ADVANCES IN MICROBIAL FUEL CELL

Dr. Nayana Borah
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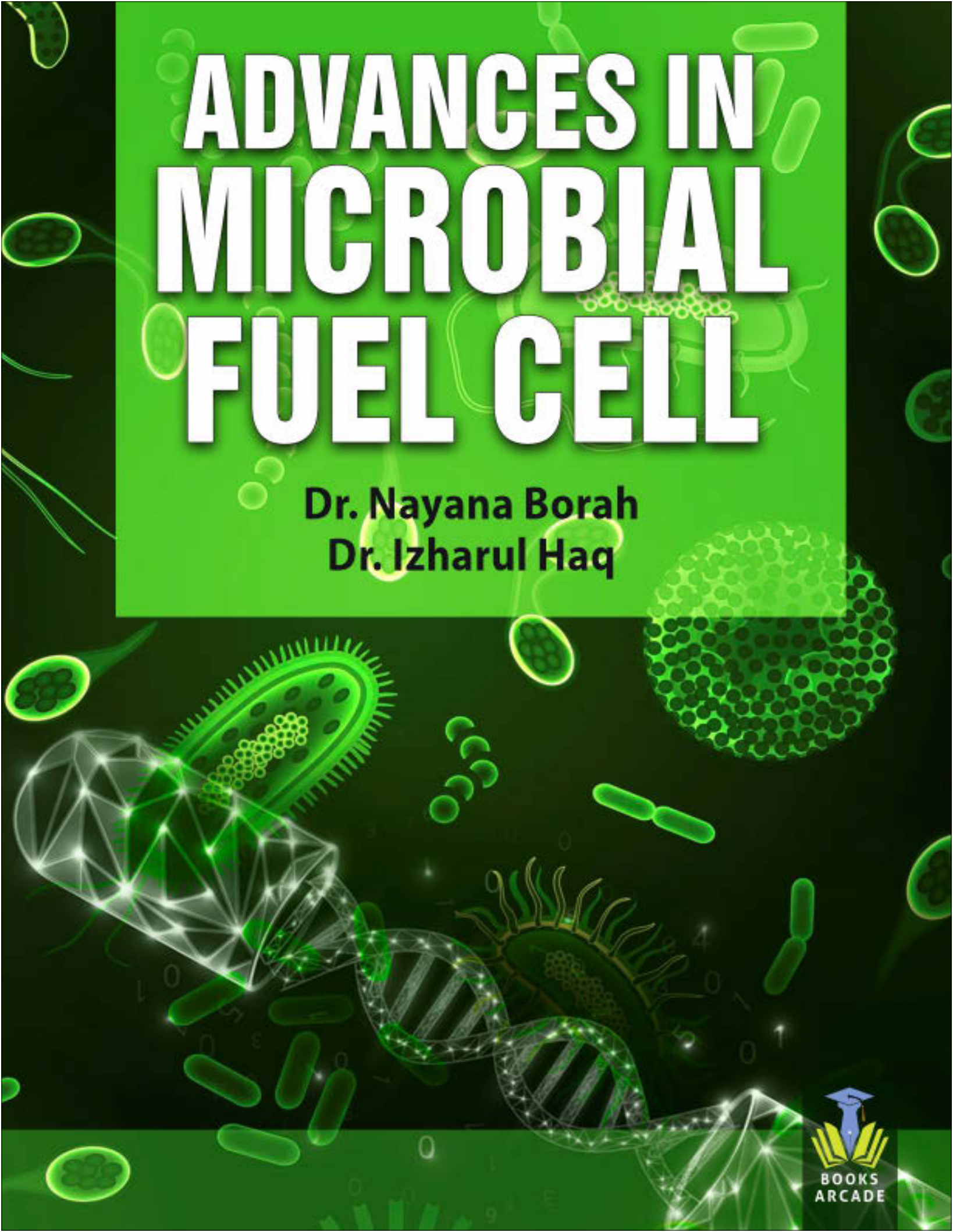

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KRISHNA NAGAR, DELHI

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Website: www.booksarcade.co.in

Year of Publication 2023

International Standard Book Number-13: 978-93-90762-73-6

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CHAPTER 1

INTRODUCTION TO MICROBIAL FUEL CELL

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One of the most alluring technologies available today for the creation of renewable energy and concurrent wastewater treatment is the microbial fuel cell (MFC) technology. Using microorganisms as biocatalysts, MFCs are bioelectrochemical devices that transform the chemical energy found in organic or inorganic compounds into electric current. The anode and the cathode are the two chambers that make up a conventional double-chamber MFC. The protons generated at the anode may often flow between these two chambers thanks to a proton exchange membrane (PEM). One option that has drawn interest is the microbial fuel cell (MFC), which produces power directly from organic materials. A microbial fuel cell is a kind of bioreactor that, under anaerobic circumstances, uses the catalytic processes of microorganisms to transform chemical energy found in the chemical bonds of organic substances into electrical energy. Research into free, renewable energy is now important because of the global energy problem brought on by an over reliance on fossil fuels (such as crude oil, coal, natural gas, etc.). The demand for environmentally friendly energy sources is justified by the future energy crisis and climate change. Nuclear energy, solar energy, wind energy, and other non-traditional, carbon-neutral energy sources are being researched and used to a great degree. We can least afford to unleash stored carbon at this time, when the atmosphere is already suffused with greenhouse gases. The following article describes the fundamental concepts behind the operation of microbial fuel cells as well as the main current uses of microbial fuel cell technology.

The use of energy sources including solar, wind, hydroelectricity, and geothermal, among others, has gained attention. More research has been conducted and will continue to be done in order to make the area of microbiology and microorganisms a significant participant in the energy generation of the new era in this so-called mad dash to fulfil the rising energy worries for today and future. In the future, a larger part of the world's energy consumption and production will come from renewable sources. The search for alternate energy supplies has been prompted by recent projections for the world's energy. The pace at which nonrenewable energy sources are being depleted suggests that there is an urgent need for energy transformation technologies that are very efficient and for methods of using alternative renewable energy sources. The use of microbial fuel cells (MFC) technology, which generates power from what would otherwise be considered trash, opens up new avenues for the generation of energy. The majority of the anaerobic bacteria used in this technology, which acts as catalysts to cleanse waste water while producing energy, may be found in the waste water. A variety of organic wastes may be used by microorganisms to produce power while oxidising the waste into less hazardous forms. A combination of energy production with waste water treatment might lower the cost of initial treatment of effluent waste water, even though MFCs produce less power than hydrogen fuel

cells. A rise in the use of fossil fuels as a consequence of rising energy demand has brought about potentially catastrophic climate change and pollution. Water and electricity security problems are consequently major challenges right now. Organic waste and wastewater constitute a potential renewable feedstock for the production of various forms of bioenergy by managing the biological process, in addition to the remediation process. Bioenergy has received a lot of interest as a sustainable or cutting-edge alternative to fossil fuels. The use of waste remediation for bioenergy has drawn a lot of interest and opened up a new route for the use of renewable and unrestricted energy sources.

Therefore, wastewater management as well as alternative energy are the most unexplored fields in biotechnology or science. The microbial fuel cell (MFC) is gaining popularity as a potential tool for the simultaneous generation of power and the environmentally friendly treatment of waste. The full degradation of a range of organic substrates to both water and carbon dioxide is often only possible via a number of straightforward enzymatic reaction steps, which may be carried out in MFCs.

A Synopsis of MFC and Fuel Cell Development the energy trapped in gasoline may be instantaneously converted into electricity using a fuel cell. A fuel cell can constantly generate power as long as the fuel source is there. This makes it a better alternative for providing electricity than a battery that has to be recharged. Most often, hydrogen is used at the anode side, in contact with a catalyst, as a substrate for oxidation. A proton or an electron may be created from H2. The electron and proton are transferred to the cathode chamber through an ion exchange membrane as well as an external circuit, respectively.

The oxygen molecules combine with the proton during reduction on the cathode to form water. The result of an electrochemical reaction in which current may be produced in a single step is water. Fuel cell research quickly gained prominence due to its superior thermodynamic efficiency, environmental friendliness, or sustainability. When the price of oil increased between 1960 and 1970, scientists devoted more attention to fuel cell development. The fuel cells may be divided into two groups: abiotic fuel cells and biotic fuel cells. For instance, solid oxide fuel cells, molten carbonate fuel cells, proton exchange membrane fuel cells, and other traditional abiotic fuel cells employ the inorganic catalyst. They typically have priceless catalysts for the oxygen reduction of the cathode and the hydrogen oxidation of the cathode. When compared to an abiotic fuel cell, an abiotic fuel cell produces large quantities of power.

An anode, a cathode, or an electrolyte make up an MFC, a kind of fuel cell (s). The cathodic and anodic chambers may or may not be separated by an ion exchange membrane (IEM). Live microorganisms in the anodic chamber oxidise substrates while planktonic or by forming biofilms. Protons, electrons, and other metabolites are therefore created as byproducts. The bacteria release electrons, which are collected by the anode and then pass via an externally applied electrode and into the cathode. While the protons merely diffuse to the cathode or pass through the IEM, where they are reduced by the incoming electrons to complete the circuit, the protons in the other case pass through the IEM. Electrons pass through the external load to create

electric current. As the protons are reduced in the presence of oxygen at the anode, water is created as a byproduct, making the process ecologically safe. Both large (liter-scale) and small MFCs are available (microlitre-scale or milliliter-scale). The three primary components of MFCs are the cathode, anode, membrane, and separators. Graphite plates, stainless steel mesh, carbon paper, rods, glassy carbon, cloth, foam, carbon felt, platinum, platinum black, or reticulated vitreous carbon have all been utilised as anode materials.

The anode material should be conductive, noncorrosive, and conducive to the growth of biofilms by bacteria. The cathode often contains catalyst materials such as platinum, manganese oxide, platinum black, or polyaniline for an improved oxygen reduction process (ORR). Graphite, carbon paper, carbon felt, glassy carbon, carbon cloth, carbon foam, stainless steel, or titanium mesh are a few materials that may be used as cathodes. In a dual-chambered MFC, a separator or an ion exchange membrane is used.

The cathode membrane assembly was prepared for passive aeration in a single-chambered MFC so that ORR may immediately get oxygen by being hot-pressed or chemically attached to the cathode. The cathode and anode chambers were first separated by a salt bridge in research. IEMs increasingly replaced them. Membranes may either be CEM or anion exchange membranes, according to research.

An innovative form of alternative energy with little to no net CO2 emissions is fuel cells. The earliest reports of microbial cultures producing electricity appeared around the turn of the century. "Bioreactors that transform the energy in the chemical bonds of organic molecules into electrical energy by catalytic activity of micro-organisms under anaerobic circumstances" are how microbial fuel cells (MFCs) have been defined. Utilizing bacteria to produce bioelectricity from the oxidation of organic waste and renewable biomass is a unique strategy made possible by MFC technology.

MFCs have the following operational and functional advantages:

They employ easily accessible microorganisms as catalysts and organic waste as fuels. MFCs have a higher conversion efficiency than enzymatic fuel cells, capturing up to 90% of the electrons from the bacterial electron transport system. MFCs do not need highly controlled distribution systems like those required for hydrogen fuel cells.

A multidisciplinary approach to the search for alternative energy sources is represented by MFC technology. It represents the intersection of the physical, biological, and chemistry sciences and serves as a hub for fundamental and practical research. The fundamentals of microbial physiology combined with electrochemistry serve as the foundation for the operation of MFCs. The intricate details of electrical and materials engineering are highlighted by the MFCs' structural architecture. The applications of this technique fall within the categories of bioremediation and environmental engineering. Thus, MFC technology is multidisciplinary in the truest meaning of the word and offers opportunities to advance research across fields.

Biological foundation

Under various circumstances, chemotrophic microorganisms use organic and other biodegradable substances. The terminal electron acceptor molecule determines the proper electron carriers to use to transfer the oxidised electrons to an electron transport chain. This terminal acceptor in aerobic organisms is oxygen, which absorbs the electrons and is reduced to water.According to the chemiosmotic theory, the translocation of protons across bacterial membranes and the subsequent coupling of ATP production to proton electrochemical potential across the energy-transducing membrane are two related processes.The chemiosmotic principle is used by the bacterial cell membrane to operate as an energy-transducing membrane. A proton electrochemical gradient is created as a consequence of the translocation of protons away from the membrane. This membrane potential is multiplied by the pH gradient to produce the proton motive force. ATP production occurs in conjunction with the re-entry of these protons through the ATP-synthase enzyme. The bacteria need the ATP that is created in this way to survive

Now that we have this theoretical foundation, let's examine how an MFC works. The anodic and cathodic half-cells, which are divided by a selectively permeable, cation-specific membrane or a salt-bridge, make up a typical MFC. Microorganisms floating in the anolyte under anaerobic circumstances make up the anodic chamber, whereas the electron acceptor is found in the cathodic chamber (oxygen). In essence, the two chambers act as a physical barrier separating the terminal electron acceptor from the electron source. The anode receives the majority of the released electrons from the oxidation process. Electron mediators or shuttling agents cells themselves, or nanowires may all move electrons to the anode. Through an external circuit, these electrons are directed to the cathode, and for each electron that is conducted, a proton is transferred over the membrane to the cathode in order to complete the reaction and maintain the electric current.

