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Treatment of Dairy Wastewaters: Evaluating Microbial Fuel Cell Tools and Mechanism

Aman Dongre, Monika Sogani, Kumar Sonu, Zainab Syed and Gopesh Sharma

Abstract

Pollution caused by chemical and dairy effluent is a major concern worldwide. Dairy wastewaters are the most challenging to treat because of the presence of various pollutants in them. The characteristics of effluent like temperature, color, pH, Dissolved Oxygen, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), dissolved solids, suspended solids, chloride, sulfate, oil, and grease depend solely on the volume of milk processed and the form of finished produce. It is difficult to select an efficient wastewater treatment method for the dairy wastewaters because of their selective nature in terms of pH, flow rate, volume, and suspended solids. Thus there exists a clear need for a technology or a combination of technologies that would efficiently treat the dairy wastewaters. This chapter explains the energy-generating microbial fuel cell or MFC technologies for dairy wastewaters treatment having different designs of MFCs, mechanism of action, different electrode materials, their surface modification, operational parameters, applications and outcomes delivered through the technology in reducing the COD, BOD, suspended solids and other residues present in the wastewaters. The chapter also elaborates on the availability of various natural low-cost anode materials which can be derived from agricultural wastes. The current chapter elaborates on MFC technology and its tools used for dairy wastewater treatment, providing useful insight for integrating it with existing conventional wastewater treatment methods to achieve the degradation of various dairy pollutants including emerging micropollutants.

Keywords: dairy wastewaters, chemical oxygen demand, microbial fuel cell, electrode materials, surface modification

1. Introduction

In most countries, the dairy industry has shown tremendous growth in size and volume and is considered to be one of the largest sources of wastewater production [1]. With the swift industrialization that took place in the last century [2] and with the increased milk production rate (approximately 3% annually), dairy processing is generally regarded to be the biggest industrial wastewater source based on food production, especially in European areas [3–5].

The dairy industry is regarded as one of India's prime food industries and India ranked 1st among all the nations for milk produce [6]. The nuanced essence of

wastewater from the dairy industry lies in the presence of carbohydrates, proteins, and fats. 2–2.5 L of wastewater is generated during the processing of every liter of milk [7]. A large number of industries are located around river banks and due to lack of stringent rules and regulations, a large volume of dairy wastewater is released without treatment which goes unutilized and pollutes the environment [8]. Dairy industries are also the potent source for various emerging contaminants specifically estrogens which find their way into the environment through wastewater effluents coming out from dairy industries and livestock activities. The fate of these emerging contaminants is recognized as an issue of public health and environmental concern. The current wastewater treatment technologies are not efficient enough for the removal of these pollutants as these are not monitored regularly due to the lack of stringent rules and regulations for these contaminants. Therefore there is a need to find an innovative technology that serves the purpose. Microbial Fuel Cell (MFC) treatment has gained appreciable interest because of its ability to treat wastewaters and simultaneously leading to the generation of power. This property of the MFC technology makes it suitable for the elimination of such recalcitrant pollutants from dairy wastewater making it sustainable in nature.

2. Characteristics of dairy wastewater

Dairy wastewater comprises of compound organic substances like carbohydrates, amino acids, and lipids which get converted into sugars, acids, and fatty acids upon hydrolysis [9]. Milk is a natural supplement for humans and animals. This consists of various nutrients including protein, vitamin, carbohydrate, and fat [10]. Milk is one of the most valuable items that join commerce, and it is vital as an object of food in daily life. Dairy wastewater contains large amounts of milk components like casein, lactose, fat, inorganic salts excluding detergents and sanitizers that accord greatly towards high BOD and COD [11]. In order to increase milk volumes and improve meat quality antibiotics and antimicrobials have been used in dairy animals at the sub-therapeutic level. This does not only harm the animal's health and well-being, but also significantly affects the health and well-being of humans through the intake of animal products like milk and meat, thereby affecting

Sr. no	Details	Value (in mg/L) except for pH
1.	pH	5.4–9.1
2.	Total solids	<2200
3.	Total dissolved solids (TDS)	<2100
4.	Suspended solids (SS)	<100
5.	Total chlorides	<600
6.	Sulfates	<1000
7.	Phosphates	<5
8.	Oil and grease	<10
9.	Chemical oxygen demand	<360
10.	Biological oxygen demand	<30
11.	Nitrates	<10

Table 1.
Standard norms of Central Pollution Control Board of India for dairy effluents (Environment (Protection) Rules, 1986).

public health [12]. According to the Environment (Protection) Rules, 1986 of India, the standard norms and limiting characteristics of dairy effluents as mandated by the Central Pollution Control Board (CPCB) of India are mentioned in **Table 1**.

Effluents from milk production have increased temperatures and varying pH, TSS, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen, total phosphorus and fat, oil and grease [3, 5, 13–16]. Generally, dairy wastewater has a white color with an undesirable odor and turbidity [4, 17, 18]. With 16–25°C annual temperatures, dairy effluent waste flows are hotter than urban wastewater (10–20°C), leading to accelerated biological deterioration correlated to other sewage treatment plants. Industrial dairy effluent average temperatures range from 17 to 18°C in winter, and from 22 to 25°C in summer.

3. Factors affecting characteristics of wastewater

3.1 Volume of wastewater

Water has an important role in milk processing. It involves cleaning, washing, disinfection, heating, and cooling in every step of the technologies used. There is a massive requirement for water [19]. A large amount of wastewater is generated through manufacturing processes [20]. Contaminated water from sanitary practices amounts to 50–80% of the actual water utilized in the dairy industry, while the rest of the 20–50% is clean water [20, 21]. It has been measured in volume units stating the quantity of wastewater is around 2.6 times more of the processed milk. The characteristics and the amount of the wastewater generated rely mainly on the size of the factory, technology used, efficacy and convolution of clean-in-place methodologies, good manufacturing practices, and so on [2, 5]. However, the world's mean wastewater volume can be decreased from 0.49–36.0 m³ to 0.5–2.0 m³ of effluent per m³ of milk processed with the introduction of GMP [5, 22]. Nowadays, the volumetric charge designed is 1 m³ of effluent per ton of milk produced. The instant discharges installed in the washing of tank on transport trucks, mediator pipelines, or machinery after every cycle are a significant aspect of the volume-based loading of wastewater treatment plants designed for dairies. In these cases, the effluent volumes are greater than those of the milk produced [23]. On average, the amount of wastewater discharged is 70% of freshwater being used at the plant [20]. Effluents from dairy products primarily include milk and its products misplaced in the processing cycles (milk spills, skimmed milk, spoiled milk, and curd remnants), inoculums used in processing, byproducts generated by manufacturing techniques (whey, milk and there permeates), and several additives used in manufacturing [16, 21, 24, 25]. Milk lost in wastewater treatment is about 0.49–2.5% of milk processed, which may rise up to 4% [26].

3.2 Categories of wastewater

3.2.1 Processing water

Cooling the milk in separate coolers along with condensation from the evaporation of whey and milk leads to the production of water for fermentation. Vapors are extracted from the milk and whey drying process that after condensation produces the cleanest effluent, but they can also consist of volatile compounds, whey, and milk droplets. Processing waters eliminate toxins, and after minimal pretreatment may be stored or released with stormwater [3]. Water can be reused for systems where the derivative materials are not in close contact. Typical applications involve

hot water, steam manufacturing, and membrane washing. After the final flushing of bottles and condensates from secondary vapors created in vacuum installations, water from liquid cooling during pasteurization can be used for room washing, irrigation, and so on.

3.2.2 Cleaning wastewater

Wastewater purification typically benefits from cleaning machinery within close contact with dairy goods. This involves spillage of milk and substance, whey pressing or brine, malfunctioning of the clean in place effluents, or machinery errors. More than 93% of the organic contents contained in the effluent are partly the remnants of milk, cheese, whey, butter, sugar, honey, and fruit concentrate or stabilizers. These effluents are found in significant concentrations and are extremely toxic thereby needing more care.

3.2.3 Sanitary wastewater

Sanitary wastewater is utilized in washrooms, toilets, etc. Sanitary wastewater has parallels with urban wastewater composition and is typically piped straight to sewage facilities. It may be used as a supply of nitrogen for irregular dairy effluents after a secondary aerobic treatment. Furthermore, by-products from agricultural processes like milk, whey, and their permeate can be classified independently if they are segregated individually from other wastewater sources [27, 28].

