourable for microbial growth and its catalytic activity. A urinal system based on the use of undiluted urine for COD removal and current production was powered by taking on a stack of MFCs and the power density was observed to be enhanced on increasing the temperature [141].

5.3 Effect of pH

pH is another key parameter playing a vital role in the performance of MFC. The main challenge is to maintain both anodic and cathodic compartments at a specific pH. The generation of protons in the anodic chamber leads to a decrease in pH and these protons then migrate from anode to cathode through proton exchange membrane. It is important to be stated that this pH difference will affect the stability and performance of the system [66]. The higher the pH, the lesser the

number of protons will migrate towards cathode chamber leading to the inefficient reduction reaction hence affecting the performance of MFC. Weakly alkaline and neutral pH is regarded as beneficial for biofilm growth on the surface of the anode electrode. It was found that the biofilm growth and the power output can be promoted through short-term pH adjustment of anolyte solution [123]. Another key factor contributing to the working of MFCs is the composition of the cathode electrode responsible for reduction reaction by capturing the protons. Oxygen is the major electron acceptor used in MFCs. Different types of cathodes have been used by scientists including biocathodes which catalyze the reduction via microorganisms in the cathode chamber and directly capture the electrons [142]. A biocathode MFC depicted brilliant performance in decolourization of Congo red dye within 48 h with a maximum power output of $9mW/m^2$. The highest decolourization of 94% and power output of 4100 $\mu W/m^2$ was observed in the case of methyl orange in MFC containing graphite electrodes [107]. Table 2 summarizes cathodes with variable composition generating power output using biocatalyst in various conformations of the microbial fuel cell.

503 **Table 2.** Summary of various developed cathodes generating power output.

Conformation of MFC	Strain	Cathode composition	COD removal (%)	Power output	References
Single-chambered	-	Plain carbon rods	92	165 µA 302 mV	[143]
Single-chambered	*Bacillus firmus* – NMBL-03	Graphite	-	2.9 mW/m^2	[144]
Double-chambered	-	PVDF coated activated carbon cloth	64	110 mW/m^2	[63]
Single-chambered	-	Activated carbon cathode having carbon black	> 60	430 mW/m^2	[64]
Dual-chambered	*Pseudomonas*	Carbon felt	-	0.32 mW	[145]
Dual-chambered	-	Biocathode	-	Lower than air cathode	[121]
Single-chambered	-	Platinum coated on air-cathode	-	-	[121]
Double-chambered	-	Plain porous carbon papers	-	0.25 V	[130]
Double-chambered	*Geobacter*	Carbon cloth	66 - 86	3.5 A/m^2 1.62 W/m^2	[146]
Serpentine up-flow	-	PVDF-based activated carbon air-cathode	92	4 W/m^3 5 V	[147]
Double-chambered	-	Graphite plates	-	16 W/m^3 1V	[148]
Single-chambered	-	PVC-co-PVP based activated carbon air-cathode modified with carbon black	79	1330 mW/m^2	[149]
Single-chambered	-	PVDF based activated carbon	-	1470 mW/m^2	[150]
Stacked MFC	-	Granular activated carbon bed	97% within 48h	50 W/m^3	[151]
Modularized MFC	-	Polyvinyl chloride	70–90%	Upton 60 W/m^3	[152]
-	-	Carbon felt	-	4 W/m^3	[153]
Stacked mono-chamber	-	Carbon paper with Platinum	>90%	1150 mA/m^2	[154]
Double-chambered	-	Compact graphite	-	47 mW/m^2	[155]
Stacked single-chambered MFC	-	Carbon felt	-	Up to 181 mW/m^2	[156]

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In MFCs, proton exchange membranes like Nafion, ultrafiltration membrane, act as a barrier between anodic and cathodic compartment hindering the flow of oxygen and substrates but helps in the diffusion of protons towards the cathode chamber with more than 95% MFC performance in terms of power output [118, 121]. Power generation in microbial fuel cells is dependent on the nature of anolyte and catholyte employed and pH. For the treatment of textile effluent, dye-bearing wastewater with injected biomass is the most commonly used anolyte solution used in MFCs [15]. When exoelectrogenic bacteria is inoculated, the pH of buffered anolyte should be close to neutral or a little alkaline for an excellent performance [146]. An improvement in columbic efficiency (43–60%) was observed when saline solutions having high ionic strengths were taken [122]. However, the observations indicated changes in pH but not in the conductivity of the solution. The cost-effective design of MFC was established by Thung et al. [77] comprising of single-chamber up-flow devoid of the membrane and the scaling-up process was easy to operate with this.