The microbial fuel cell (MFC), which generates electricity directly from organic sources, is one alternative that has attracted attention. A microbial fuel cell is a form of bioreactor that converts chemical energy from organic molecules' chemical bonds to electrical energy by catalysing microbial processes under anaerobic circumstances.Energy is one of the most important components in the development of civilisation, with fossil fuels being largely responsible for the industrial revolution. Fossil fuels are not abundant enough to provide all of the world's energy needs until the end of the twenty-first century. As a result, there is a present need for alternative renewable energy sources. In recent years, microbial fuel cells (MFCs) have been a promising and challenging technology. Microbes interact with electrodes in an MFC by trading or providing electrons via an electrical circuit. MFCs are the most prevalent kind of bioelectrochemical systems (BESs), which employ microbial metabolism to transform biomass into energy spontaneously.

Microbial fuel cell research has become interesting and productive. MFCs have a variety of applications that may help reduce the use of fossil fuels and facilitate energy recovery from waste. Microbial fuel cells cannot be the one technology that changes the world since they will never be able to produce enough energy to replace a coal-fired power plant. However, they will help make the world a more sustainable and eco-friendly place. It is now known that the microbial fuel cell's ability to directly produce electricity may result from the organic material's breakdown. Like a normal fuel cell, an MFC has either an anode or a cathode chamber. The anoxic anode chamber and cathode chamber are connected internally by an ion exchange membrane, and an external wire completes the circuit. MFCs may be used in breweries, residential wastewater treatment systems, desalination plants, hydrogen production facilities, remote sensing, environmental remediation, and as a long-distance power source, to name a few applications. The extensive use of MFCs in these areas enables the creation of power from garbage.

CHAPTER 2

HISTORY OF MICROBIAL FUEL CELLS

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Electrical energy is also produced during the microbiological activities that decompose organic materials. The idea for trying to utilise this freshly found source of energy for human purpose originated from Potter. He was able to construct a basic microbial fuel cell, but the understanding of bacterial metabolism was inadequate to allow for design advancement. Recently, researchers have worked to enhance the kinds or combinations of bacteria used in microbial fuel cells, the electrode materials used, as well as electron transport. Although the idea of utilising bacterial energy has been known for more than a century, researchers have only just begun to fully understand the MFC and how to optimise its potential.A device known as a microbial fuel cell catalyses the transformation of chemical energy into electrical energy using microorganisms. In a traditional microbial fuel cell, the anode and cathode compartments are separated by a cation (positively charged ion) specific membrane, as shown in figure 2.1. Fuel is oxidised by microorganisms in the anode compartment, resulting in the production of CO2, electrons, and protons. While electrons are sent to the cathode compartment by an external electric circuit, protons are transferred there via the membrane. Protons and electrons undergo oxidation in the cathode compartment, where they mix with oxygen to form water, as shown in below figure. Broadly speaking, there are two types of microbial fuel cells: mediator-less and mediator-containing.

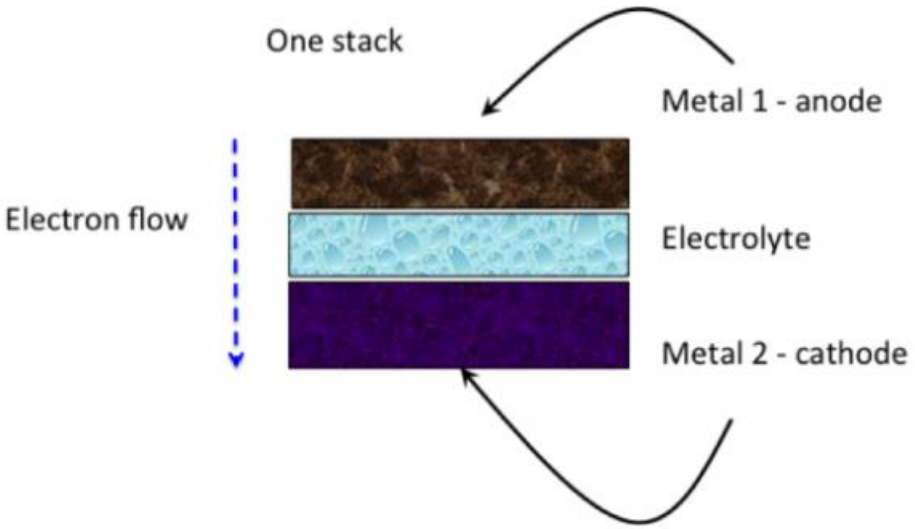

Figure 2.1: Showing the direction of electron flow.

Molecular Fuel Cell Mediator

Most microbial cells are not electrochemically active. The passage of electrons from microbial cells to the electrode is aided by mediators such as thionine, neutral red, methyl blue, methyl viologen, humic acid, and others. Most readily available mediators are costly and hazardous.

A Microbial Fuel Cell

Mediator-free microbial fuel cells use electrochemically active microorganisms to transfer electrons to the electrode in place of a mediator (electrons are carried directly from the bacterial respiratory enzyme to the electrode). Examples of bacteria that are electrochemically active include *Aeromonas hydrophila, Shewanella putrefaciens,* and others. Some bacteria's pili on their outer membrane may be used to convey their electron production. Because MFCs are a relatively new area of study, it is difficult to know the factors that impact ideal efficiencies, such as the kind of ion-exchange membrane used in the system, the type of bacteria used in the system, or system parameters (temperature, pH, etc.).

Some plants may be utilised as a direct source of energy for mediator-less microbial fuel cells in addition to utilising wastewater as fuel. The system in question is known as a plant-microbial fuel cell. Potential plants include reed sweet grass, lupines, rice, cord grass, tomatoes, and algae.

Cell of Bacterial for Electrolysis

A variation of the mediator-free cells are the microbial electrolysis cells (MEC). MFCs generate electric current by the bacterial decomposition of organic compounds in water, whereas MECs partially reverse the process that produces hydrogen or methane besides applying an electrical to bacteria to supplement the voltage generated by the microbial degradation of organics sufficiently leading to the electrolysis of water or the production of methane. A complete inversion of the MFC idea is microbial electrosynthesis, in which microorganisms decrease CO2 by using an external electric current to produce multi-carbon organic compounds.

Built-In Microbial Fuel Cells in the Soil

Soil-based microbial fuel cells may use the same basic MFC concepts. The soil is made up of the inoculum, the proton-exchange membrane, and the nutrient-rich anodic media (PEM). While the anode is buried at a certain depth in the soil, the cathode is on top of the soil and exposed to the oxygen in the air. Soils naturally contain a wide range of different bacteria in addition to the electrogenic microorganisms needed for MFCs.

These soils are also rich in complex sugars and other nutrients that have built up through millions of years of the breakdown of plant and animal matter. The soil's redox potential also decreases with depth as a result of the aerobic (oxygen-consuming) bacteria acting as an oxygen filter, much like the expensive PEM materials used in laboratory MFC systems.

A Microbial Fuel Cell with a Biofilm Phototrophic

Since photosynthetic microorganisms such as Chlorophyta, Cyanophyta, and other photosynthetic organisms may undertake photosynthesis, functioning as both producers of organic molecules and electron providers, phototrophic biofilm MFCs (PBMFCs) employ anodes with phototrophic biofilms on them. A subtype of phototrophic microbial fuel cells called biological photovoltaic systems uses solely oxygenic photosynthetic material at the anode.

The Basic Components in Microbial Fuel Cells

In a typical MFC, a proton-exchange membrane separates the cathodic chamber from the anodic chamber (PEM). The cathodic chamber is not required in a one-compartment MFC since the cathode is exposed to the air directly. MFC technology is still not marketed after 10 years of thorough research on the subject. Before MFCs may be employed in real-world applications, a number of technical problems must be overcome. The main problem with the MFCs is their insufficient power output. One of the major limitations is the high cost of the cathode catalyst, membranes, or electrode materials. In the future, MFCs may use high surface area electrode materials to boost power output, albeit (on a large scale) MFCs without PEM may be more cost-effective. The MFCs used for wastewater treatment still need to be adequately changed in order to properly clean the water. Very little research has been done for applications using biosensors. Since MFC-based biosensors are biofilm-based, their response times are greater.

The efficiency of MFCs will be greatly influenced by the kind of material used to manufacture the electrode. Using better electrode materials would always improve MFC performance since different anode materials yield different activation polarisation losses.

pH Buffer and Electrolyte

If no buffer solution is used in a working MFC, there will be a pronounced pH difference between the cathodic and anodic chambers even though theoretically there won't be a pH shift when the reaction rate of protons, electrons, and oxygen at the cathode equals the production rate of protons at the anode. The pH difference increases the driving force for proton transport from the anode to the cathode chamber, and finally a dynamic equilibrium takes place.

Proton Exchange System

An MFC system's internal resistance or concentration polarisation loss may be influenced by a proton exchange system, which in turn impacts the MFC's power output. The very selective proton permeability of Nafion (Wilmington, DuPont, Delaware), which is the basis for its popularity.

Use Requirements in the Anodic Chamber

The main factor influencing an MFC's performance is the kind of substrate, concentration, or feed rate. Power density varies dramatically with different substrates and the same microbe or microbial consortia. The amount of substrate used determines whether electricity is produced in

batch or continuous flow modes. In MFCs, oxygen is the most typical electron acceptor for the cathodic process. Cathodic chamber operational parameters. An MFC's ability to produce power is significantly influenced by the density of electron acceptors.

Mechanisms for Transferring Electrons

The produced electrons should be transported from the anodic chamber to the anode via shuttles or electron mediators. The direct electron transmission to the anode is slowed down by peptidoglycans and lipopolysaccharides in the outer layer of the non-conductive lipid membrane of various microorganisms, including several Saccharomyces species and E. coli strains used in MFCs. In most cases, the mediators include coloured materials such neutral red, methylene blue, humic acid, thionine, or methyl viologen.

Microbial Metabolism and the Potential of Cells

The microorganism's metabolic pathway and the anode's subsequent potential have a major role in defining the cell potential. Bacterial catabolism is the rate-limiting step in MFCs. Heterotrophic organisms get their energy from the oxidation of organic materials. Exogenous oxidants, or external terminal electron acceptors, participate in the anodic chamber via two important metabolic pathways: the respiratory chain and fermentation. Various redox potentials for the MFC processes. The electrical potential of the MFC is determined by the potential discrepancies between the anode and the cathode.

Anode Materials Changes

Given its reasonable cost, manageability, and specific surface area, carbon is the most flexible electrode material. MFCs are a cost-effective and energy-sustainable technology because they can support bacterial multiplication and proliferation using inexpensive ingredients. Carbon-based materials such graphite fibre brushes, rods, felt, plates, sheets, and fabric are now the most preferred anode materials because to their high electrical conductivity, excellent biocompatibility, and cheap cost. Additionally, it has been shown that changing the electrode surfaces is a good way to improve an MFC's efficiency since it modifies the physicochemical properties of electrodes to encourage microbial adhesion or electron transfer. It is possible to change the anode surface or add new materials to the electrode composition.

Electrode design is the main barrier to making MFC an economical and scalable technology. The electrode material and its configuration have recently attracted increasing attention in MFC research. Over the last 10 years, several electrodes have undergone in-depth MFC research. The alteration of electrode surfaces has shown to be a viable strategy for improving the performance of an MFC because it modifies the physicochemical characteristics of electrodes to encourage microbial adherence and electron transmission. It has been successful to use cutting-edge materials as anodes in MFC, including melamine sponges, Carbon nanotubes, natural polymers, Berl saddles, and high capacitance electrode materials. The anode materials' surfaces were modified by oxidation, electrochemistry, or acidic processes, which improved the performance of

MFC. The performance of an MFC is greatly influenced by the cathode materials, which need to have a high redox potential in order to accept the electrons. Bio-cathodes are getting increasing consideration due to their many advantages over abiotic cathodes. The production of electricity, wastewater treatment, and bioremediation of contaminated groundwater sources may all be accomplished using MFCs. High levels of COD removal are possible using MFCs. Several factors have an effect on how well MFCs work. By taking the aforementioned factors into account, designing the perfect MFC is possible. Most MFCs used for wastewater treatment were inoculated by either aerobic or anaerobic sludge and did not need a mediator. Ideal pH and temperature encourage bacterial growth, which improves MFC efficiency, much as in other microbial systems.

MFCs generate energy

Fossil fuels were responsible for a large portion of the industrial revolution, making energy one of the key factors in the growth of civilisation. Fossil fuels cannot provide all of the world's energy demands until the end of the twenty-first century because they are not plentiful enough. Therefore, the necessity for alternate renewable sources of energy production is increasing.

Microbial fuel cells (MFCs) have been a promising and difficult technology in recent years. In an MFC, microorganisms communicate with electrodes by exchanging or supplying electrons through an electrical circuit. MFCs are the most common form of bioelectrochemical systems (BESs), which use the metabolic activity of microorganisms to convert biomass spontaneously into electricity.