4. Dairy wastewater treatment

For the dairy industry, common wastewater treatment strategies involve grease traps, oil-water separators to remove floatable solids, flow equalization, and clearers to isolate suspended solids. Biological treatment consists of the aerobic and anaerobic methodologies. Anaerobic treatment accompanied by aerobic treatment is also used to minimize soluble organic matter (BOD), and the reduction of biological nutrients (BNR) is used to increase nitrogen and phosphorus levels. Biological aerobic treatment requires cellular destruction in the presence of oxygen. Conventional aerobic treatment of dairy manure includes procedures such as activated sludge, batch sequencing generator, revolving biological contactors, trickling pipes, aerated lagoons, or a variation of these.

Treatment of anaerobic wastewater has emerged as a feasible and inexpensive alternative particularly for high BOD removal over conventional aerobic treatment. Anaerobic methods of treatment involve up-flow anaerobic sludge blanket or UASB, anaerobic batch sequencing reactors or ASBR, continuous-flow reactor, hybrid anaerobic digesters, up/downflow anaerobic filter, and various 2-stage processes that use acid and methane forming bacteria. **Figure 1** shows the sequential treatment of dairy wastewater through mechanical, physical, chemical and biological treatment methods [20].

4.1 Mechanical treatment

This is the initial phase of dairy wastewater treatment and this includes grit pool, skimming tank, and main clarifiers. During further effluent processing, the large floating material is removed by screens, in-turn avoiding the chocking of pipes. Chambers are used for extracting heavier inorganic substances like sand, gravel, etc. The aim of installing skimming tanks is to extract oil, grease, pieces

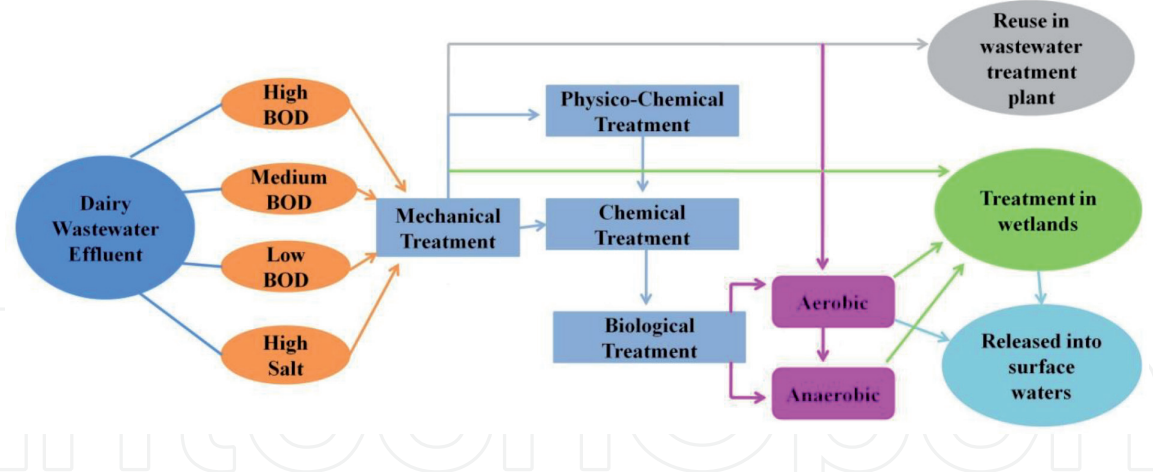


Figure 1.
Dairy wastewater treatment alternates (adapted from [20]).

of wood, skins of fruit; etc. The clarifier helps matter to settle at a very slow rate or sediment at the bottom in the tank. The substance accumulated underneath is known as sludge [29].

4.2 Chemical treatment

Chemical treatment is also recognized as precipitation. This is performed by adding flocculants to wastewater and vigorous mixing with agitators. This method precipitates insoluble phosphate into larger flocks, in the form of small pellets. In pre-sedimentation basins, the greater flocks settle as the main sludge, whereas a clear supernatant fluid overflows into a lake for biological therapies. Sedimentation lagoons are armed with tools to continuously scrape the sediment towards a sump or oblique gutters to keep water away from the clarified surface layers [29].

4.3 Biological treatment

Milk effluent includes organic waste; therefore most viable methods for the elimination of organic content are biological degradation. However, sludge generated may lead to serious and costly problems towards disposal, particularly during the processes of aerobic biodegradation. This can be further worsened due to the tendency of sludge to absorb various organic compounds and poisonous heavy metals also. Nonetheless, biological treatment has the profits of dynamic organic microbial processes and the ability for adsorption of heavy metals effectively. Biological waste management strategies have an immense capacity to incorporate diverse types of biological schemes for selective elimination [30].

4.3.1 Aerobic treatment

Microorganisms cultured in an O₂-rich environment degrade organics by oxidizing matter to CO₂, soil, and cellular material. Aerobic treatment methods include activated sludge reactors, rotating biological reactors, conventional filters for trickling, and so on [30].

4.3.2 Anaerobic treatment

Anaerobic method of treatment is mainly intended for the biological processing of high strength wastewater. It is a process by which microbes are used in the absence of O₂ to digest organic matter by converting it to biogas (CH₄ and CO₂)

and some inorganic contents. 6% of the organic load can be converted into biogas from the wastewater and the rest can be used for cell growth and maintenance. The process reactors are shielded to avoid air obstruction and the release of odors [30].

5. Advanced technologies for the treatment of dairy effluent

5.1 Physio-chemical process

5.1.1 Electrocoagulation (EC)

The electrocoagulation (EC) method could be the alternative treatment option for dairy wastes. Electrocoagulation is an electrolysis process that uses specific electrodes by transferring electrical current via the effluent to extract dissolved organic waste, turbidity, and coloring matter. The method assists in the substantial removal of suspended colloidal particles.

5.1.2 Adsorption

Adsorption was found beneficial among the various physio-chemical treatment methods for removing organic compounds in wastewaters. Activated carbon is mainly used in treating wastewater, among other types of adsorbent materials. Although certain additional adsorbents can also be used to treat streams of wastewater and are cost-effective as well. For instance rice husk ash, coal fly ash, etc. [31].

5.1.3 Membrane treatment

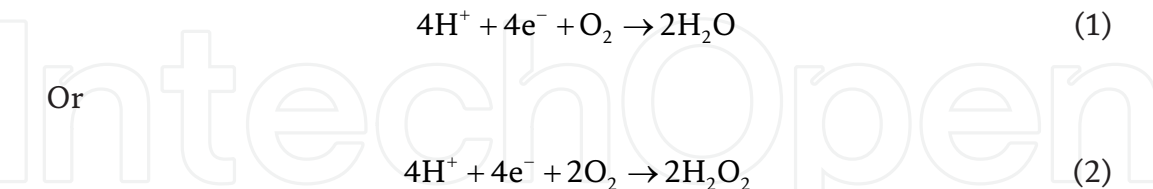
Microfiltration, nanofiltration, ultrafiltration, reverse osmosis, and electrodialysis are typical membrane separation processes. Highly feasible product recovery is possible using these methods and the effluent generated is of high quality which can be used directly [31].

6. MFC in dairy wastewater treatment

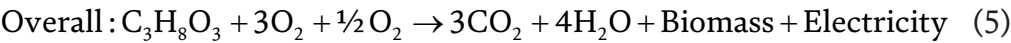
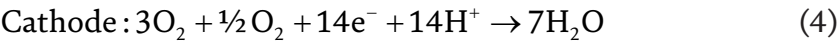
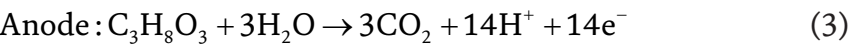
The organic contents in wastewater make it a convenient substrate for MFC applications [32]. Various studies have shown that wastewaters from the dairy industry generate significantly less power as compared to the other wastewaters in MFC [33, 34]. Carbohydrates and proteins are among the main components of dairy wastewater. Their influence on the generation of power in MFC along with COD removal by using dairy wastewater as a substrate was mentioned by [35], and was reported that reduction in proteins and carbohydrates does not have a virtuous relation with power generation. The presence and elimination of antibiotics found in dairy wastewaters is a major problem. New technologies need to be employed to solve this problem. Researchers working with dairy wastewaters have concentrated on developing the MFC design that will boost the power generation (**Table 2**). Various surface modifications of the electrode material have improved MFC efficiency by increasing the power output [36].

MFCs are distinctive biofuel cells among the various bio-electrochemical systems that generate electricity by employing microorganisms [41]. For electricity production, hydrogen fuel and oxygen are utilized by the microbial fuel cell. Using bacteria as biocatalyst, MFC converts organic matter into electrical energy [42, 43]. An ideal MFC contains two chambers (cathode and anode), both separated

via a proton transfer membrane. The anode chamber consists of the electroactive microorganism, thereby making the chamber biotic whereas the cathode chamber remains abiotic. The available microorganisms in the anode chamber act as the biocatalysts, thereby leading to the degradation of organic matter in order to generate electrons that are transferred to the cathodic chamber via an electric circuit. The free electrons present on the cathode leads to the reduction of oxygen for processing of water as shown in Eqs. (1) and (2).