Electricity generation, characterization and structure of the microbial community in MFC was investigated by executing long-term (400 days) and short-term (10 days) operation and the results revealed an increased relative abundance of microbes responsible for organic degradation while those contributing towards electricity generation decreased. Besides that, the removal efficiencies and the power output also decreased during long-term operation because of increased resistances internally [124]. Decolourization of reactive blue 19 (RB19) dye and bioelectricity generation was assessed using MFC, which stated the improvement in microbial enzymatic activity through the addition of a co-substrate, but the efficiency of degradation decreased with increasing concentration of dye owing to the formation of some toxic intermediates which inhibited electricity production. However, the degradation efficiency of 89% was achieved within 48 h in comparison

to 51% degradation rate in open circuit when no resistance was applied and 55% in aerobic conditions [16].

Prasad et al. [75] reported that in contrast to butyrate, propionate and glucose, acetate is regarded as the most efficient organic compound giving 66–72% yield. The factors which should be under observation while working on MFC are the size of electrodes, the surface area of electrodes and distance between the electrodes. Greater the surface area of electrodes, more microorganisms will find a place to attach and hence more activity [157]. Many methods have been devised to remove the organic and inorganic contents but all those need greater cost and high external energy. With the help of MFC, the operational cost is reduced, and renewable energy is generated along with the treatment of wastewater. Although a promising approach, its application on large scale is still a challenge to be accomplished because of the low generation of electrical energy [62].

6 Conclusion and future perspectives

Microbial fuel cell technology is a promising approach for simultaneous wastewater treatment and electricity generation. During the past few years, several advancements have been made in MFC technology. The optimization of various parameters i.e., temperature, substrate and pH were done to enhance the efficiency of the cell. By controlling these factors, maximum power output was obtained. The central issue in microbial fuel cells is electricigens that must be of high quality to enhance the performance of MFCs. Recently, many biological tools have been developed for the modification of existing electricigens bearing tremendous electrochemical activities. The toxicity of chemicals in MFC can also be sensed with the aid of electricigens. The power densities were upgraded not only by the nature of microbial catalysts but also a significant change had been

observed by the selection of materials used for electrode fabrication, the spacing between the electrodes and the conductivity of electrolytes within the chambers.

The power output and efficiency of the cell can be enhanced by using different ion-exchange membranes and efficient binders in the fabrication of electrodes. The selection of substrate for bacterial growth and terminal electron acceptor (TEA) in cathode chamber plays a significant role. Sodium acetate as substrate and potassium permanganate ($KMnO_4$) solution as TEA has proved to provide the increased electric current. In addition to that, this technology becomes economically feasible when integrated in combination with other processes. There is a dire need to reverse the energy, the foremost contributor to climate change. More than 3 billion people yet relying upon unsustainable sources like timber, char, wood coal or dry manure fuel for cooking or heating and renewable energy contributes only less than 20% to the total energy consumption. According to an estimation, 1 MW of electrical energy and 2 MW of thermal power per hour can be produced from one ton of biomass. With such promising projections, one of the objectives that demand microbiology the most is escalating share of renewable energy for the global sustainable energy sector.

Agricultural substrates/wastes and microbes are now used for the manufacture of third generation biofuels specifically bio-electricity. Exoelectrogens; microorganisms capable of extracellular electrons transfer are extensively explored in the field of MFCs. MFCs are a bio-electrochemical system possessing the potential for sustainable energy production by making use of degraded organic matter. Owing to low-energy output with a single microorganism in MFCs, a consortium is now used for higher-energy output. The best promising exoelectrogens are presently being

employed for bioelectricity production, foreseeing powering a NASA space mission. Renewable energy is fundamentally associated with carbon capture along with storage and bioenergy is substantial technology that will play a vital role in coping up with sustainable development goals in the forthcoming decade. Another hopeful application of MFC is bioremediation used to monitor the environmental pollutants and recovery of heavy metals. The dream of launching a large-scale application of MFC can be accomplished by combining it with other processes.

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