Research on microbial fuel cells has grown to be intriguing and fruitful. There are several uses for MFCs that may assist cut down on the use of fossil fuels and enable energy recovery from waste. Microbial fuel cells can never generate enough energy to replace a coal-fired power station, hence they cannot be the technology that transforms the world on their own. But they will contribute to making the globe more ecologically friendly and sustainable. It is now understood that the breakdown of organic material in a microbial fuel cell may directly create power. An MFC has both an anode and a cathode chamber, much like a typical fuel cell. An ion exchange membrane connects the anoxic anode chamber to the cathode chamber internally, and an external wire completes the circuit. Breweries, home wastewater treatment, desalination facilities, hydrogen generation, remote sensing, environmental remediation, and usage as a distant power source are just a few of the practical uses for MFCs. The widespread usage of MFCs in these locations may turn our waste materials into electricity.

A fascinating and rapidly developing area of science and technology known as microbial fuel cells (MFCs) combines biological catalytic redox activity with traditional abiotic electrochemical reactions and physics. More recently, this field has expanded to include various bio-electrical systems (BESs). These systems become potentially more complicated than previously complex electrochemical systems due to the presence of living organisms that catalyse electrochemical processes (e.g. batteries, fuel cells and supercapacitors). I The electrocatalyst is biotic

(electroactive bacteria or proteins) at the anode, which is one of the fundamental distinctions between MFCs and traditional low temperature fuel cells (direct methanol fuel cell or proton exchange membrane fuel cell). ii) the temperature can be anywhere between 15 and 45 degrees Celsius, with close to ambient levels being ideal iii) the working conditions should be neutral and iv) the use of complex biomass (often different types of waste or effluent) as anodic fuel has a promising but moderate environmental impact as determined by life cycle analysis. Even though the notion of "animal electricity" goes back to the 18th century, when Galvani was testing out frog legs, Potter is credited with coming up with the initial concept of using bacteria to create electricity in 1911. Since Cohen's 35-unit setup in 1931, catalyst investigations in the 1960s, and more recently with the work on synthetic mediators in the 1980s and 1990s, additional concepts and practical developments have been investigated, leading to the development of the so-called "analytical MFC" that is still in use today. Before MFCs are industrialised, there is still a lot of room for improvement and work to be done. Based on those early examples, significant progress has already been made in the understanding of electron transfer mechanisms, development of effective bio-electrocatalytic interfaces, and development of novel, low cost, and durable electrode materials.

Applications are used to categorise the many BESs that have been put out. According to the ISI of Web of Science, MFC is the top and most researched, accounting for almost 75% of articles in 2016. This kind of BES is capable of converting chemical energy from intricate organic substrates into usable power. Other BESs have been created that can desalinate water or produce valuable chemicals like hydrogen, formate, acetate, or methane. Even if several efforts have been reported, there are still many issues that need to be resolved in the MFC sector for effective implementation in actual situations. With synthetic substrates, model microbial catalysts, or microbial consortia produced in the lab, significant progress has been achieved starting with the anode. However, when dealing in more complex situations like genuine industrial effluents or natural habitats, research problems of implementation are naturally more difficult (sediments , marine environments lagoons etc.). For instance, many kinds of organic waste have been employed as microbial anode fuel, but electroactive bacteria kinetics are still subpar, and the relationship between the electrode and the bacteria is still not completely understood. Furthermore, interactions and/or cohabitation between bacteria and solid electrodes in electron transfer processes are poorly understood, particularly in complex situations where a wide variety of microbial species (electroactive or not) may be found on the electrodes. Finally, because it is challenging to combine the intricate processes of microbial electrochemistry with the available imaging technology, it is unknown how microbial cells are drawn to the electrodes, how biofilms form and develop on anode surfaces, how interactions and interspecies cooperation occur, and how environmental factors affect microbial colonisation. By changing the surface shape and chemistry, the interaction of bacteria with electrode surfaces has been investigated to some degree. Due to its availability and significant reduction potential, oxygen has been utilised as the cathode's main oxidant for the majority of time. Studies have also suggested the use of metallic oxidants that can be converted to a less harmful oxidation state. Due to the large over-potentials and poor kinetics encountered, the oxygen reduction reaction (ORR) continues to be one of the

major bottlenecks of this technology. The low energy generated by MFCs, which is now orders of magnitude lower than that of chemical fuel cells, presents another difficulty. In order to control and collect the low power produced by MFCs, new hybrid systems have been developed. These systems combine MFCs with external, commercially available harvesting devices, which are mostly based on supercapacitors and have a variety of claimed uses.

Recently, researchers have looked into the electrodes' capacitive characteristics. Additionally, supercapacitive electrodes have been employed as internal supercapacitors, and researchers have looked into the characteristics of such materials. Finally, successful research into a number of organic compounds originating from various municipal and industrial types of wastewater has demonstrated the viability of BES in producing power while simultaneously removing pollutants, making it an alternative technology for water filtration with a negative or positive energy budget.

In this chapter a brief outline the key processes are described that led from the electrochemical abiotic field to the hybrid biological-electrochemical system. This chapter provides the understanding of MFC development in the following areas:

1. Evolution of bio-electrochemical systems from MFC to MXC.
2. Knowledge of the anode's microbiology and the mechanisms for electron transfer in electro active microbial biofilm.
3. Electrochemistry with regard to the anode and cathode
4. Research and development on electrode materials;
5. Practical applications involving MFCs.

Microbial fuel cell types

A microbial fuel cell is a device that uses microorganisms to catalyse the conversion of chemical energy into electrical energy. Ananode and cathode compartments are often separated by a membrane that is unique to cations (positively charged ions) in microbial fuel cells. Microorganisms in the anode compartment oxidise fuel, producing CO2, electrons, and protons in the process. Protons are transported to the cathode compartment via the membrane, while electrons are delivered to the cathode compartment by an external electric circuit. In the cathode compartment, protons and electrons are oxidised, where they combine with oxygen to produce water.Microbial fuel cells come in two general categories: mediator-less and mediator-containing.

Microbial fuel cell mediator

The majority of microbial cells lack electrochemical activity. The mediator’s thionine, methyl viologen, methyl blue, humic acid, neutral red, and others enhance the transport of electrons from microbial cells to the electrode. The majority of the accessible mediators are pricy and harmful.Microbial fuel cell without a mediator Instead of using a mediator, mediator-free microbial fuel cells employ electrochemically active microorganisms to deliver electrons to the electrode (electrons are carried directly from the bacterial respiratory enzyme to the electrode).

Shewanella putrefaciens, Aeromonas hydrophila, and other bacteria are examples of those that are electrochemically active. Some bacteria may transmit their electron production via the pili on their exterior membrane. The usage of mediator-less MFCs is a relatively recent field of study, and as a result, parameters influencing optimal efficiency, such as the kind of ion-exchange membrane employed in the system and system conditions (temperature, pH, etc.), are not well known.

In addition to using wastewater as fuel, certain plants may also be used as a direct source of energy for mediator-less microbial fuel cells. This arrangement is referred to as a plant microbial fuel cell. Reed sweet grass, cord grass, rice, tomatoes, lupines, and algae are examples of potential plants. There are several different varieties of mediator-free microbial fuel cells, including: microbial electrolysis cells, soil-based microbial fuel cells, and phototrophic biofilm microbial fuel cells.

Cell for microbial electrolysis,

The microbial electrolysis cells are a variant of the mediator-less MFC (MEC). MECs partially reverse the process to generate hydrogen or methane by applying a voltage to bacteria to supplement the voltage generated by the microbial decomposition of organics sufficiently lead to the electrolysis of water or the production of methane, whereas MFCs produce electric current by the bacterial decomposition of organic compounds in water.

Microbial electrosynthesis, in which CO_2 is reduced by bacteria utilising an external electric current to create multi-carbon organic molecules, is a full reverse of the MFC concept. Microbial fuel cell built on the soil. The same fundamental MFC principles apply to soil-based microbial fuel cells. The inoculum, the proton-exchange membrane, and the nutrient-rich anodic medium all function as the soil (PEM). The cathode lies on top of the soil and is exposed to the oxygen in the air above while the anode is buried at a certain depth in the soil.

The electrogenic bacteria required for MFCs are among the many microorganisms that naturally abound in soils, which are also rich in other nutrients and complex sugars that have accumulated during millions of years of plant and animal material breakdown. Furthermore, the soil's redox potential decreases with depth due to the presence of aerobic (oxygen-consuming) bacteria, which function as an oxygen filter, much like the pricey PEM materials used in laboratory MFC systems.

A proton-exchange membrane separates the anodic chamber from the cathodic chamber in a conventional MFC (PEM). By placing the cathode in direct contact with the air, a one-compartment MFC does away with the necessity for the cathodic chamber.

Microbiological fuel cell with phototrophic biofilm

Phototrophic biofilm MFCs (PBMFCs) utilise anodes with phototrophic biofilms that include photosynthetic microorganisms including *chlorophyta, cyanophyta*, and other photosynthetic organisms because they can perform photosynthesis, acting as both makers of organic

compounds and electron suppliers.Biological photovoltaic systems are a subset of phototrophic microbial fuel cells that utilise only oxygenic photosynthetic material at the anode.

Advantages

When employing fuel cells, burning fossil fuels creates no pollution; the only result is water.If the hydrogen is created by electrolyzing water, using fuel cells reduces greenhouse gas emissions.Given that hydrogen can be produced everywhere there is access to water and electricity, the production of potential fuel might be distributed. A smaller-scale installation of stationary fuel cells leads to a more decentralised and reliable power grid.Fuel cells are more effective than gas or diesel engines.Most fuel cells are rather quiet while they're running when compared to internal combustion engines.There is no "memory effect" while refuelling fuel cells.Fuel cells need less maintenance since there are few moving parts in them. Fuel cells generate superior DC power.Since fuel cell technologies don't use combustion or have moving parts, they should be far more dependable than traditional combustion engines.Use a variety of fuels, such as renewable energy and clean fossil fuels.There are high power densities.Fuel cells are flexible enough to meet changing electricity demand.

This overview includes the many cathodic electron acceptors that have been used in MFCs. Several of these electron acceptors are pollutants in aquatic systems. Therefore, a therapeutic technique is also made feasible by employing MFC. The list is by no means exhaustive, however, since new cathodic catalysts, electrode materials, or developments in solution chemistry may lead to the emergence of more electron acceptors. In the early applications of MFC, oxygen was widely used as a terminal electron acceptor in the cathode chamber. To boost MFC voltage potential while simultaneously treating specific wastes or recovering significant compounds, scientists have recently started looking at more novel cathodic electron acceptors. The production of electricity with the reduction of specific electron acceptors in the cathode offers great potential for producing bioenergy and lowering the cost of specialised pollution treatment (e.g., persulfate, mercury, chromium, nitrogen species, copper, and perchlorate). As a result, reduction in the cathode compartment may be able to remove pollutants with significant redox potential. Using certain electron acceptors may improve MFC's performance. Ferricyanide, hydrogen peroxide, or iron may be used to release certain valuable compounds from wastewater, such as phosphate, for high-power generation.

CHAPTER 3

APPROPRIATE ANODE MATERIALS FOR MICROBIAL FUEL CELLS

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Poor wastewater treatment poses a severe risk to the preservation of a natural environment for people. A critical and worrying scenario is developing for human beings all over the world as a result of water pollution, water scarcity, and energy problems. The microbial fuel cell (MFC) strategy successfully addresses the aforementioned crises. Because of its distinctive approaches to achieving energy and wastewater treatment, the MFC technique has attracted a lot of attention in the current period. MFC is a new and important method that uses microorganisms as catalysts to cleanse harmful contaminants while also converting chemical energy into electrical energy. There have been notable reports of developments in wastewater treatment and electricity density. However, because of their inefficient energy generation and low removal efficiency, MFCs have not yet been used on a commercial scale.

Microbial fuel cells (MFCs) are a new technology that may turn organic waste into power, potentially solving energy problems while also offering environmentally acceptable wastewater treatment. The primary variables influencing electricity output in MFC are electrode characteristics and biocatalysts. The anode traps the electrons produced by microbial metabolism, which are then transported to the cathode through an external circuit and start the flow of electricity. Since the parameters of the electrode have a significant impact on this electron flow, electrode modification has received a lot of attention in attempts to boost MFC performance. As they have a high specific surface area, strong biocompatibility, chemical stability, or conductive qualities, various semiconductors, and nanostructured metal oxides, as well as their composite materials have been employed to change the anode. To improve the cathode materials' redox potential, electrical conductivity, or surface area, metals like platinum and nanocomposites were also used. As a result, this research examines recent advances in the alteration of electrodes to enhance MFCs' ability to generate electricity.

The world's shortage of clean water is also growing in importance as a result of the world's increasing population, as well as the energy problem and the development of technology and the economy. The world water development Report by the UN states that the water demand is rising globally at a pace of 1% per year and is anticipated to do so in the future. Water is a precious resource that is harmed by many types of human meddling in the form of contamination and deterioration. Wastewater treatment is a key problem for everyone due to its considerable effects on both the environment and human well-being. Physical, chemical or biological treatment procedures are now employed to treat wastewater. However, due to the significant infrastructure

and operation needed, several methods, such as membrane biotechnology, ion exchange, or electrolytic reduction, are expensive. Many of the methods, including chemical precipitation, coagulation, or flocculation, result in secondary pollutants and harm the ecology.