Considering glycerol as an electron donor and oxygen as a terminal electron acceptor, the following reactions occurring in MFCs, shown in Eqs. (3-5).



In biological fuel cells, the catalyst is either an enzyme or the microorganisms as simple as Baker’s yeast. Microbial fuel cells convert the chemical energy

S. no.	Types of MFC	System configuration		%COD removal	Maximum surface/volume power density	Refs.
		Anode	Cathode			
1.	Single chamber MFC	Graphite coated SS anode	Carbon cloth	91%	20.2 W/m ³	[36]
2.	Single chamber MFC	SS mesh anode with graphite coating	Carbon cloth	80%	27 W/m ³	[37]
3.	Dual-chamber MFCs	Plain graphite plates	Plain graphite plates	91%	3.2 W/m ³	[8]
4.	Dual-chamber MFCs	Graphite-sprayed SS mesh	Graphite-sprayed SS mesh	91%	5.15 W/m ³	[38]
5.	Dual-chamber MFCs	3D laminated composites	3D laminated composites	81%	122 W/m ³	[39]
6.	Dual-chamber MFCs	Carbon fiber brush	Platinum/ carbon	NA	1056 mw/m ²	[40]

Table 2.
Performance of different types of MFC using dairy wastewater as substrate (authors created).

of carbohydrates present in the substrate, such as alcohol and sugars directly into electrical energy. Currently, efforts have been made towards using MFCs for domestic wastewater treatment and at the same time point, electricity production considering the environmental issues and further reuse of waste [44]. Sewage sludge of anaerobic nature is used to inoculate MFCs, as it is conveniently used from a wastewater treatment plant and it has largely diverse bacterial communities containing electrogenic bacterial strains [45]. MFCs have functional and operational benefits compared with the presently used technologies for producing energy from organic content [46].

7. Comparison of anodic metabolisms in bioelectricity generation by dairy wastewater treatment in microbial fuel cell

The growing concern about environment safety and rapid depletion of energy reserves have made it imperative to update the waste management methods from the mere waste treatment to a novel prospect of waste to energy [47]. Microbial Fuel Cell is a novel technology for electricity generation from organic matter present in wastewater, treating wastewater simultaneously solves energy crisis and environmental damage issues [48]. To generate electricity, Microbial Fuel Cell (MFC) is a

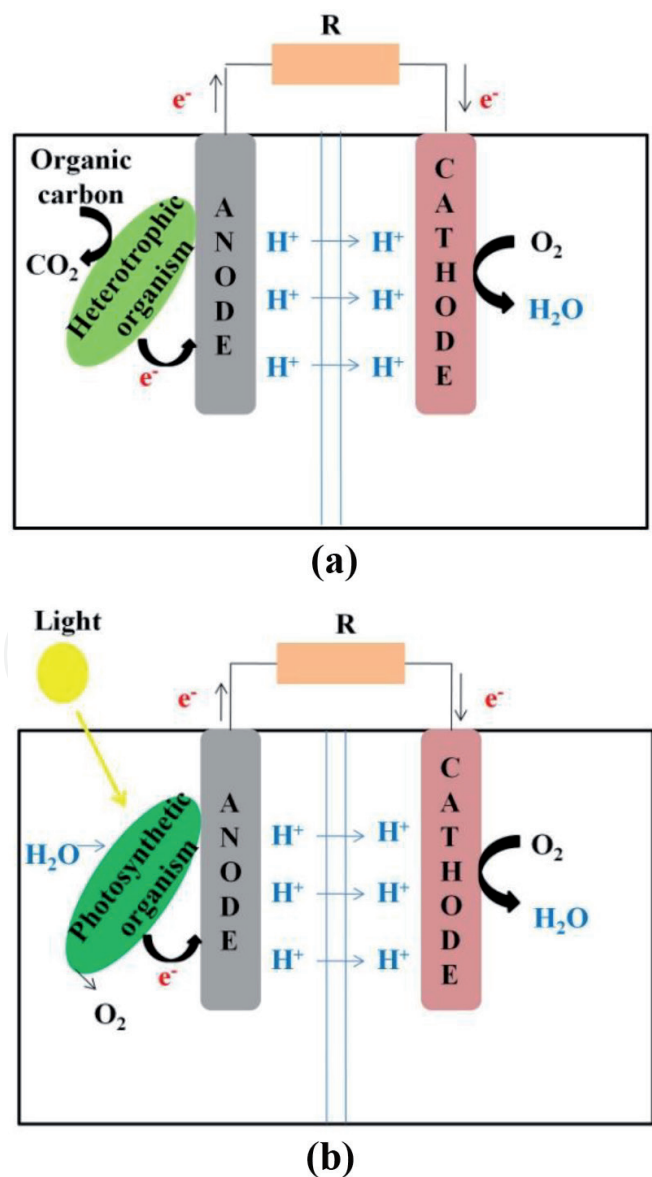


Figure 2. (a) Anaerobic anodic metabolism in MFC (adapted from [51]). (b) Aerobic anodic metabolism in MFC (adapted from [51]).

bio-electro-chemical system that uses bacterial oxidation of biodegradable organics. The development of bio-potential takes place when organic substances get oxidized to electrons and protons through microbial metabolism. The bacteria transport the electrons to the anode via a variety of mechanisms such as electron shuttles or solid conductive matrix. Then electrons get transported to the cathode via circuit externally [45]. The protons from the anode chamber are transferred to the cathode chamber via passing through the proton exchange membrane, where they form water by combining with the electrons and O_2 in the presence of a mediator. The potential difference between the bacteria's respiratory metabolism and the electron acceptor creates the voltage and current required to produce electricity [45, 49]. In a study, the MFC system is scaled up, consisting of 40 individual cells that have been constructed and evaluated which can generate 4.2 W/m^3 of energy and capable of powering LED panel [50]. Extensive research and scaling up of MFCs will further enable adequate conversion of waste to energy. For long term use, MFCs can be clubbed with the existing technologies for wastewater treatment and electricity generation. MFC uses anaerobic anodic metabolism where it employs bacteria as a substrate for the reduction of COD from wastewaters as shown in **Figure 2(a)**. This technology is further followed by aerobic anodic metabolism where it employs algae as a substrate and under photosynthetic conditions causes the reduction of nitrates and phosphates from wastewaters as shown in **Figure 2(b)**. This combination of treatment with an effective and proper choice of anode material will help in the generation of power followed by the degradation of wastewaters. Further studies need to be carried out for the degradation of antibiotics in such an innovative integrated MFC model for dairy wastewater.

8. Degradation mechanism

The bacteria transfers the electrons to the anode through different mechanisms, including (i) direct bacterial contact via cytochrome, endogenous redox-active based self-mediated electron transfer, such as pyocyanin and conductive pili; (ii) artificial electron shuttles or mediator. Electrons then get transported to the cathode by passing via an external circuit, while the protons are passed through from the anode chamber to the cathode chamber by proton exchange membrane or PEM. At the cathode, the concoction of electron, proton, and O_2 occurs for the production of water. The potential difference between the respiratory metabolism of bacteria and the electron-acceptor creates the voltage and current necessary for electrification.

9. Electron transfer mechanism

The power output of an MFC rests on different aspects including the type of organic content available in wastewater, electron transfer rate from bacteria to the anode, and the membrane ability to carry hydrogen ions [52]. Some micro-organisms are known to transfer electrons to their external environment from their oxidative metabolic pathways, which are called exoelectrogens [53]. *Geobacter* and *Shewanella* are the two prime bacterial genera that are known with this ability; the extracellular transportation of electrons to the electrodes occurs through three different ways namely:

1. Direct transfer of electrons
2. Mediator based electron transfer, and
3. Nanowires based electron transfer.

9.1 Direct transfer of electrons

Geobacter and *Shewanella* sp. use a direct electron transport mechanism where the electrons are dispatched directly to the electrode surface. The outer C-type cytochrome membrane is associated with the direct dispatch of NADH-produced electrons [54].

9.2 Mediator based electron transfer

Some species of bacteria like *Shewanella* and *Pseudomonas* secrete certain shuttle molecules like flavins, to pass electrons to electrodes via the cell membrane of the bacteria [55, 56].

9.3 Nanowires based electron transfer

Genera of *Geobacter* and *Shewanella* are evident to use conductive auxiliaries for transporting the electrons outside of the cell [57, 58]. Such conductive networks, called nanowires, are cellular outgrowths for as long as 20 μm . These nanowires are claimed to have a substantially higher electrical conductivity than the synthetic metallic nanostructure [59].

10. Anode materials for MFCs

Choosing and designing an anode has a direct effect on the performance parameters which includes the microbial adhesion, transfer of electrons, and oxidation of fuels. An MFC system's achievable power density depends on the selection of an anode that significantly affects the output of an MFC system [60]. As a consequence, achieving higher power density requires the ability to facilitate the improved transfer of electrons from the bacterial cells to the external circuit, thus the anode is of prime importance towards attaining this objective [61]. The electron transfer process necessitates the donation of an electron using extracellular electron transfer (EET) towards the anode surface by the anode respiring bacteria or ARB and, consequently, the current flow in the circuit externally. This mechanism has been interpreted as being similar to transfer electrons to the anode surface from the cell through direct electron transfer mechanism, soluble electron shuttles diffusion, and the transfer of electrons from biofilm via solid component (pili) [61]. Essential features for the anode to attain the best performance include biocompatibility [62–64], corrosion-resistant, low electrical resistance, and high conduction of electricity [62]. The anode must also be of chemically inert in nature that can function in an environment containing diverse biodegradable wastewater composed of variety of organic and inorganic components that are able to react with the anode material causing its deterioration inefficiency.

Lots of anode materials have been used in the last five years to create various anodes for MFCs. The choice of material for the construction of anode, in particular, is significantly influenced by improvement in different MFC system structures. On a particular note, various exotic carbonaceous materials' use is on a hike. This new category includes stainless steel, stainless steel with modified surface, and anodes based on graphene-based carbonaceous anodes. In many recent studies the graphene-based anodes are found very encouraging [65–68]. The grapheme composite anodes have been stated for higher power production [69–71]. Similarly, the use of carbon nanofibers, carbon nanotubes single and multi-walled anodes has also been documented for high-performance MFCs [72]. This chapter has categorized a few

of the recent approaches in the configuration of anode materials dividing them into four vast categories, namely modern carbon-based anodes, carbon-based composite anodes, surface-modified and metal-based anodes, and each of these categorized materials are discussed individually in the proceeding sections.

10.1 Modern carbon-based anodes

In MFC systems, various anode materials based on carbon have been used over the last decade. These include carbon cloth, carbon paper, or sheet or graphite plates and graphite rod. Using carbon-based anode materials has the advantages of cost-effectiveness, biocompatible nature, efficient electrical conductivity, and chemical stability [73]. Due to their potentially high-performance enhancement and excellent properties, these have been recognized as being very useful for building MFCs. Accessible surface area is an essential factor that affects the efficiency of these anode materials [74, 75]. Such anodes comprises of natural or synthetic anode materials which are as follows:

10.1.1 Natural anode materials

Synthesis of high-efficiency anode components, by using renewable and recyclable components, provides an outstanding ecological solution including both deriving reusable energy from nature and maintaining biodiversity. An interesting example is the layered corrugated carbon anode production from low priced packaging material through carbonization (LCC). It is important to remember that the LCC's 3D surface is normally tunable by differing the height and layers of the flute. A six times increase in the number of layers resulted in a successive rise in current density because of the potential for biofilm formation in wider surface areas. It is evident that the LCC anode has four times the current density as correlated with the graphite felt anode. Natural anode materials prove to be an ideal option for low priced microbial fuel cells due to their 3-dimensional microporous structures, increased electron transfer rate, and high kinetics of the electrogenic bacterial population. A variety of recently produced highly 3-dimensional porous anode material uses LCC as a low-cost high-performance substitute, usually manufactured from carbonized recycled paper [76, 77]. High performance was obtained from the use of 3-dimensional anodes, based on exoelectrogens' 3-dimensional growth. Stronger anode kinetics can be attained by using maximal anode surface area, but the efficiency only rises gradually as the reaction reaches the triple-phase boundary, i.e. lower inner resistance among anode, cathode, and electrolyte. Interestingly, in comparison with the plane graphite electrode, 8 times better performance is seen with carbonized corn stem. However, few benefits of the aforementioned electrode material include increased biocompatibility, less internal resistance, and rougher surface that facilitated linkage to biofilm. A coated rough electrode, constructed from the carbonization of common packaging materials, was observed to be the highest rated anode of all carbon-based modifications. The current densities achieved were 201 A/m² and 391 A/m², respectively, from three and six corrugated layers. This is a low-cost material with higher performance for the construction of MFC.

10.1.2 Synthetic anode materials

It is quite evident that 3-dimensional carbon fiber (non-woven) can achieve a maximal current density of up to 31 A/m² which is prepared by electrospinning and blowing the solution. The performance and efficiency of MFCs also depends on the system architecture, based on these 3D materials [78]. Double-sided air cathode reduces the boundaries of mass transfer. The stainless steel frame was

used for this design as a current assimilator and a carbon fiber support in the 3D matrix. In another study [79], it has been shown that an upgraded adaptation of the carbon-based multi-brush anode achieved admirable power generation. The power generated is similar to that obtained with a carbon anode with a single brush design. Because of cathodic limitations, the MFC system [80] gave a comprehensive comparison of carbon-based material for anodes, like graphite, carbon fiber veil, polycrystalline carbon rod, glossy carbon rod, graphite foil. The maximal current density attainable was calculated using a standardized biofilm grown in domestic wastewater. At 30°C, graphite, and polycrystalline carbon-based rods, both reached catalytic currents peaks of around $501 \mu\text{A cm}^{-2}$. By comparison, carbon fiber veil or paper-based material delivered a 40.1% higher current than graphite anode due to its large, microbial rich surface area [80]. In comparison with steady-state reactor, the rotational motion of carbon brush anodes in the tubular microbial fuel cell resulted in a 2.6 times rise in performance. The rotation was adequately mixing the nutrient and minimizing the limitation of mass transport. In general, several studies have shown that the existence and electrode content affected the kinetics of the biocatalyst. It has also been shown that the internal resistance is a major aspect affecting the overall performance. The use of 3-dimensional anode models, like carbon nanotubes (CNTs), nanofibers (CNF), gold/poly (ε-caprolactone) micro-fibers (GPM), and gold/poly (ε-caprolactone), to reduce the internal resistance increasingly preferred in microbial fuel cells. 3-dimensional anode material has less internal resistance than two-dimensional anodes. Such anode materials serve to increase the efficiency of nutrients, H^+ , and O_2 transfer via biofilm as compared to macroscopic carbon-based paper and planar gold-based anodes. Chemical assisted surface alteration of the CNT/CNF-based anodes has been demonstrated to reduce kinetic losses and cellular toxicity. Ren et al. [81] investigated vertically aligned CNT, randomly aligned CNT, and spin-spray layered CNT. The studied nanotube-materials have a 4000 m^{-1} very large surface area to volume ratio which is very huge. The results showed that CNT-based anodes attracted more electro-genic microbes than bare gold, resulting in a thicker and more stable formation of biofilms. Using CNTs in a miniature MFC device, a maximal power density of 3321 W/m^3 was achieved [81]. This was 8.5 times greater than that attained with the 2D-electrode systems.

10.2 Composite anodes

Composite anodes have intrigued extensive interest recently. These materials were utilized to attain synergistic effects with two or more materials to alter original content, resulting in increased anodic kinetics efficiency.

10.2.1 Graphite-polymer composites

Tang, Yuan, Liu, & Zhou prepared a nano-structured capacitive layer of modified 3D anode consisting of core-shell nanoparticles derived from titanium dioxide (TiO_2) and egg albumin (EWP). This was built into a loofah sponge carbon (LSC) to achieve an efficient 3-dimensional electrode. The LSC's coating with TiO_2 and heat treatment caused tiny particles to cover its entire surface. The resulting altered anode supplied greater power than a graphite anode. The increased power was associated with the increased electrochemical capacity of 3-dimensional anodes and to the synergistic effects of carbon derived TiO_2 and EMP with good characteristics like more surface area, improved biocompatibility, and favorable surface functionality for easier extracellular electron transport [82]. The anodes of open-celled carbon scaffold (CS) and carbon scaffold graphite (CS – GR) were created by