A bio-electrochemical system based on redox reactions called a microbial fuel cell (MFC) can be constructed as a single (SCMFC) or two-chambered device (DCMFC). In an MFC, the organic matter contained in wastewater or externally introduced substrates is oxidized by microbial activity to create electrons and protons in an anoxic environment (anodic chamber). CO2 is generated as an oxidation byproduct. Contrary to a direct combustion process, the electrons are moved from the anode to the cathode and then moved to. Electrode material Choice of material to be used as the electrode is critical for power generation in an MFC concerning electron transfer, electrochemical properties, and microbial adhesion. The most commonly used electrode materials include different carbon-based materials such as carbon cloth, carbon paper, carbon fiber, carbon felt, carbon nanotube-based composites, and graphene-based nanocomposites.

Electrodes for Anodes

Key factors in choosing an anode material include biocompatibility, easy microbial adherence, electrochemical efficiency, and a method for transferring electrons from the microbial cell to the electron acceptor. To optimize both current collection and the attachment of electroactive microorganisms to the electrode surface, the materials used to make anode electrodes should possess some special qualities.

Electrode Cathodes

Redox potential is the most significant cathode property that affects an MFC's performance. Low corrosion, strong electrical conductivity, high porosity, or high specific surface area are some more desirable cathode characteristics. The performance of MFCs is enhanced by cathode materials with high redox potential because they can more easily catch protons. O2 is a crucial molecule for the cathode chamber of an MFC to function as an electron acceptor.

Pure bacterial cultures are known to less efficiently contribute to the generation of electricity in MFCs than mixed consortia of electrogenic or electrotrophic microorganisms. This distinction may be explained by the production of electrochemically active biofilms as a consequence of synergistic interactions between syntrophic bacteria species, which effectively use the substrates that are available. Electroactive biofilm development and performance may be improved by selectively regulating growth conditions using synthetic biology and engineering methods. In order to get insights from a structural and functional standpoint, communities of microbial consortia have also been analysed and described utilising 'omics' technology and statistical analysis.

Separators and electrodes

Essentially, biocompatible, electrically conductive, non-corrosive, and electrochemically stable electrode materials are required for MFCs to function well. analyse the benefits and drawbacks

of various materials used as MFC electrodes in their thorough review paper and go over the future of electrode development. Designing effective MFCs necessitates evaluating the performance of the electrodes and separators as well as the utilisation of inexpensive materials such ceramics, ligno-cellulosic material, and biochar without considerably sacrificing efficiency. Discuss important factors for boosting bioelectrodes in MFCs and provide a novel approach to employing surfactants to enhance the performance of microbial electrochemical systems.

In MFCs, anodes act as both a current collector and a substrate for the development of biofilms. Carbon is the most recommended material among those that have been reported for anodes due to its adaptability, non-reactivity, high electrical conductivity, and biocompatibility. Because they are more porous than graphite sheets or carbon paper, carbon cloth and carbon felt provide bacteria more opportunity to colonise, but the inventive development of graphite brush anodes allowed for the insertion of electrodes with a higher surface area for a given volume of the reactor. Describe the characteristics of the anode materials used in MFCs and several processing processes that may increase the effectiveness of bacterial adhesion, electron capture, and transfer. The high conductivity and surface area given by nanomaterials led to their application in the anode chamber of MFCs. The creation of next-generation MFC anodes is facilitated by a comparison of conventional and modified anodes, which provides a fresh perspective on the properties of anode materials.

In an MFC, cathodes provide a single interface for the microbial electron transfer process's conclusion, which brings together electrons, protons, and the terminal electron acceptor. Cathodes have been regarded as a crucial factor in determining the efficiency of MFCs due to their complicated function. Cathodes may be categorised as chemical or biological depending on the kind of electron acceptor employed. Because it is so common and has a tendency to be converted to water, oxygen is often used as a terminal electron acceptor. However, because to the low kinetics of the oxygen reduction process, platinum was used as a costly precious metal catalyst at the cathode. Studies aimed at lowering operating costs finally paved the way for the creation of more cost-effective, alternative cathode materials based on carbon nanocomposites free of precious metals for enhancing oxygen reduction reaction efficiency. the use of microorganisms to catalyse the oxygen reduction process at the cathode, which is a rate-limiting step. In order to address many of the drawbacks associated with chemical cathodes, electrotrophic microorganisms that can directly receive electrons from the electrode are currently being aggressively studied as a research subject in biocathodes.

In an MFC, the anode and cathode are separated by a separator, a physical obstruction that let charges to flow through but acts as a barrier to avoid direct electrical contact.Proton exchange membranes, like were first utilised in MFCs to restrict only protons to the cathode chamber. The removal of the proton-specific, separating membrane from MFCs was a significant advancement in terms of lowering operating costs, but it also brought with it the twin drawbacks of oxygen diffusion into the anoxic anode chamber and short circuiting of electrons between the anode and cathode, both of which, when unchecked, negatively affect performance efficiency. In the years

that followed, expensive membranes were replaced with less expensive materials that had more general transport properties, such as, ion exchange membranes, ceramic filtration membranes, polymeric membrane separators, sand/activated carbon separator

Characterization of performance

With the aim of reducing electrochemical losses and improving performance efficiency, alterations to MFCs are examined for their impact using electrochemical methods and instruments. A thorough overview of the elements to be taken into account when developing anodes for microbial electrochemical systems. Cyclic voltammetry electrochemical impedance spectroscopy, confocal resonance Raman microscopy, interdigitated electrode array, and other methods have been used to characterise the electroactivity of biofilms. To reduce losses and improve the performance of MFCs, technical aspects like internal resistance anode potential and voltage reversal must be understood. Other frequently encountered problems include power overshoot, as well as common issues like power Tutorial articles provide newcomers the assistance they need to comprehend basic ideas in electrical circuits, the intricacies of procedures like cyclic voltammetry, and the selection of electrode configurations and operating conditions for electroanalysis together with electrochemical impedance spectroscopy. Other helpful studies define terminologies and performance measures for microbial electrochemical systems. Having a standardised framework and fundamental principles to plan experiments, analyse observations, and report results in a more meaningful manner can address challenges brought on by the various configurations of MFCs and the various techniques available for characterising activity of electroactive microbes.

MFCs have shown improved performance in terms of electricity generation. Despite the fact that L and mL scale laboratory experiments offer cues and hints regarding various mechanisms involved in the functioning of MFCs, systemic understanding gained from such studies must be transferred and translated in order to enable the creation of pilot-scale systems. It is essential to have knowledge of the many processes and components involved in order to make scaling up of MFCs actually viable. Over the last ten years, significant progress has been made in creating scaled-up MFC systems for use in real applications.

An innovative form of alternative energy with little to no net CO2 emissions is fuel cells. The earliest reports of microbial cultures producing electricity appeared at the beginning of the previous century. According to their definition, microbial fuel cells (MFCs) are "bioreactors that transform the energy in the chemical bonds of organic substances into electrical energy by catalytic activity of micro-organisms under anaerobic circumstances." MFC technology is a unique method of employing microorganisms to oxidise organic waste and renewable biomass to produce bioelectricity.MFCs have the following operational and functional advantages: They employ easily accessible microorganisms as catalysts and organic waste as fuels.Unlike hydrogen fuel cells, which need highly controlled distribution networks, MFCs do not.Compared to Enzymatic Fuel Cells, MFCs have a higher conversion efficiency and can capture up to 90% of the electrons from the bacterial electron transport system.

A multidisciplinary approach to the search for alternative energy sources is represented by MFC technology. It represents the intersection of the physical, biological, and chemistry sciences and serves as a hub for fundamental and practical research. The fundamentals of microbial physiology combined with electrochemistry serve as the foundation for the operation of MFCs. The intricate details of electrical and materials engineering are highlighted by the MFCs' structural architecture. The applications of this technique fall within the categories of bioremediation and environmental engineering. Thus, MFC technology is multidisciplinary in the truest meaning of the word and offers opportunities to improve cross-disciplinary research.

Biological foundation

How can microorganisms get nutrients to sustain themselves? Under various circumstances, chemotrophic microorganisms use organic and other biodegradable substances. The terminal electron acceptor molecule determines the proper electron carriers to use to transfer the oxidised electrons to an electron transport chain. This terminal acceptor in aerobic organisms is oxygen, which absorbs the electrons and is reduced to water.

According to the chemiosmotic theory, the translocation of protons across bacterial membranes and the subsequent coupling of ATP production to proton electrochemical potential across the energy-transducing membrane are two related processes.The chemiosmotic principle is used by the bacterial cell membrane to operate as an energy-transducing membrane. A proton electrochemical gradient is created as a consequence of the translocation of protons away from the membrane. This membrane potential is multiplied by the pH gradient to produce the proton motive force. ATP production occurs in conjunction with the re-entry of these protons through the ATP-synthase enzyme. The bacteria need the ATP that is created in this way to survive.

Now that we have this theoretical foundation, let's examine how an MFC works. The anodic and cathodic half-cells, which are divided by a selectively permeable, cation-specific membrane or a salt-bridge, make up a typical MFC. Microorganisms floating in the anolyte under anaerobic circumstances make up the anodic chamber, whereas the electron acceptor is found in the cathodic chamber (oxygen).

In essence, the two chambers act as a physical barrier separating the terminal electron acceptor from the electron source. The anode receives the majority of the released electrons from the oxidation process. Electron mediators or shuttling agents, cells themselves, or "nanowires" may all move electrons to the anode. Through an external circuit, these electrons are directed to the cathode, where for each electron carried, a proton is delivered over the membrane to the cathode to complete the reaction and maintain the electric current.

The usage of MFCs is currently limited to a few specialised industries, despite the fact that they have been researched as an alternative energy source. Based on these near-term applications, further improvements in design, cost effectiveness, and performance efficiency would make scaling up and employing MFCs as a renewable energy source feasible.

Water treatment for waste

The ability of microorganisms to produce power and decompose effluents. MFCs are now being taken seriously as instruments for producing energy while treating municipal, industrial, or agricultural wastewater. A continuous flow of electrical current is produced when microbes oxidise organic materials contained in waste water. If power output in these systems can be increased, sophisticated waste water treatment might become more affordable in both developing and industrialised nations, offering a new means to offset operating expenses of waste water treatment plants. In comparison to aerobic treatment, MFCs are said to create less additional sludge.

Maintaining undersea surveillance equipment's power

The operation of the sensors positioned throughout the environment requires power, despite the fact that information from the natural environment may be beneficial for understanding and forecasting ecosystem responses. MFCs may be used to power these devices, particularly in river and deep-water environments where it is difficult to routinely access the system to replace the batteries.

Sediment fuel cells are being developed in order to monitor natural systems including creeks, rivers, and oceans. Low organic matter concentrations and significant inherent internal resistance in sediment fuel cells lead to inadequate power densities. However, poor power density may be made up for by energy storage devices that provide data to central sensors in bursts.

Power supply for remote sensors

Thanks to developments in microelectronics and related sectors, electronic gadgets now consume substantially less electricity. Batteries are often used to power chemical sensors and telemetry systems, although in certain cases, doing so might be costly, time-consuming, or even impossible. One solution to this problem is to use self-renewable power sources, such as MFCs, which can operate for a long time utilising local resources. The major focus of significant research into the creation of reliable MFCs in this respect is the selection of appropriate organic and inorganic materials that may be used as energy sources.

BOD recognition

Another potential use for the technique is as a sensor for in-situ process monitoring and control and pollution assessment. The biological oxygen demand (BOD) is the amount of dissolved oxygen required to meet the metabolic needs of aerobic organisms in water that is high in organic waste, such as sewage. Due to the proportional link between the coulombic production of MFCs and the amount of assimilable organic contaminants in wastewater, MFCs may be employed as BOD sensors. An MFC-type BOD sensor has a service life of over 5 years without any further maintenance, which is much longer than that of other types of BOD sensors mentioned in the literature.

Producing hydrogen

It would be beneficial to use modified MFCs that generate hydrogen from organic waste. In such systems, the cathode chamber is maintained anaerobic and given an additional 0.25 V of power. Under these conditions, protons are transformed to hydrogen on the cathode. These modified MFCs are referred to as "bio-electrochemically assisted microbial reactors".

Applications

The main use of MFCs is the treatment of wastewater while simultaneously producing power. The goal of description of the energetics of MFCs is to create a self-sustaining home wastewater treatment process. Utilizing MFCs' potential as a power source and for the production of valuable products through microbial electrosynthesis necessitates a thorough comprehension of performance-limiting factors as well as the principles of energy capture and storage.

Microbiological evolution has favoured the diversification of MFCs into a number of technologies with a variety of applications, leading to the more general name "microbial electrochemical cells" (MXCs). a summary of the many uses for microbial electrochemical technology is given, along with references to freshly released review studies. Torres places a strong emphasis on the need to "detect, analyse and forecast" the many events that influence how these systems function. The diversification of microbial electrochemical cells, as shown in figure 2.1.