carbonizing the microcellular polyacrylonitrile (PAN) and composite PAN/graphite (PAN – GR). The PAN-GR was created by utilizing supercritical carbon dioxide (Sc-CO₂), as a practical foaming agent. The maximal current density achieved with a CS altered anode was 102% greater than that with carbon felt. Improved performance has been referred to as enhanced hydrophilicity and biocompatibility caused by carbonization. Carbon nanofibers with improved graphite fibers and reduced nanotube-coated graphene oxide/carbon scaffold promise new composite anode materials. Using carbon nanofibers as anodes for MFC modified graphite fibers achieved a maximal current density at a peak of 35.8 A/m². The nanotube-coated scaffold anode device with reduced graphene oxide/carbon obtained a power density of 335 mW/m³. Composite graphite fiber brush anode (MFC-GFB) was used in combination with granular graphite (MFC-GG) in a tubular setup to boost the power density 5.2 and 1.3 times greater than that obtained with MFC-GG and MFC-GFB. The improved efficiency of the system was referred to the thick biofilm of the system, and scant internal resistance [83]. Six types of micro or nano-structured anodes utilized in micro-sized MFCs have been compared. The anodes under consideration included carbon nanotubes (CNTs), carbon nanofibers (CNFs), gold or poly (ε-caprolactone) microfibers (GPM), nanofibers (GPN), planar gold (PG), and traditional carbon paper (CP). All anode's effectiveness was tested with the use of small and micro-liter sized MFC. A homemade 3-dimensional anode coating has been developed using the iron net as the structural anchor and fastened to a carbon felt layer [82]. The combination of carbon powder and a solution mixture of 30% polytetrafluoroethylene (PTFE) have greatly affected power generation. The performance was assessed using an acetate-fed MFC and the anode coating which improved the power generation considerably. The internal resistance measured in the MFC system was decreased by 59.3% compared to the non-coated iron net, whereas the power density improved by 1.49 times.

10.2.2 Carbon nanotubes composite

Due to their special intrinsic properties, including high conductivity, rust tolerance, high surface area and electrochemical inertness, the usage of CNTs has drawn significant attention lately.

10.2.3 Multi-walled carbon nanotubes composite

Multi-walled carbon nanotubes (MWCNTs) with carboxyl functional groups were utilized for MFC air respiration. It demonstrated a 2-fold improvement in power density relative to the carbon cloth electrode [84]. In a recent report, multi-walled carbon nanotubes/SnO₂ nanocomposite coated on the glass fiber electrode is used [85] producing maximal power densities of 1422 mW/m² and 457 mW/m², respectively [86]. The use of graphite coated with manganese oxide/multiwalled carbon nanotubes composites has greatly elevated benthic microbial fuel cells in another study. The composite provided greater hydrophobicity, kinetic movement, and power density when opposed to the standard graphite electrode. The shift seen was attributed to the consolidated impact of the Mn ions electron transfer shuttle on the reaction site and its redox reactions (i.e. anode and biofilm) [87].

10.2.4 Graphene anodes

Graphene is an allotrope of 2D crystalline carbon with unusual characteristics such as large surface area (up to 2600 m²/g), exceptionally high electrical conductivity (7200 S/m), and exceptional tensile strength up to 35 GPa [88]. Graphene-modified

stainless steel mesh (GMS) power density was recorded to be 18 times higher than that of a stainless steel mesh anode (SSM) and 17 times higher than that of polytetrafluoroethylene modified SSM (PMS) [68]. The significant improvement was recognized due to increased surface area of the electrodes, improved adhesion of bacterial biofilms, and efficient extracellular electron transfer. The current stainless steel collector (SS) boosts electrical conductivity for electrode, and the overall efficiency of the system is enhanced by the current SS assimilator which reduces internal resistance. Chen et al. [69] used an ice template as an anode to create a versatile macroporous 3D graphene sponge. The microporous 3D graphene allowed the random propagation of bacteria and resulted in a high biofilm span and increased performance [69]. From another study, tin oxide (SnO₂) nanomaterials were utilized on the reduced graphene oxide surface (R-GO-SnO₂) able to generate electricity that was approximately 5 times higher than the use of an unaltered graphene oxide (reduced). Collegial effects among SnO₂ and graphene and strong biocompatibility were liable for the much stable formation of bacterial biofilms and the efficiency of charges transfer [86]. Reduced graphene oxide/carbon nanofibers (R-GO-CNTs sponges) melamine sponges based on dip-coating technique tend to cater to a huge electrically conductive surface area for *Escherichia coli* growth as well as electron transport in MFC [65]. Four R-GO-CNT sponges were tested with varied thicknesses and configurations, but the thinnest one (with a thickness of 1.5 mm) displayed prime efficiency, generating a maximal current density of 336 A/m³ [65]. The usage of a redesigned anode built from graphene-polyaniline nanocomposite was also found to produce power three times greater than carbon cloth [70]. Often used as an anode for MFC was a 3-dimensional reduced graphene oxide-nickel foam (R-GO-Ni) by accurate deposition of R-GO sheets to the nickel foam substratum. The R-GO thickness may be modified in comparison to the surface region of the electrode by initiation cycles. This macro-porous scaffolding design not only offers a 3-dimensional surface for microbial growth but also promotes the mobility of substrates inside the culture medium. The efficiency was extensively better than with the usage of nickel foam and various graphite materials dependent on anodes [63, 64]. The formation in MFC of highly crystalline graphene or nickel electrode with *Shewanella putrefaciens* provided the power density of typical MFC carbon cloth anode 13 times greater. Because of the minimal cost of hollow Ni and the low weight percent of graphene (5% w), this composite electrode provides good potential in the development of efficient MFCs for greater power generation [71].

10.3 Surface modified anodes

The electrode surface has a tremendous role in the total anode's efficiency. Currently, several reports have stated that surface alteration is advantageous in actuating increased bacterial adhesion and better biocompatibility that favors electron transfer kinetics. The surface alterations using TiO₂-carbon fabric-based nanofiber usually attain the highest current density of 7.99 A/m², whereas a changed surface with carbon nanotubes and coated with conductive polymer had a maximal power density of 1573 mW/m². The two broad surface treatments that are most generally used are silicone coating and graphite or carbon surface application. Each of these surface alteration forms is discussed in subsequent subsections.

10.3.1 Conductive polymer coatings

Provided their high conductivity and biocompatibility, conductive polymer coatings have drawn considerable interest [85]. Composite polyaniline (PANI)-mesoporous tungsten trioxide (m-WO₃) had been formed and utilized as a catalyst, free of precious metals [63, 64]. PANI was mounted onto m-WO₃ by

the chemical oxidative process. The composite's catalytic nature was elaborated through the application of electrochemical techniques. Significant efficiency changes were observed with the composite based on the- WO_3 and PANI combinations. The m- WO_3 has excellent biocompatibility while PANI has strong electrical conductivity [63, 64]. PANI networks' electrode location on graphene nanoribbons (GNRs)-coated carbon paper (CP/GNRs/PANI) has been found to increase power generation as opposed to GNR and CP usage. The improvement was due to the positively charged PANI backbone which increased the affinity of interaction with negatively charged microbial cells and thus favored direct transfer of electrons through cytochromes. Conductive GNRs significantly enhanced CP/GNRs/ PANI electrode conductivity in neutral environments. This discovery explicitly shows that the synergistic impact of both components was responsible for major energy production changes. In another report, carbon nanotubes/polyaniline carbon paper (CNT/PANI carbon paper) is used and correlated with other conventional carbon paper [63, 64]. The findings revealed that the CNT/PANI carbon paper has obtained a lower ohmic loss and improved power generation. The use of CNTs enhanced the surface area for the biofilm span, as well as achieved a higher electrical conductivity. The achieved maximum power density of 257 mW/m^2 corresponds to an increase of 343% and 186%, respectively, when compared with those achieved with the pristine GF MFC and the PANI/GF MFC, respectively [89].

10.3.2 Graphite/carbon surface modifications

Vertically targeted TiO_2 modified carbon paper shapes vertically breaching pores which offer the bacteria a large area of contact for direct electron transmission. This was particularly useful in a recent study for improving the delivery of nutrients, attaining high biocompatibility, and supporting the electron transport routes [90]. Through using a TiO_2 -NSs or CP as a bio-anode, a mixed consortium inoculated MFC's average power production density was improved by 64% relative to using a pure CP as a bio-anode. In a different study, dual nanofiber mats TiO_2 (rutile)-C (semi-grafito)/C (semi-grafito) were used for MFC anode, one fiber consisting mainly of O, Ti, and C, while the content of the other fiber was predominantly Carbon. The dual nanofiber had stronger efficiency than a single nanofiber. The highest existing density obtained in that analysis was 8 A/m^2 [91]. The activated carbon (AC) with SSM (AcM) and Fe_3O_4 anode was also investigated for MFCs, and capability enhancement was related to device efficiency [92]. Nano-goethite was added with 0, 2.5, 5.0, and 7.5% (mass percentage) to the activated carbon (AC) powder and pressed onto the stainless steel wire. The composite material anodes produced 35 percent more power than a non-modified AC anode. The improved performance was achieved due to reduced transfer charge resistance (R_{ct}) and strengthened the current exchange rate (I_o) [92]. Several experiments have shown that start-up time for MFCs in nitric acid or ammonium nitrate can be reduced by electrochemically oxygenated carbon wire. It has been replicated in one report [93] that the coulombic performance of the anodes adjusted by this process was 71 percent. Responsive groups containing oxygen on the carbon surface could be liable for the improved overall efficiency of the system [94].

10.4 Metal-based anode

Many metals such as gold, titanium, and copper have been used as anodes in MFCs for use in the last ten years. Because of their corrosive nature, most of those metals were unsuitable. Conversely, the use of stainless steel as an anode for microbial fuel cells has attracted increasing interest [95].

11. MFC components

A MFC consists of an anode chamber divided by a PEM, and a cathode chamber. By exposing the cathode into the air directly, a mono-compartment MFC eradicates the need for the cathode chamber.

12. Two-compartment MFC systems

Two-compartment MFCs are frequently run in a batch before equilibrium is established to produce energy in the MFC device with a well-defined chemical media such as glucose or acetate. Once the stability is maintained the dairy wastewater is pumped into the anodic chamber continuously through a peristaltic pump, which is currently only being used in the laboratories. A standard two-compartment MFC has two chambers one for the anode and the other for the cathode linked by a PEM or a salt bridge, to enable protons to travel to the cathode whilst preventing oxygen diffusion towards the anode. The compartments would be taking numerous functional forms. Mansoorian et al. [96] constructed non-catalyst and non-mediator membrane microbial fuel cell (CAML-MMFC), as seen in **Figure 3**, for simultaneous treatment of wastewater and bioelectricity production. The CAML-MMFC was equipped with two chambers with an anaerobic anode and aerobic cathode container and divided from one another by a proton exchange membrane. The chambers were constructed of plexiglass sheets 2 cm in diameter, each with an effective volume of 2 L with the gaskets tightly sealed. The anode and cathode electrodes were formed from a graphite plate $14 \times 6 \times 0.5 \text{ cm}^3$. The electrode in the anode was 5 cm from the membrane, and the electrode in the cathode was 2 cm from the membrane. Via a resistance, the electrodes were attached to copper wire 2 mm in diameter and 35 cm in total.

Jadhav et al. [97] used a cow urine administered another type of dual-chambered MFC with an outer cathodic chamber volume of 2.5 L, made of a plastic bucket and

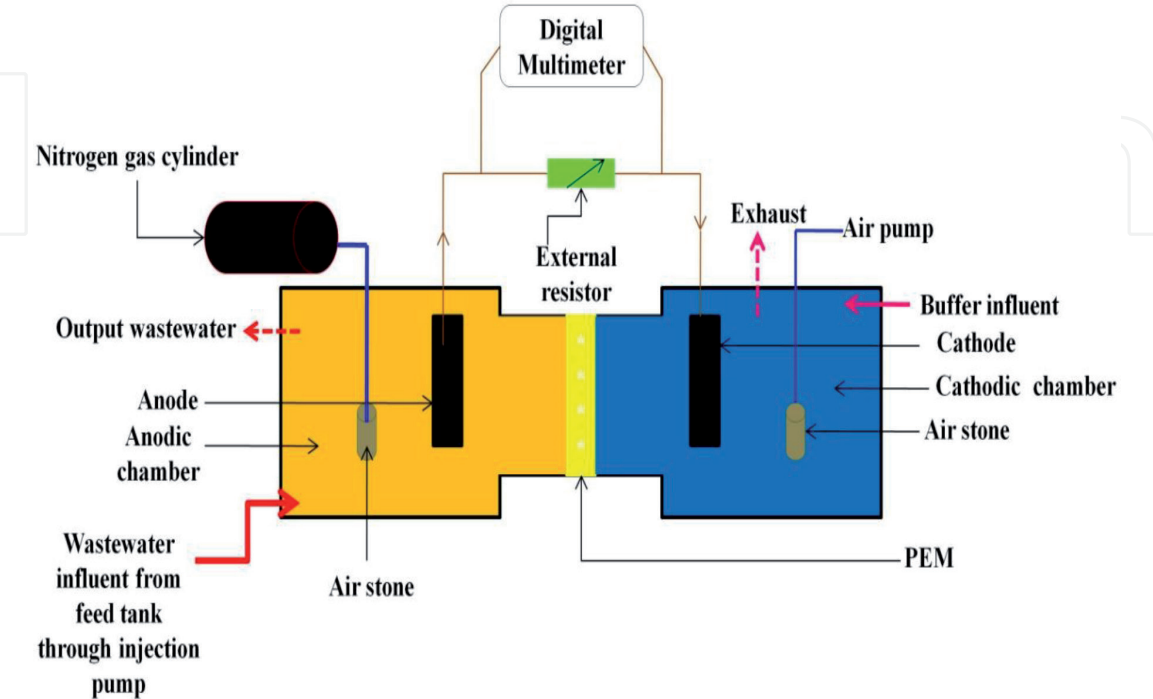


Figure 3. The schematic view of the CAML-MMFC reactor (adapted from [96]).

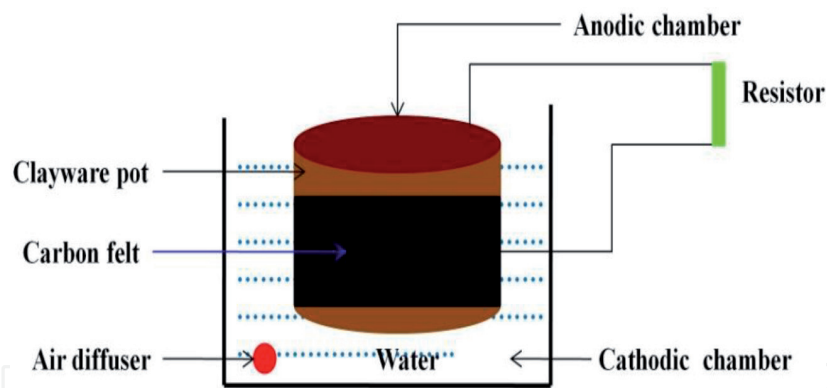


Figure 4.
Dual chambered MFC treating cow urine as a substrate in the anodic chamber (adapted from [97]).

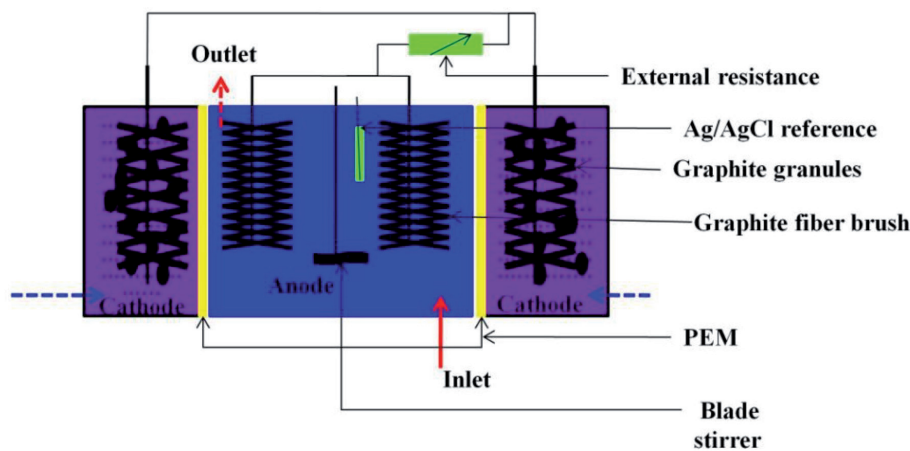


Figure 5.
Schematic drawing of the MFC reactor. (1) Graphite fiber brush; (2) graphite granules; (3) proton exchange membrane (PEM); (4) Ag/AgCl reference; (5) blade stirrer; (6) air; (7) air bubbles; (8) external resistance; (9) inlet; (10) outlet (adapted from [98]).

inner clay container as an anodic chamber with a working volume of 0.4 L as seen in **Figure 4**. The substance of the clayware pot wall itself worked as a separator between the anodized chamber and the cathodic chamber. The anode and cathode are constructed of carbon felt with 394 cm² and 755 cm² of estimated surface area, respectively.