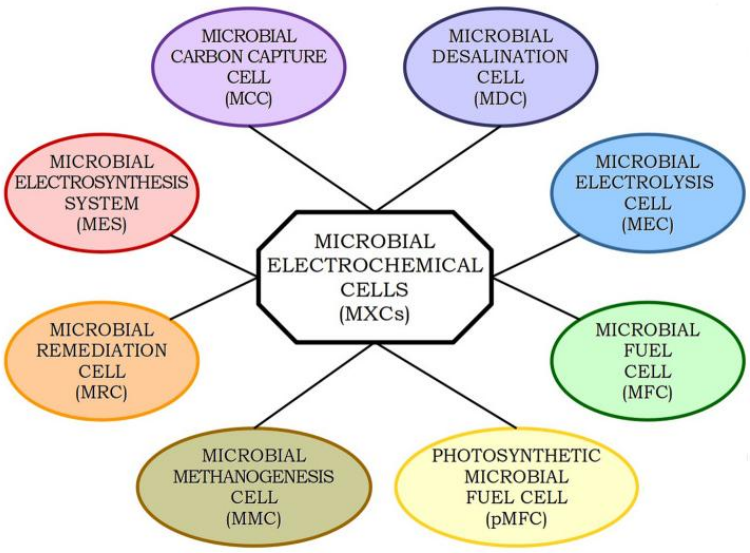

Figure 3.1: Illustrate the representation of Microbial Electrochemical Cells.

Researchers' attention has been drawn to MFCs, which effectively play a dual-role of wastewater treatment and clean energy generation, as a result of the quest for alternative energy sources owing to the effects of an unchecked growth in human population. Over the last 15 years, the

number of research publications published on MFCs has steadily increased (Md Khudzari *et al.* 2018), which is a sign of the scientific community's perseverance and dedication. In addition, among the many books written or compiled on MFCs, the following three stand out for encapsulating important developments related to the development, characterization, applications, and diversification of this technology: Microbial fuel cells , Microbial Electrochemical Technology: Sustainable Platform for Fuels, Chemicals, and Remediation (Elsevier), and Microbial Electrochemical Technologies.

Unnoticed, however, is the rise in students choosing MFCs and related technologies for their high school and college projects because of the social significance of these subjects. It would be interesting investigating the development of a platform to record and compile promising outcomes from such initiatives, given that the data offered in such project reports often prompt more specialised and resource-intensive investigations. Additionally, highlighting the contributions of these young authors in the research papers that follow up on these findings will motivate students to do more in-depth investigations.

Due to the apparently endless options for observing the impacts of adjusting the many physico-chemical and biological factors that either directly or indirectly affect MFC performance, planning a student project or creating a research experiment may appear to be a simple procedure. However, in order to make a significant contribution to the body of knowledge, it would be preferable to align the scope of such investigations to the aforementioned four major objectives of MFC research—maintaining electrochemical losses under control, increasing electron transfer efficiency, lowering operational costs, and upscaling systems for practical applications.

Due to the limited resources and high energy needs, the globe is now undergoing an energy crisis. Therefore, it is vital to find a different way to produce power. Microbial Fuel Cell (MFC) technology is one such development. This method converts chemical energy into electrical energy using microorganisms to produce power. After much research and cooperation, it is now recognised that microbial wastes, including a variety of carbon sources, are also used to generate power. The fundamentals of the Microbial Fuel Cell (MFC) and how it operates are briefly described in this chapter.

Microbial Fuel Cells are bio-electrochemical devices that use microbial catalysis at the anode to transform chemical energy from organic molecules or renewable energy into electrical or bio-electrical energy (MFC). Electricity production using waste products from industrial processes or wastewater treatment is an innovative and appealing technique. It directly converts organic matter to electrical energy by using microbes to do it. It is regarded as a novel technique for recovering renewable energy. Utilizing the oxidation process and electrochemically active microorganisms, the MFC technology transforms chemical energy from organic wastes or carbon sources into electrical energy. By using the electrons created by the anaerobic oxidation of materials, it produces power. It has two chambers, such as the cathode and the anode. The exchange membrane, as its name suggests, separates them. The MFC technique makes use of

bio-electrochemically active bacteria as its microorganisms. One kW per cubic metre of reactor volume is the power density produced by MFC.

Microbial Fuel Cell Operation

Redox reactions are the foundation for how microbial fuel cell (MFC) technology works. The bacteria oxidise the organic material to release protons, electrons, and carbon dioxide (CO2). Electricity is produced using the bacteria' natural metabolism. Bacteria transform the substrates into electrons. As shown in the above diagram, the MFC is made up of anode and cathode chambers that are divided by a proton exchange membrane (PEM). Protons, electrons, and CO2 are produced at the anode by the microbes or microorganisms oxidising the fuel or substrate. While the exchange membrane transports the protons to the cathode chamber.

In order to create electrical energy, the electrons are sent from the anode chamber to the cathode chamber via an external electrical circuit. Protons and electrons are used up at the cathode, where they react with oxygen (O2) to make water. These are the anode and cathode reactions during the whole process. The limitations of oxygen use in microbial fuel cell technology are caused by contact failure of electrodes with oxygen and sluggish oxygen reduction on the carbon electrode. Even though utilising electrodes coated with catalysts might boost the cathodic chamber's response. Because rare and costly metals are used in the catalysts.

Free of Mediator Microbial Fuel Cell (MFC)

For the transport of electrons to an electrode, this form of MFC employs bacteria that have undergone bio electrochemical activation. It has cytochromes, which are electrochemically active redox enzymes found on the outer membrane and aid in the transmission of electrons. The anode chamber's surface develops a biofilm, which facilitates direct electron transmission through conductance to the anode. Examples of this sort of bio-electrochemically active bacteria are Shewanella putrefaciens and Aeromonas hydrophila.

Microbial Fuel Cell Mediator (MFC)

The MFC in question is not electrochemically active. This implies that without assistance from a mediator like humic acid, the bacteria in the fuel cannot transmit the electrons. This form of mediation uses unfavourable, harmful, and costly mediators. By snatching the electrons and transferring them to the anode for the reoxidation process, the mediator lowers the oxidising state. MFCs have been prototyped in a variety of forms and sizes, with each new configuration showing a performance gain over the others. In the ongoing search for models that can be successfully deployed on a broad scale, MFC setups will be changed. The knowledge of factors affecting MFC performance has undoubtedly increased over time, and the supply of products that enhance MFC performance is also steadily growing. These must coexist with initiatives to reduce the cost of scaled-up systems. For instance, carbon-rich agricultural wastes may be carbonised and used as an inexpensive electrode material. Such replacements, however, may entail a trade-

off in terms of performance effectiveness, providing additional opportunities for in-depth optimization studies employing statistical techniques like response surface analysis.

The best design, parts, and operating parameters to use for a given set of circumstances for particular applications may be estimated with high accuracy using mathematical modelling and computer simulation. Because of the interrelationships among the physico-chemical and factors affecting MFCs, designing high-throughput techniques for screening performance of components and operating parameters is a task that is still important and requires attention.

However, the biological component will always be a complicated variable that is difficult to predict exactly, necessitating more concentrated study to uncover unidentified aspects of bacterial metabolism and energetics, particularly in the context of bioelectrochemical systems. It is still unclear how microbial consortia in electroactive biofilms that power microbial electrochemical devices behave as a community. It is possible to gain insights into the mechanistic aspects of bacterial electron transfer systems and processes through in silico analyses of genomic and proteomic data in publicly accessible repositories like the European Bioinformatics Institute, Worldwide Protein Data Ban, National Center for Biotechnology Information, and many others. Since they take into consideration microorganisms that are difficult to cultivate under controlled laboratory settings, metagenomic techniques for microbial community profiling are becoming more and more important.

Today's sophisticated protein modelling and visualisation technologies may reveal characteristics of bacterial respiratory proteins and biofilm-associated proteins that were previously unknown. The protocols used to regulate biofilms, particularly in the food and healthcare industries where they are recognised to be a nuisance, may provide helpful guidance for formulating strategies to encourage their development in bioelectrochemical systems.

The setting up of the working environment will be aided with tutorial articles on a design of experiments strategy to successfully plan experiments and on electrochemical techniques for performance characterization. A significant barrier to getting trustworthy findings might be the lack of inexpensive access to electrochemical characterisation equipment and the lack of established funding sources in institutions. Academic research using MFCs might provide higher-quality findings if efforts were made to reduce the price of fundamental instrumentation utilising microcontrollers.

Literature demonstrates how what started as an intriguing phenomena over a century ago has developed into a fruitful space for academics from many fields to congregate and contribute.

The voyage of MFCs seems to be comparable to the folktale of the six blind men who attempted to describe an elephant, each of whom based his assessment on a specific aspect of the animal that he physically sensed. They only saw the wider picture and realised that an elephant is much more than simply fan-like ears, pillar-like legs, spear-like tusks, a tube-like trunk, a rope-like tail, and a wall-like body when all of their points of view were properly considered. Due to the use of a simplex strategy for extracting energy from wastewater employing microbial catalysts, old

obstacles and gaps have been destroyed and crossed by multidisciplinary approaches and transdisciplinary efforts.

The vast array of applications that have been thought of, shown off, and predicted present microbial electrochemical technology as a "miracle solution" to looming sustainability challenges. However, MFCs can only effectively solve global sustainability challenges if the efforts are gathered, organised, and focused on a single goal of actually using these technologies. Single-minded efforts in several areas would only lead to a struggle amongst research teams with different levels of expertise. Instead, a regionally-based cooperative strategy may make the most use of the pool of available knowledge to raise the production of MFCs to useable levels for large-scale applications at reasonable prices. For other emerging organisations to participate in their different specialised fields toward a shared goal of societal benefit, established groups must take the initiative in their own regions by developing a framework and outlining a roadmap. This will undoubtedly be made feasible in large part by the International Society for Microbial Electrochemistry and Technology's tireless work in that regard. The saying "coming together is a beginning" is true: Together we can make progress. Collaboration leads to success.

The microbial fuel cell is a cutting-edge and sustainable technique for creating bioelectricity and purifying wastewater. Several researchers have focused on the creation of electrode materials, membranes, and construction materials to show that this technology is economical, ecologically friendly, and has a very little carbon footprint. Carbon-based electrode materials are commonly employed in the area of environmental engineering due to their widespread availability, high conductivity, or chemical durability.

A full system is created by connecting the cathode to the anode through an electrical circuit, such as copper or titanium wires. The microorganisms in the anode chamber oxidise the organic substrates to create electrons, protons, and carbon dioxide. Redox-active proteins or cytochromes initially transmit the electrons generated by microbial metabolic activity to the anode surface before passing them via an electrical circuit to the cathode. The reduction of electrons happens in the cathode chamber. At the cathode, an electron acceptor is often present, such as oxygen or ferricyanide. At the cathode, protons and oxygen mix with electrons to make water. An additional catalyst, such as platinum, may be used to speed up this process. Fig. 1 depicts the overall operating procedure and the elements of an MFC.

Anode response:

$$CH_3COO^- + 2H_2O = 2CO_2 + 7H + 8e$$

Reaction at the cathode:

$$O_2 + 4e^- = 4H + 2H_2O$$

MFC technology is a promising method for producing electric current from many substances, including complicated organic waste and natural organic matter, and it may be advantageously combined with uses in wastewater treatment. MFCs have a few special qualities that set them

apart from other technologies and provide them an advantage. The conversion efficiency of chemical energy to electric current is greater with MFCs. The MFC technique is distinct from other current bioenergy approaches in that it may provide successful results in a range of temperature ranges (from 20 to 40 C). Since the cathode may be passively aerated, MFCs do not need an external electric source of aeration to deliver oxygen (as an electron acceptor) during operation. MFCs are built utilising a variety of designs and materials. These systems may be run under multiple settings, such as low or high temperatures, acidic or basic pH, with different electron acceptors, etc. These systems are often operated at optimal parameters to extract more energy from the system.

Construction Materials for MFC

Anode. The components utilised to create the anode must be conductive materials. Additionally, the substance should be environmentally safe and chemically inert to the anolyte, the electrolyte in the anode chamber. Carbon-based materials, including as graphite plates, rods, felt electrodes, granules, fabric, brushes, stainless steel, and more, are often used to make electrodes for MFCs. In general, MFCs are quite interested in the cheaper, greater surface area electrode materials. For better MFC performance, the low surface area bare electrodes may be simply changed with conductive nanomaterials with greater surface areas, such graphene. The nanomaterial may be coated on the bare electrodes using a variety of ways. In the MFCs, the anodic alterations using nanoparticles have so far shown successful results. Such nanomodifications often encourage the growth of biofilms on the anode, shorten the startup time of the MFC, simplify the electron transfer process, lower internal resistance in the system, and ultimately boost the MFC's overall performance.

Cathode the cathode material, an electron acceptor, and a catalyst to speed up electron reduction are all present in the cathode compartment. The same electrode materials that were utilised as the anode in the previous sentence may also be employed as the cathode. Additionally, when oxygen is utilised as the electron acceptor, a catalyst (such as platinum) is added to the cathode electrode to speed up the rate of oxygen reduction. Given its low cost, wide availability, and strong redox potential, oxygen is one of the finest options for the electron acceptor. In contrast, ferricyanide (K3 [Fe (CN)6]) is a frequently utilised MFC because it is a precise common electron acceptor and has good enactment. When ferricyanide is used as the electron acceptor in MFCs, a catalyst is not necessary at the cathode. This is especially true since it displays a little overpotential when used with a simple carbon electrode. However, there are significant drawbacks to using ferricyanide in MFCs, such as insufficient oxygen reoxidation, which necessitates regular catholyte replacement.