Zhang et al. [98] constructed a novel design for the treatment of dairy manure as shown in **Figure 5**. The MFC consisted of one cylinder (Ø100 mm × 90 mm, anode compartment with two identical square vision windows (80 mm × 80 mm)) and two rectangular cubes (80 mm × 80 mm × 50 mm, two cathode compartments attached to a Plexiglas conduit (Ø20 mm) and a cathodic compartment passing freely between them). The anode and the cathode compartments were divided by two proton exchange membranes (PEM) with the same cross-sectional region (80 mm = 6400 mm²). The cathode chambers were constantly aerated at 300 ml min⁻¹, to maintain dissolved oxygen at the cathode, and the anolyte was agitated with a blade stirrer (300 rpm) every other hour. The anodic and cathodic chamber had appropriate volumes of 617 ml and 321 ml.

13. Single-compartment MFC systems

Owing to their complicated architectures, two-compartment MFCs are challenging to scale up, but they can be run either in batch or continuous mode. One

compartment of the MFCs provides simplified layout and cost savings. Typically they provide just an anodic chamber in a cathodic chamber without aeration need. Mohanakrishna et al. [35] fabricated single-chamber MFC with “perspex” material with a total working volume of 0.54/0.48 L operated under fed-batch mode in an anaerobic microenvironment (**Figure 6**). Plain graphite plates (5 cm × 5 cm; 1 cm thick; surface area 70cm²) were used as electrodes without coating along with NAFION 117 (Sigma–Aldrich) as proton exchange membrane sandwiched between anode and cathode duly after pre-treatment. Whereas the bottom portion was connected to PEM and exposed to liquid, the top section of the cathode was exposed to sunlight. The anode was mounted below the PEM and submerged in the wastewater absolutely. After sealing with epoxy sealant copper wires were used for contact with electrodes. In order to maintain the anaerobic microenvironment in the anode compartment, leak-proof sealing was provided at the joints. Provisions for the sampling ports, wire input points (top), inlet and outlet ports have been developed.

Mardanpour et al. [36] fabricated a unique annular single chamber microbial fuel cell (ASCMFC) with the spiral anode (**Figure 7**). They used stainless steel mesh coated with graphite as an anode material. The dimensions of the chamber were 3 cm in height, 7.1 cm internal diameter, and 8 cm external diameter. The volume of the anaerobic chamber was 90 cm³. The anode electrode (63 cm × 2 cm) was composed of stainless steel mesh coated with graphite (mesh 300).

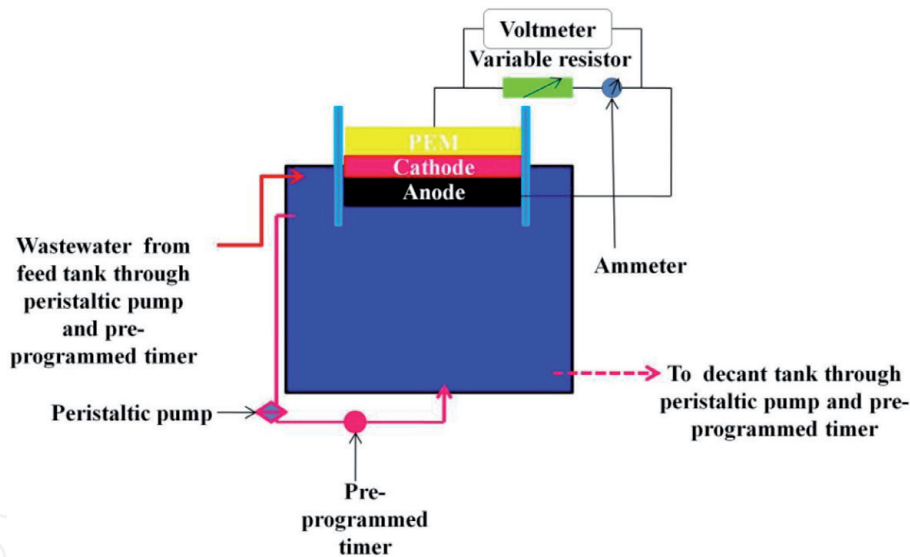


Figure 6. Schematic details of non-catalyzed single-chambered microbial fuel cell (MFC) used in this study with measurement circuits [FT; wastewater feeding tank; DT; decant tank; VR, variable resistor; A, ammeter; V, voltmeter; T, pre-programmed timer; P, peristaltic pump; PEM, proton exchange membrane (NAFION 117)] (adapted from [35]).

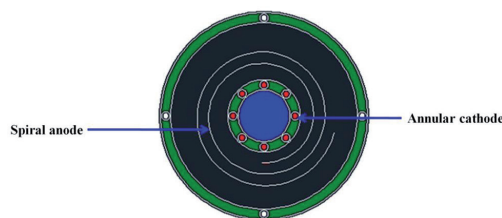


Figure 7. Schematic diagram of annular single chamber microbial fuel cell (ASCMFC) with the spiral anode (adapted from [36]).

14. Operation parameter

14.1 Performance of MFC under different anodic metabolism

In MFC performance, microbial metabolism at anode plays a significant role. Each metabolism follows its metabolic pathway for generating energy, varying the capacity to generate power. The MFC was maintained at an initial concentration of 1601 mg/L COD and a pH 7 anolyte. Phosphate buffer at 10 mM working concentration was used to control anolyte pH. The voltage could be quickly produced in the MFC during the treatment of aerobic as well as anaerobic anodic metabolism in dairy wastewater. Nearly 760 and 780 mV of OCV was recorded for anaerobic and aerobic metabolism, respectively. Considering both aerobic and anaerobic anodic processes, the maximal OCV was observed from the first cycle of operation. Various studies [36, 99] showed the need of lag phase by microbes after which maximal OCV was obtained. The eradication of requirements for the lag phase may be a determinative result of using inherent microorganism of dairy wastewater which limits the microbial growth adaptation phase. MFC's behavior marks a chance to generate current from the first cycle of operation. However, in power generation there was a clear difference when specific anodic metabolism was used. The polarization data suggests that both the MFCs produced maximal power density of external resistance at 470 ohm; for aerobic and anaerobic metabolism it was recorded as 196 and 162 mW/m² respectively. The COD removal efficiency obtained was 91% and 92% for anaerobic and aerobic metabolism in a week's time respectively. The efficiency of conversion of chemical to electrical energy was 3.7 folds lower than anaerobic metabolism with 17.15% efficiency making it the major flaw in the aerobic system. In aerobic mode, oxygen was used by the microbes as terminal electron acceptor, which resulted in the loss of electrons reducing CE. While the CE for aerobic metabolism was much lower than anaerobic metabolism it could generate higher power density, this may be the product of aerobic bacteria's fast growth and rapid metabolic activity, resulting in a higher concentration of protons and production of electrons. The speedier removal of COD by aerobic metabolism results from rapid use of substrates [99].

14.2 Effect of anolyte pH

14.2.1 MFC operation without pH buffer

Anolyte system using a 10 mM concentration phosphate buffer (pH 6.9) reduces the initial anolyte concentration to 7.2 showing a gradual reduction of pH to 6.9 in 8 days. Utilizing orthophosphoric acid, the pH was set to 7 when the device was run in the absence of buffer. The pH variations were found to be crucial in the absence of buffer. In the absence of a buffer system, the MFC pH gradually increased to 7.51 on the 3rd day, and then fell to 7.03 on the 6th day. Though the efficiency of treatment and OCV was the same, a clear difference was observed in system polarization. The MFC's average power density without buffer was 85.97 mW/m² which was almost half the system output using a buffer configuration for 161 mW/m² of pH maintenance. The requirement of 8-day batch time for both reactors for 90% COD reduction demonstrated that the pH buffer removal did not affect the bacterial activity. In MFC, the citrate and phosphates remain as proton carriers. While for these carriers the diffusion coefficient is smaller, the concentration gradient is higher across the membrane. In cathode chamber, the concentration gradient is higher due to the deficiency of citrate and phosphates. Due to improved

proton transfer, the internal resistance was typically reduced due to polarization of the concentration of protons, thereby increasing the output of power in the system using pH buffer. Phosphate buffer system has a wonderful impact on the electricity generation by altering the electrochemical reactions although it has not affected MFC's microbial growth and efficiency in COD removal. The higher anolyte power density may be attained at pH 7 [8].

14.3 Substrate concentration

The concentration of the substrate in the anode chamber has a significant effect on microbial development. The MFCs were run using an anaerobic metabolism buffer system with an initial pH of 7 anolytes. The substrate concentration varied as a function of COD concentration (800, 1600, and 2800 mg/L). A remarkable variation in the overall OCV obtained by the MFC could be observed. MFC having COD concentration of 1600 mg/L reported a maximum OCV of 760 mV. Operating system with 800 and 2800 mg/L COD concentration achieved maximum OCV of 656 and 612 mV. MFC working with COD concentration of 800, 1600, and 2800 mg/L had a batch time requirement of 6, 7, and 11 days. The peak power density (161 mW/m^2) was reported at 1600 mg/L COD concentration and is 2.5 and 1.8 fold lower for 800 and 2800 mg/L COD operating MFCs. The columbic efficiency was 2.6 and 1.7 folds lower for MFC with 800 and 2800 mg/L, respectively, compared to MFC at 1600 mg/L COD concentration having 17.16%. The use of wastewater with higher COD results in a reduction in electricity generation, which may be due to microbial growth inhibition mediated by substrates. A dramatic decrease in power output occurred when 800 mg/L of initial COD concentration was used. Power generation decreased with a decline in the initial concentration of the substrate [99]. The initial COD variance did not influence the effluent quality of the MFC, although the duration of treatment improved with an increased substrate concentration.

14.4 The effect of temperature

The operating time was longer at low temperatures than that at high temperatures, but the voltage generation at high temperatures (30 and 35°C) was higher [100, 101]. The peak current and voltage intensity was measured at 35°C. Decreasing voltage, output and current intensity may occur for a variety of reasons. As temperature rises, the biochemical reactions, bacterial metabolism, and bacterial growth rate increases, leading to rapid bacterial growth and better voltage efficiency. Nonetheless, during long processing periods while bacteria are at high temperatures, essential cell's compounds like nucleic acid and other temperature-sensitive material can be irreversibly impaired, resulting in extreme cell function degradation or death. The voltage and current strength decrease drastically in this case. The slow bacterial growth rate at low temperatures often leads to a reduction in the bacterial population and activity and voltage output decreases [102].

14.5 The effect of organic loading rate (OLR)

A number of studies on the generation of electricity by MFCs have also shown that amount of current generated in both closed and continuous MFC depends upon organic loadings. The MFC research analyzed various organic loadings and measured their effects on current and power during service. During the 30 days of operation, the maximal current and power density was achieved in OLR equal to $53.21 \text{ kg COD/m}^3\text{d}$. This is because the MFC requires more time at low OLR to achieve the optimum current and power density. But in greater OLR, maximal current and power density

will be attained in a shorter time frame. The other reason is that the microorganisms in inoculated sludge and wastewater are compatible [15, 96].

14.6 The effect of external resistance

Higher external resistance results in diminished power density. Therefore, MFC has to be constructed with lower external resistance for better performance. In other words, the voltage rises as the resistance increases, and the current decreases. The voltage produced decreases from 0.855 to 0.319 V when the external resistance increases from 1 to 25 K Ω . The decrease in voltage indicates that processes other than cathodic reactions used some electrons [1]. Low voltage may be due to a reduced rate of usage of electrons in the cathode with high electrical resistance relative to the rate of transfer from the external circuit. It is acceptable that the ejection of electrons via the circuit reduces as the resistance of a circuit increases. Electrons in the cathode have been used to eliminate other electron receptors from the cathode, like sulfate, permeable oxygen, or nitrate. Electrons quickly pass through the external circuit at lower external resistance and oxidize the electron carriers in the anodic chamber on the external membrane of the microorganism. Maximum power density is also obtained in MFC systems where internal and external resistances are equivalent. Differences in MFC output with varied external resistances can be due to differences in activation losses at the anode, which is a result of the electrochemical behavior of the microorganism-reducing anode [103].

14.7 The effect of hydraulic retention time (HRT)

Hydraulic retention time (HRT) is a critical parameter in the treatment of wastewater and regulates the residual substrate concentration and the amount of dissolved oxygen (DO) in the reactor. When HRT decreases, the concentration of the substrate increases, leading to the utilization of the entire substrate with an improved voltage and power density. On the other hand, higher concentrations of DO in the influent wastewater lead to an increase in the potential for oxidation-reduction (ORP), resulting in a reduction in the voltage and power density generated in the MFC. To understand the impact of HRT on bioelectricity generation, the MFC was run continuously with dairy wastewater at seven different HRTs (2, 3, 4, 5, 6, 7, and 8 days) [96].

The explanation for the rise in voltage as HRT rises may be usually due to the long interaction time between biofilm and organic matter, which may demonstrate the benefit of biofilm, degradation of a substrate, electron output, and transition to the surface of the anode. Despite this, the voltage decreases slightly when HRT increases (8 days). These observations are compatible with the conclusions of single-chamber MFC energy production with the aerial cathode in the existence and exclusion of proton exchange membrane, and also the results of electricity generation and the wastewater treatment utilizing single-chamber MFC [104].

15. Application of MFC

Although a centuries-old technique, initially recognized in the treatment of dairy wastewater, MFC is taking an interest in bioelectricity generation, bio-hydrogen, Nitrogen, and Phosphorus recovery and also used as a biosensor [33, 105–108]. Several issues such as expensive materials, complicated design, and low power output at higher internal resistance needed to be tackled before utilizing MFC for large scale applications.

15.1 Treatment of wastewater

During the early stage of MFC technology, it was considered that this technology could only be used for the treatment of the limited wastewater, but in the recent years, it has been seen that it could be used in the treatment of almost all kinds of industrial, agricultural and municipal wastewater. The most suitable temperature studied for electricity generation via MFC is about 30°C in a regulated climate. Glycerol wastewater, the main source of pollution in the biodiesel industries, has reported a maximum surface power density 600 mW/m² [109]. The low cost and the operational stability is an important characteristic for an effective and efficient treatment technology. An earlier study has reported the simultaneous methane and bio-electricity production in the anaerobic digestion process for higher concentrated wastewater at a longer detention time [62]. MFC with certain microbes have the ability for removable of organic matter, sulfides, nitrides, phosphorous, salinity, etc. Do et al. [110] reported the maximum of 90% COD removal and 80% columbic efficiency.

15.2 Bio-electricity

MFC is a wonderful technology in transferring the chemical energy inside the wide varieties of the waste organic matter with the help of the microorganism into bio-electricity. The current MFC technology is capable of producing only low power outputs which are suitable for small telemetry and wireless sensor system with a small power requirement in the remote areas. However, [39] achieved a peak power density of 122 W/m³ with 81% COD removal using dairy wastewater as a substrate with 3D laminated composites as electrodes [39].

15.3 Biohydrogen

With a minor adjustment, MFCs could also be used to generate biohydrogen instead of bio-electricity that could be extracted and processed for later use. The anode potential is improved with an external voltage of 0.23 V for overcoming the thermodynamic barrier which is much lesser than the conventional fermentation process. The electron and hydrogen ion produced by the microbial activities at the anodic chamber combines at an oxygen devoid cathode chamber generating biohydrogen. MFC has a potential of about 8–9 mol H₂/mol glucose in comparison to 4 mol H₂/mol glucose produced from a conventional fermentation process [52]. In order to produce hydrogen gas in a standard MFC, the anodizing potential for an additional voltage must be increased roughly 0.23 V or more.

15.4 Bio-sensor

The MFC is also utilized as an electrochemical biosensor for pollutant analysis. The metabolic activities of the electrogenic microorganisms are highly affected by the sudden change in the concentration of the targeted analyte in the exposed aquatic environment and are reflected as a change of the output electric signal. MFC sensor is a self-sustained sensor unlike other types of the biosensor which require an external source of power. The biofilm-electrode is used as the sensing element in the MFC sensor [67].

16. Conclusion

Anaerobic treatment is most commonly used to treat dairy wastewaters, mainly hybrid anaerobic and UASB digesters. Upstream anaerobic sludge blanket reactors

are more commonly used and ideal for the wastewater treatment from the dairy sector since they can handle large amounts of influents within a short period. But, as dairy wastewater, these processes partially degrade wastewater that contains nutrients and fats. Further treatment for anaerobically treated wastewater from the dairy is therefore necessary. The proper selection of anode material it is made from is a key factor in attempts to obtain high-performance MFCs. Selecting the incorrect anode content would make this option obsolete. Since the kinetics of the microbes used in MFCs are far slower than that which can be accomplished with cathode content or cathode catalyst, the use of 3D anodes has so far been seen to be very advantageous and capable of raising power generation by many magnitudes. Developing countries like India who are the leading producers of milk and are among the top world dairy industries rely on the use of antibiotics for enhancing the production of milk in the cows but these antibiotics when finding their way into the water streams, these are very detrimental. Therefore, the adoption of circular practices for the management of the environment is increasing in order to promote the circular economy. From a future perspective, MFCs are the most promising and environmentally friendly approach to the management of environmental pollution. However, scaling up of this technology is an obstacle due to low power outputs but this could be overcome by integrating MFC with other wastewater treatment technologies and a centralized system will solve the problem. Also, various low-cost electrode materials such as ceramics and biological materials make this technology economically sound.

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