Additionally, ferricyanide may permeate into the anode chamber through the ion exchange membrane, which means that its usage might impair MFC performance. Membrane The proton exchange membrane (PEM), which permits the transfer of protons or certain cations from the anode to the cathode compartment, is often utilised in an MFC between the anode and the cathode chamber. The most often used PEM is nafion. Ultrex CMI-7000, however, is much more

cost-effective than Nafion and is as suited for MFC applications. Additionally, PEM may be oxygen-leak-prone, and anolytes or bacteria may diffuse to the cathode while catholytes like ferricyanide may also travel to the anode, which may significantly reduce an MFC's performance. However, in order to assess the impact of the PEM on performance and permanent permanency, more effective adjustments are necessary.

Two MFC Types

Doubly-Chambered MFC

The simplest MFC design is the double-chamber MFC. A common configuration uses two bottles, separated by PEM, with one serving as the anode and the other as the cathode. Energy is typically produced in two-chamber MFC using a predetermined catholyte solution and a specific medium (or substrate) in the anode. In other words, batch mode is often used to run the double-chamber MFC. The double-chamber MFC may be shaped as a cube or like a bottle. The name of the design may be determined by the catholyte selected in the MFC. For instance, if oxygen from the air is utilised as the electron acceptor in the cathode, the MFC is referred to as a two-chamber air-cathode MFC Such MFCs could be useful for producing power in distant sensing areas.

The anode and cathode are both included in the same chamber in this form of MFC. Either distant or close to the cathode, the anode is separated from it by PEM. It has been claimed that internal ohmic resistance may also be reduced by reducing interelectrode distance. By connecting two chambers, it is possible to avoid the need of catholyte and increase power density. Compared to double-chamber MFC, this MFC is straightforward, affordable, and provides a lot of power.

However, the main issues with SCMFC, such microbial adulteration and oxygen flowing backward from cathode to anode, happen regularly. SCMFCs suggest less complex and expensive designs. These MFCs typically just have an anodic chamber and do not need a cathodic chamber to contain air.

MFC Upflow

A rise in interest in MFC research led to the creation of the new design. The upflow MFC is formed like a cylinder. The cathode chamber is located at the top of the MFC, while the anode is located at the bottom. Glass wool and glass bead layers distribute the space between the two chambers. From the anode's base, the substrate is supplied, moving up to the cathode and out the top. The formation of a gradient between the electrodes also aids in the fuel cell's favourably acting. There are no distinguishable anolyte or catholyte in his design. Furthermore, it doesn't physically separate. Consequently, proton transmission-related problems are exceedingly rare.

Since upflow mode MFCs scale up more readily than other MFC designs, they are intriguing for the treatment of wastewater. The energy expenses to pump the substrate are the fuel cell's primary downside, since they are much higher than their power output. Consequently, we may draw the conclusion that the primary goal of an upflow MFC is wastewater treatment rather than electricity production. These MFCs are often used in fundamental research, and studies suggest

that their poor power densities are caused by their high internal resistance, electrode-based losses, and intricate designs. According to the UN DESA Population Division's 2019 estimate, there are now close to 8 billion people living on Earth. By the second part of the twenty-first century, the population is predicted to level out. Due to ambitious social and economic objectives, the sustainability of natural resources has been a subject of worry. Over 80% of the world's main energy usage is derived from depleting fossil fuel stocks. The greenhouse effect, a natural occurrence that is mostly responsible for keeping the world habitable, seems to be growing out of control. Unchecked emissions of carbon dioxide and other greenhouse gases brought on by human activity have increased the earth's ability to absorb infrared radiation from the sun, causing surface temperatures to rise above average (IPCC 2014). The need to reduce these emissions highlights the need for sustainable, carbon-free energy sources (Arent *et al.* 2011; Villano *et al.* 2012). Rittman (2008) especially discusses the potential of microbes as a source of energy, reiterating the need of switching to renewable energy.

According to Buhaug and Urdal (2013), urbanisation is increasing in developing countries, and the resulting rise in average wealth has improved food tastes while placing stress on water supplies. Water supply has been influenced by the rise in water demand and reuse of wastewater for landscaping and irrigation purposes has been encouraged. However, population expansion has often outpaced improvements in sanitary infrastructure and wastewater treatment in emerging nations. As a consequence, many of them are searching for dependable, affordable ways to clean home, agricultural, and industrial wastewater so that it may be reused explore the necessity to embrace new tactics for finding resource-efficient solutions for problems connected to urban water management in an interesting and well-illustrated review study.

MFCs' history and present

Over a century ago, Potter provided the first description of the electrical effects brought on by the microbial breakdown of organic molecules. There were just a few sporadic accounts of efforts to apply this exciting finding to practical uses in the decades that followed and into the next century. Schröder (2011) traces the development of microbial electrochemical systems over a century, highlighting key turning points, briefly outlining the initial lack of enthusiasm for furthering this technology, and finally outlining the relevance and potential of this field following its resurgence at the turn of the century.

so nicely put it, "bioreactors that transform the energy in the chemical bonds of organic substances into electrical energy via catalytic activity of microorganisms under anaerobic circumstances" are microbial fuel cells (MFCs). Figure A conventional two-chambered MFC with an anode and a cathode chamber divided by a membrane that allows for selective permeability is shown graphically. The anode of an MFC easily satisfies the microorganisms' need for a compatible electron acceptor to deposit electrons in the absence of a more appropriate acceptor. To generate useful energy, the anode's electrons are directed across a resistor, an

external load. At the cathode, where there is a terminal electron acceptor, the electron transport process concludes. The core of an MFC is therefore a "quasi-engineered" electron transport chain that resembles the bacterial respiratory chain. Schröder explains fundamental MFC ideas in a well written course material provide thorough reviews of the technological underpinnings and ideas that underpin this technology. These microbe-catalyzed electrochemical devices are seen as a possible source of sustainable and clean energy as well as a wastewater control solution. Research on microbial electrochemical technologies has generally concentrated on four elements, including decreasing electrochemical losses, enhancing performance efficiency, lowering operating expenses, and scaling up systems for practical application, in order to make this solution realistically feasible.

Electroactive microorganisms

By catalysing the release of electrons from organic substrates' energy-rich bonds in anoxic environments, microbes play a crucial part in MFCs. Different pure substrates and varieties of wastewater that have been employed as a carbon source for microorganisms in MFCs. Before being donated to the anode of the MFC, the electrons released during this oxidation process pass through a variety of microbial electron transport chains, which are composed of serially arranged conductive protein complexes, cytochromes, nanowires, and redox proteins. In a classic review, Schröder outlines the basic mechanics and energy concerns of anodic electron transport. Indirect electron transfer, which is mediated by naturally occurring or artificially introduced redox shuttles, or direct extracellular electron transfer may move electrons directly from microorganisms to the electrode. While Lovley outlines the procedures connected with direct interspecies electron transfer, which facilitates long-distance transport of electrons in bioelectrochemical systems, provide insightful information on endogenous extracellular electron shuttles.

A stacked MFC is often made up of a number of MFCs that are connected either in series or parallel to increase power output. By connecting many MFCs, the output of each MFC is multiplied, increasing the output of the MFC. Typically, one MFC unit (using oxygen as an electron acceptor) may produce a OCV (open circuit voltage) maximum of 0.8 V. As a consequence, a number of MFC unit cells may be connected in series or parallel, and the combined power output is obtained by multiplying the individual power output of each cell. However, several other operational elements are also crucial for current generation in a stacked MFC and may either improve or worsen the MFC's overall performance. Additionally, the final voltage may not equal the sum of the individual cell voltages after joining each individual MFC in a stacked MFC since there will be a voltage drop when each cell is linked either in series or parallel. When MFCs are stacked in a series connection, it has been shown that the parallel-connected stack MFCs generate higher current than their rivals. Therefore, we may conclude that a greater bioelectrochemical reaction rate is attained in parallel-connected stack MFCs than in series-connected stack MFCs. Additionally, if the MFC units are not functioning independently, a parallel connection is preferred to increase chemical oxygen demand (COD) elimination for

improved wastewater treatment efficiency. Six MFCs were wired either in series or parallel in a successful experiment. Acetate served as the anode's substrate, ferricyanide served as the catholyte, and graphite rods served as both the anode and the cathode in the MFC. The study's findings showed that when linked in series, stacked MFC produced a volumetric power density of 59 W/m3, but when connected in parallel, 51 W/m3 was produced.

The MFC's coulombic efficiency (CE) may provide an estimate of the electrons transported from the anode that contribute to the current's generation (from the total electrons created theoretically via substrate oxidation). Different rates of CE may be produced in stacked MFCs by connecting the cells in various ways. According to the earlier research, the stacked MFC generated a larger rate of CE when it was run in parallel, or 78%, than when it was operated in series, which produced a CE of just 12%. Voltage reversal is the main barrier preventing stack MFC from producing higher voltage outputs. The voltage reversal may be caused by the cell's substrate being depleted, which would indicate that the bacteria's capacity to create greater voltage has been reduced. Most MFC research employ the basic designs, and little attention is given to creating new designs that address the shortcomings of the present designs. Min and Logan created a flat-plate MFC (FPMFC) in 2004 to lessen one barrier by lowering the ohmic resistance brought on by increased interelectrode spacing (Min and Logan 2004). This kind of design is often employed in chemical fuel cells, where it also produces greater power than earlier styles. Flat plates served as the anode and cathode in the FPMFC (each plate with a projected surface area of 225 cm2). Between the two plates was a Nafion membrane. This reactor produced 56 mW/m2 of power density using domestic fuel.

The research used wastewater as a substrate and produced a COD of 58%. Although the reactor generated less power than the other designs, such as the cube reactor, it was also utilised to generate electricity from different substrates, such as acetate, glucose, starch, etc. The microbial community's ability to flourish may be hampered by oxygen passing through the membrane to the bacteria in the anode chamber as a result of the electrodes being too tightly connected.

Elements That Impact MFCs' Performance

Electrode Substance

Both the anode and the cathode in MFCs are built of electrode materials that must be inexpensive, non-corrosive, and conductive in nature (in the case of the anode). The MFC performs well thanks to the electrode material with a large surface area. The advancement of the MFC electrodes has reached a greater degree. Additionally, compared to simple electrodes, many electrodes modified with nanoparticles have generated far more electricity. For greater power production, the anode's electrode should be modified with a catalyst or nanomaterial that can encourage the growth of biofilms and speed up electron transfer rates. For instance, employing G. sulfurreducens as an inoculum, magnetite nanoparticles improved the current output in the MFC. According to the research, the nanoparticles made the biofilm-electrode contact more electrically conductive, which improved the electron transfer process. Pt or Pt-coated cathodes

created more electric current in the cathode than plain cathodes without a catalyst. Pt has been recognised as the best catalyst for oxygen reduction in MFCs (when employed as an electron acceptor), but its greater cost is also a barrier to the technology's use in large-scale applications.

Proton Exchange System

A proton exchange membrane (PEM) connects the anode and cathode chambers in an MFC's proton exchange system. Protons may flow from the anode to the cathode more easily thanks to holes in the membrane that have charged side walls. As a result, this membrane plays a crucial role in MFCs and may influence their ability to produce electricity. The anolyte may diffuse across the membrane to the catholyte while the MFC is in action. This might result in the membrane being fouled, which would then prevent protons from reaching the cathode. It reduce the MFC's power output. As an alternative, the performance of the bacteria will be impacted by catholyte entering the anode chamber. The formation of the anaerobic bacteria's biofilm, for instance, might be impacted by oxygen's diffusion to the anode, which in turn can impair the MFC's overall performance. Additionally, the membrane raises the internal resistance of the MFC, and the concentration polarisation loss of the system caused by the passage of ions or electrolytes through it has a direct impact on the fuel cell's ability to generate current. The MFC technology's most popular PEM is nafion membrane. This is due to the membrane's protons-only selective permeability. However, throughout the MFC procedure, a few additional cations were also transported via Nafion. However, using membrane to keep the anolyte and catholyte's charges in balance is quite advantageous. Additionally, it is discovered that the MFC's membrane surface area to volume ratio is crucial for the system's electrical output. According to research findings, MFCs with PEMs with large surface areas produce less internal resistance and so have better power production. According to several research, MFCs without a PEM provide greater power production than MFCs without a membrane for a certain amount of time. One of the PEM's drawbacks is also its greater price. As a result, we may see membrane-less MFCs being used in large-scale applications in the future.

Operational State

Only the fundamental MFC operating variables, such as pH, temperature, organic loading, feed rate, and shear stress, are covered in this section. It has been discovered that MFC performance at small scales, such in the lab, is still much below optimal performance. Optimizing the operating conditions will thus greatly benefit the MFCs' overall performance.

The impact of pH

The microorganisms oxidise the substrates in the MFCs' anodic chamber, generating protons that go to the cathode via the PEM and combine with electrons and an electron acceptor (if oxygen) to make water. Because of the sluggish or constrained flow of protons through the PEM over a prolonged period of MFC operation, the concentration of protons in the anolyte rises. Despite the fact that bacteria are more active when the pH is close to neutral, the concentration of protons makes the anode chamber more acidic and much less favourable for bacterial development. On

the other hand, since there is insufficient replenishment of protons from the oxidation process and continual depletion of protons by the reduction reaction, the cathode chamber becomes more alkaline. A pH concentration differential between the two chambers affects MFC performance electrochemically and thermodynamically.

The cathode chamber's high pH may significantly lower current production. With a drop in pH, the possibility for oxygen reduction rises (according to the Nernst equation). As a result, the oxygen reduction and consequent increase in electric current from MFCs benefit from the low operating pH. Bacteria typically need a neutral pH for their best development, and they adapt to changes in internal and external pH by controlling their activities. The pH in the anode chamber may fluctuate according to the needs of the bacterial growth, which may then lead to changes in basic physiological characteristics as membrane potential, ion concentration, biofilm development, and proton-motive force.

One of the important elements that influences microbial metabolic activity and, as a result, upsets the process for producing electrons and protons is pH maintenance in the anodic compartment. The anolyte's lower pH reduces bacterial activity, which has an impact on the growth of biofilms and the MFC's current production.

According to a research, MFCs operating at low anodic pH showed increased proton transfer rates, which led to more protons accumulating on the cathode side and reducing the current density. The pH range of 6 to 9 is suitable for microbial development and attaining somewhat larger power outputs, according to a different research.

In a dual-chamber MFC as opposed to a single-chamber MFC, it is easier to maintain two separate pH values to maximise the anodic and cathodic processes. However, since there is only one electrolyte present in the anode chamber of an air-cathode MFC device, referred to as anolyte. It is more challenging to maintain the pH in this device. Catholyte is the name given to the electrolyte found within the cathode (in two-chamber MFC).

The air-cathode MFCs have a more straightforward construction and have shown to produce more power than conventional MFCs (the cathode electrode is exposed to the air). According to a research, an anolyte with a pH range of 8 to 10 can run the air-cathode MFC. The findings showed that although the oxygen reduction process was altered at a higher pH, the anodic bacterial activity was best at a neutral pH.

Effect of Temperature

One of the most significant variables affecting the kinetics of the whole system in MFCs is temperature. Any significant change in temperature when MFC is in operation may have a significant impact on its performance. It primarily influences thermodynamics, mass transport, and microbial metabolism (electrode potentials and Gibbs free energy). For the MFC to work well in terms of energy production and wastewater treatment effectiveness, the temperature has been included as a crucial component. Typically, MFCs are run at a temperature of around 25 to

30 C, which is close to room temperature. However, MFCs have also performed effectively (in terms of power density and COD elimination) at higher temperatures. The enhancement of bacterial metabolism and membrane permeability may be to blame for the rise in power density. Furthermore, a high temperature may boost the catholyte's and anolyte's conductivity, which lowers the MFC's Ohmic resistance and raises power density. It has been noted that a minor rise in temperature has no effect on the capacity of the membrane to permeate. The results of the experiments indicate that the relationship between temperature and bacterial activity is exponential. Therefore, the increased bacterial activity may be directly linked to the higher power output caused by a rise in temperature. The development of a biofilm at the anode, which has an effect on the current generation, may be used to measure the bacterial activity. According to the research, the temperature is crucial for the MFC to start up and, in turn, for the early production of the biofilm. Higher temperatures have been shown to reduce the MFC's startup time and promote the production of stable biofilms.

According to a research, the operating temperature ranges for MFCs to achieve greater power outputs are between 30 and 45 C since the bacterial biofilms had their highest levels of catalytic activity at these temperatures. To get the most from the MFC, it is necessary to change the temperature range during operation since certain specific bacterial species can only thrive in a certain temperature range. Additionally, the varied microbial populations in the MFC's anodic chamber may be caused by temperature variations. Once a stable biofilm is formed, the bacteria may modify their metabolism in response to minute changes in temperature.

Feed Rate and Shear Stress

The MFCs may be used in two different operating modes: batch mode and continuous mode. In the batch mode, the substrate is supplied at the start of the cycle; in the continuous mode, it is supplied at regular intervals during the cycle. In the continuous mode of operation, MFCs display hydrodynamic issues that further impair the system's overall performance. Because of this, it is crucial to adjust the flow rate, the following hydraulic retention time (HRT), and the shear stress for MFC operation in order to get the most out of the fuel cell.

It has been discovered that flow rate has an impact on how well MFCs operate in terms of power density and COD removal. According to the investigations, larger flow rates reduce coulombic efficiency, COD removal efficiency, and power production. In reality, the HRT is decreased by increased flow rates. It implies that the bacteria have less time to oxidise the substrate, which lowers the MFC's ability to remove COD. The hydrodynamic strength is a further crucial MFC characteristic. On the anode, it influences bacterial adhesion and biofilm development. Low shear rates, as shown by the experiments, promote the development of thicker biofilms. The presence of persistent bacterial adhesion on the electrode may explain why denser biofilms grow (anode). Different bacterial populations at the anode may develop from MFC operation at various shear rates. Higher shear rates in MFCs have been seen to reduce microbial diversity, mostly causing the production of uniform biofilms.

Applications

MFCs have previously undergone testing in laboratories for a variety of uses, including the creation of hydrogen, wastewater treatment, energy generation, and biosensing. The chapter goes into further information about each application.

Production of electricity

It is clear that the majority of research on MFCs is done for the purpose of producing energy, which is the technology's main use. Table 1 provides several examples of MFC performance for energy production. The microorganisms oxidise the substrate in the MFC's anode chamber to produce protons and electrons, which are then transferred to the cathode through PEM and an electrical connection, respectively. To measure the voltage, the two chambers of the MFC may be electrically linked to a multimeter and an external resistor box.

The power may then be determined using Ohm's law. To increase coulombic efficiency and therefore the MFCs' power production, it is crucial to choose substrates that can totally oxidise into electrons in MFCs. According to a research, Geobacter sulfurreducens may entirely convert acetate into electrons and protons. The design of the MFC, the electrode materials used, the inoculum (pure or mixed culture), the proton exchange membrane, and the operating circumstances all affect the MFC's electrical output. There are already several methods being used to boost the electrical output in MFCs. The improvements in MFCs are mainly concentrated on new MFC designs to decrease the internal resistance of the system, cost-effective electrode materials with high surface area, less expensive cation exchange membranes, modifications of the electrode material with nanomaterials (for example, gold nanoparticles, nickel nanoparticles), and other physical (for example, heat treatment of stainless steel electrode) or chemical (nitrogen-doped electrodes) treatment methods.

Water Waste Management

The ability of the MFCs to treat various industrial, urban, or home wastewaters has been shown. a few examples of MFC performance in the treatment of wastewater. Although MFCs are unable to entirely treat the extremely hazardous wastewaters, they may significantly lower the COD of the wastewaters to fulfil discharge requirements before they are discharged into the environment. The MFCs have shown to remove up to 98% of COD from wastewater. Alternatively, the organically rich wastewaters (carbohydrates, proteins, lipids, minerals, fatty acids,provide the microbial metabolism the substrate it needs to create electrons and protons. Moreover, inoculum may be found in wastewaters. Before and after the MFC operation, the treatment effectiveness of the MFCs may be assessed using the standard wastewater treatment tests (COD, BOD, total solids, and nitrogen removal). By running the MFCs at ideal circumstances, such as mesophilic temperatures, which have been found to promote the COD removal, the COD removal in MFCs may be further enhanced. Additionally, the MFC's fed-batch mode operation is beneficial for achieving high COD removal rates. The coulombic efficiency attained in such instances is rather

poor, ranging from 10% to 30% only. Typically, MFC experiments performed for wastewater treatment are paired with electricity production.

Biosensor

MFC technology is used as a biosensor to detect pollutants in water, in addition to producing power and treating wastewater. MFC is designated as a BOD sensor due to the linear connection between the coulombic yield of the material and wastewater strength. The MFC-based biosensor is superior than traditional biosensors in many ways. As opposed to traditional biosensors, which typically require a transducer, these biosensors are comparably less expensive. Additionally, they may run for a very long time—up to 5 years—without any maintenance. MFC-based biosensors are thus more stable and dependable. Numerous studies have shown that vast BOD ranges (low/high) may be monitored in MFC-based biosensors on the basis of linear correlation.

Bio hydrogen

A microbial electrolysis cell (MEC) may be modified from a normal double-chamber MFC to produce hydrogen. The fundamental workings of a MEC remain essentially the same; however, electric current is now supplied to the cathodic chamber. The anode and the cathode are the other two chambers that make up a MEC. MEC's two chambers are divided by an ion exchange membrane, same as MFC. Exoelectrogens metabolise the substrate and generate electrons and protons in the anode chamber. In MFCs, the protons are transported to the cathode similarly. However, it is thermodynamically impossible for protons and electrons to react at the cathode to form hydrogen. At the cathode, electric current is applied to cause this reaction. Most of the time, >0.3 V is sufficient to meet the electrical need. The MFCs can readily provide such low voltages. In order to meet the electrical demand, the MFCs used to create power may be paired with MEC. It is simple to store the hydrogen created by the MEC, which can then be utilised to generate energy.

Future Directions

Despite ten years of rigorous study on the MFC studies, the MFC technology is still not commercially available. Many technological issues need to be resolved before MFCs can be used in practical applications. The MFCs' inadequate power output is their primary flaw. The high cost of the cathode catalyst, membranes, and electrode materials is one of the major restrictions. In the future, high surface area electrode materials might be used in MFCs to increase power production, whereas (on a large scale) MFCs without PEM could be more cost-effective. To thoroughly cleanse the water, the MFCs used for wastewater treatment still need adequate amendment. For applications using biosensors, little work has been done. The reaction time is longer in MFC-based biosensors since they are biofilm-based biosensors.

MFC Design and Development

An MFC's design and prototype are discussed in this chapter. The construction and analysis of models then employ this MFC. The data is gathered and sent to the dSpace ControlDesk via a

potentiostat. Matlab/Simulink is used in the interface of D-Space. The nitrogen cylinder continuously bubbled nitrogen into the system, including the MFC and the storage containers. On top of the MFC and storage containers are autoclaved syringe needles that allow the oxygen to bubble out. The nitrogen gas is kept sterile via filters. Samples from the MFC were routinely taken in order to demonstrate the impact of the pH buffer. Using a sterile syringe, samples were obtained, and at MSU's Reguera lab, samples were also utilised for the HPLC analysis.

Design and Fabrication of MFC

Anode

In this work, carbon fibre (PANEX 35 50K, Zoltek) brush with two twisted Ti (Titanium) wires served as the anode material in the MFC. The brush (The Mill Rose Company, Mentor, OH, USA) has a 1.989-inch diameter a 2.75-inch brush portion length, and an overall length of 4 inches when the Ti component is included. Because a brush-type electrode has a larger surface area than other carbon felt anodes, it was selected for this experiment. Due to the anode brushes short Ti wire, only a small amount of it came in touch with the medium throughout the trials. The surface area of the active anode brush was estimated to be 0.67 m2. Cathode In many MSU labs, the cathode was created. According to the cathodes were created by coating a carbon cloth coated with teflon with platinum and four diffusion layers. The ingredients needed for this method along with further information. The carbon fabric, carbon black powder, and 40% PTFE solution were used to create the carbon base layer first. By dilution with DI, this 40% PTFE solution was created from a 60% PTFE solution.

Cathode

In many MSU labs, the cathode was created. According to the author, the cathodes were created by coating a carbon cloth coated with teflon with platinum and four diffusion layers. the tools and thorough information utilised in this procedure. The carbon fabric, carbon black powder, and 40% PTFE solution were used to create the carbon base layer first. By dilution with DI, this 40% PTFE solution was created from a 60% PTFE solution (deionized water). The plastic sample vial tube was used to store the whole mixture. To aid in the formation of an uniform mixture, solid glass beads were added to the tube. A vortexer was used to stir the mixture.

Modeling of Microbial Fuel Cells

Simply put, microbial fuel cells are machines that get their energy from the anaerobic digestion of microbes. Understanding the underlying biological and electrochemical processes is necessary to create a model for this behaviour. The suspended microorganism model is the fundamental advancement for understanding how anaerobic digestion functions and provides a more straightforward approach for MFC modelling and control. The biofilm model is important for understanding the electron transportation from the bacteria to the anode in mediator-less MFCs. For the sake of simplicity, the model of suspended microorganisms will be used in this research; however, similar strategy may also be used to the biofilm model.

Animal Kinetics

The most important element for comprehension is the relationship between the active biomass and the major substrates. Mass-balance modelling is a crucial tool since this relationship has to be addressed systematically and statistically for engineering design and operation [16]. The substrate is thought to be the limiting element for bacterial growth (electron donor). The Monod equation accurately describes the relationship between these two processes.

Here, we shall take into account the dynamics of bacteria, including both production and degradation, for a more methodical approach. In the literature, instead of using this technique, researchers often only take into account the synthesis portion of bacterial dynamics, with the decay rate being calculated as the rate at which the bacteria detach from the biofilm. The synthesis rate may be expressed as

$$\mu_{\mathrm{syn}} = \left(\frac{1}{X_{\mathrm{a}}}\frac{dX_{\mathrm{a}}}{dt}\right)_{\mathrm{syn}} = \mu_{\max}\frac{S}{K+S}$$

Due to the nature of suspended microorganisms, these kinetics may be estimated to reflect the complete bacterial community. Xa is the expression for the active biomass (for our model, this will be expressed as X as it represents the overall biomass in the system). No distinction between active and inactive biomasses will be made in this investigation, and all suspended biomass will be denoted by the letter X.

On the other side, bacterial deterioration will occur, as was already described. Active biomass has an energy need for maintenance, which includes cell processes including resynthesis and repair, transport, osmotic management, motility, and heat loss. This has been shown via research on more slowly growing bacteria. Endogenous decay is a common representation used by environmental engineers for the flow of energy and electrons needed to satisfy maintenance requirements. In order to fulfil their demands, the bacteria oxidise themselves. Endogenous decay rate may be expressed as:

$$\mu_{\mathrm{dec}} = \left(\frac{1}{X_{\mathrm{a}}}\frac{dX_{\mathrm{a}}}{dt}\right)_{\mathrm{decay}} = -b$$

Where b is the endogenous decay coefficient and b > 0. The total of growth and decay rates is known as the net specific growth rate of biomass ():

$$\mu = \frac{1}{X}\frac{dX}{dt} = \mu_{\mathrm{syn}} + \mu_{\mathrm{dec}} = \mu_{\max}\frac{S}{K+S} - b$$

Another kinetic that is worth mentioning is substrate usage. The Monod equation also has a connection to the speed at which bacteria degrade the substrate. Despite the fact that cell development results from substrate usage, the Monod equation has the following structure:

$$r_s = -\frac{q_{max}S}{K+S}X$$

Where rs denotes the rate of change in substrate concentration (substrate utilization).

Growth Yield

A biological characteristic called the growth yield enables us to measure the rate at which electron-donor electrons are transformed to biomass electrons during the synthesis of new biomass. Utilization of substrates and biomass expansion are related

$$\mu max = qmaxY$$

Where the growth yield is denoted by Y. Consequently, the net rate of cell growth becomes

$$X_s = Y\frac{q_{max}S}{K+S}X - bX$$

Where Xs is the biomass growth's net rate. The observed growth yield is useful for MFC modelling. Y may be deduced from the two variables, qmax and max. However, it is important to describe how Y is directly measured. The growth yield is calculated by dividing the rate of change in bacterial concentration by the rate of change in substrate concentration. The growth yield then manifests itself.

$$Y = rx/\ rs$$

Where rx is the rate at which the concentration of bacteria changes (the net growth rate of biomass). The growth yield in batch systems becomes,

$$Y = -\frac{\frac{dX}{dt}}{\frac{dS}{dt}} = -\frac{dX}{dS}$$

Since the substrate's consumption rate is changing downward in this case, the negative sign is derived from the equation. For a continuous system, one could simply define a time period and collect samples at the beginning and end of the period (for bacteria or substrate concentrations change), and dividing them might provide an approximation of the growth yield,

$$Y = -\frac{\Delta X}{\Delta S} = -\frac{X - X_0}{S - S_0}$$

Where X represents the change in bacterial concentration over a set time period, and S represents the change in substrate concentration over the same fixed time period. The terminal bacterium concentration is denoted by X, while the substrate concentration is denoted by S. The starting concentrations of bacteria and substrate are denoted by X0 and S0, respectively.

Possibilities of an MFC

Standard and non-standard electromotive forces of an MFC vary from one another. The difference between the standard electromotive forces of the cathode and the anode component in the MFC, provided the cell is operating under normal circumstances, may be used to determine the cell potential. Standard circumstances are those that exist at

- T= 298.15 [K]
- P= 1 [atm]
- Ms= 1.0 [M]

Based on the IUPAC (International Union of Pure and Applied Chemistry) convention, where T is the temperature, P is the atmospheric pressure, and Ms is the chemical concentration for liquid. Any of these three requirements that are broken results in non-standard circumstances, which often include a change in concentration.

The Nernst-Monod equation serves as the foundation for MFCs' OCP. The link between the rate of ED use and the two variables electrical potential and ED concentration is expressed quantitatively by the Nernst-Monod equation.

The literature contains information on the typical potential of chemical reactions.

Thermodynamic Analysis

Half-cell reactions, or the independent reactions taking place at the anode and cathode, may be used to study the processes taking place in an MFC.

For instance, the anode compartment's oxidation of acetate 47 may be represented as

$$CH_3COO^- + 4H_2O \Longrightarrow 2HCO_3^- + 9H^+ + 8e^-$$

Whereas on the cathode side,

$$O_2 + 4H^+ + 4e^- \Longrightarrow 2H_2O$$

Activity

Since the chemical potential of a species relies on the activity of a real solution in the same way that it would rely on the concentration for an ideal solution, activity in chemical thermodynamics is a measure of the effective concentration of species in a mixture. A substance's activity is a value without dimensions. It is customary to consider the activity of pure substances in condensed phases (solids or liquids) to be unity (=1). The reaction quotient has the following form for the reaction aA+bBcC+dD, where the reactants are on the left and the products are on the right:

$$Q = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

Where [C] is interpreted as either the partial pressure in the environment for a gas or the molar concentration of product C if it is aqueous. We can compute 48 the potential on the anode side based on the chemical reaction involving the substrate on the anode side.

$$E_{\text{Anode}} = E_{0_\text{a}} - \frac{RT}{nF}\ln(Q)$$

Where E0a is the anode side reference potential. The potential that exists under typical circumstances such as those described previously in this section. The ideal gas constant, the MFC's temperature, the quantity of electrons transported, and the Faraday constant are denoted by the letters R, T, n, and F, respectively. The chemical reaction that takes place on the anode will have a reaction quotient of,

$$Q = \frac{[CH_3COO^-]}{([HCO_3^-])^2([H^+])^9}$$

Where the concentrations of acetate, bicarbonate ions, and protons are represented by CH3COO, HCO3, and H+, respectively. The potential on the cathode side may also be calculated using the chemical process there,

$$E_{\text{Cathode}} = E_{0_\text{c}} - \frac{RT}{nF}\ln(Q)$$

Where E0c is the cathode side's reference potential. Consequently, it is possible to determine an MFC's total potential as,

$$E_{Cell} = E_{Cathode} – A_{Anode}$$

The fundamental contribution of this thesis is to create a model for a microbial fuel cell based on anaerobic digestion, validate the model using experimental data, and analyse the model using nonlinear systems theory. Based on the premise that bacteria are suspended, the MFC model was selected. The literature was used to determine the kinetics of both bacterial growth and substrate consumption. However, adjustments were made to the differential equations based on the chemical process to account for the activity of ions and protons. The model did not provide a solid foundation for the experimental findings. This suggests that the MFC without mediator must take biofilm dynamics into account. The tests used membrane-less single-chamber MFC structures, acetate as the substrate, and anaerobic pure culture for the bacteria. Data from the trials in the batch mode were used to estimate the model's determinable parameters. The literature was carefully consulted to choose non-identifiable factors. This ensured that the model could replicate both the state variables and the output. The model validation was carried out using the independent data sets. The anode's measured open circuit potential was somewhat lower than the output of the predicted open circuit potential. This happened as a result of the crossover potential loss being excluded from the model. After the model had been verified, a model analysis was carried out. Other researchers have performed analyses of anaerobic 77 digestion that are comparable; however, MFCs have not previously undergone this kind of investigation. A suitable dilution rate interval was found after looking at the stability of the equilibria in the continuous mode. If the input value exceeds the threshold, which was determined after the study, theoretically the biomass will be washed off. This would not be entirely accurate in a biofilm-based model due to the separation from the biofilm, however. Therefore, the suspended component of the bacteria's contribution to the voltage output might utilise this analysis.

The focus of future effort may mostly be on improving model development. In this work, the identification of the model parameters was done using just a few observations from the trials. Tests like electrochemical impedance spectroscopy, cyclic voltammetry, and polarisation may provide crucial information for more precise parameter identification. Extended kalman filtering may be used to estimate state variables like the substrate and bacterium concentrations. In the end, feedback control of the MFC may be explored to stabilise or maximise the power output for the MFC system using the estimated states.

CHAPTER 4

MICROBIAL FUEL CELLS: RECENT DEVELOPMENTS IN DESIGN AND MATERIALS

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With the population rise in recent years, there have been reports of enormous energy use. Based on their source and dependability, energy sources may be divided into three categories: fossil fuels, nuclear, and renewable. The majority of energy utilised today comes from non-renewable sources like nuclear and fossil fuels. Fossil fuel extraction and combustion releases CO2 that was previously contained within the earth's strata and contributes significantly to global warming and environmental damage. Countries from all over the globe are looking carefully for alternative renewable energy sources to address the energy issue and challenges posed by fossil fuels. Fuel cell technology, which uses precious metal catalysts to create energy, is one of the results of such attempts. Fuel cell technology offers several advantages over other energy production techniques, including lower production of hazardous inorganic oxides (CO2, SOX, NOX, and CO), improved efficiency, and others. High material and operating expenses, however, are the main drawbacks preventing the commercialization of fuel cell technology. On the other hand, it is becoming more and harder to provide enough drinkable water to the world's population, which is expanding at an uncontrollable rate. Along with the rate of urban population expansion, the issue of water pollution is becoming worse. If left uncontrolled, it will cause dangerous health problems for the general populace. Low-income nations should invest a significant portion of their resources to ensure access to clean water and sanitary facilities, nevertheless. Even though they can provide clean water, economically developed nations spend a lot of money treating their effluent. For instance, the United States uses 3-4% of its electrical energy—equivalent to the yearly energy use of 9.6 million homes—for the treatment of wastewater. The average daily power use in England and Wales accounts for 1% of the UK's total electricity consumption. Based on the quality and method used, wastewater needs around 0.5-2kWh/m3 of electrical energy, according to data on energy related with wastewater and energy needed for wastewater treatment. Wastewater also includes energy that is 3–10 times more than that needed for its treatment. Wastewater energy is primarily present in three types of energy: heat energy, degradable organic matter, and nutritional elemental energy (N2, P etc). In the form of COD and other nutrients, carbon accounts for around 26% of the chemical energy. Even though extracting heat energy from wastewater is a laborious and sparsely economically viable process, wastewater treatment can be made into a net positive energy (energy generating) process rather than an

energy-consuming one, as well as a choice that produces no environmental pollution, by harvesting the chemical energy trapped in the wastewater. The majority of the world's wastewater treatment techniques now in use are based on the traditional activated sludge process.

Even though the activated sludge process is widely known and a speedy processing technology that produces outstanding results in wastewater treatment, it nevertheless consumes a lot of energy. On the other hand, adding chemical compounds during the activated sludge process adds pollutants to the water and requires significant financial investment. According to a research by the US Environmental Protection Agency from 2008, the aeration process alone during the activated sludge process consumes almost 75% of the energy cost of a wastewater treatment facility. Additionally, the United States spends annually between $300 and $25 billion on public plants and household water treatment facilities, respectively. Additionally, it has been calculated that a typical home wastewater treatment facility needs 0.6 kWh of energy for every m3 of treated water, of which about 50% is lost as electrical energy for the purpose of supplying air to aerobic and anaerobic systems. Anaerobic digestion of organic matter in the form of sludges acquired from primary and secondary treatments is typically how energy from wastewater is recovered. The organic carbon is converted into energy-dense methane gas during anaerobic digestion. Fats are largely degraded into sugars, amino acids, and fatty acids, which are then further digested into short-chain, volatile fatty acids and hydrogen via acetogenesis and acidogenesis processes. This process entails a number of intricate events. The resulting intermediates are ultimately transformed into CO2 and methane (methanogenesis). A significant amount of CO2 is released into the environment as a result of traditional treatment procedures for organic pollutant degradation. Approximately 15,000 tonnes of CO2 are emitted into the environment for every 1,000 tonnes of treated wastewater. Research and development should be directed toward the creation of potentially effective, commercially feasible, and ecologically friendly treatment systems in order to overcome the limitations associated with current wastewater treatment methods.

Microbial Fuel Cells (MFCs): Bio electrochemical systems (BESs) are biological processes that may transform chemical energy of organic matter, ranging from complex lignocellulosic biomass to low strength wastewater, into electrical energy, hydrogen, or other useful products. In contrast to its competitors, such as traditional fuel cells, BESs may operate well in moderate settings and can employ a variety of substrates without the need of precious metal catalysts. BESs are primarily divided into two kinds based on the type of biocatalyst used: I Microbial Fuel Cells (MFCs) and ii) Enzymatic Fuel Cells (EFCs). BESs may be further divided into microbial fuel cells (MFCs), microbial electrochemical cells (MECs), microbial desalination cells (MDCs), and microbial solar cells (MSCs) depending on the application (MSCs) a certain kind of bacterium may emit electrons extracellularly (exoelectrogens) while breaking down organic material, as he found via his investigations. Later, it was assumed that the microbes' breakdown of organic substances accompanied the production of electrical energy. Through a specific BES known as microbial fuel cell technology, the development of such electron-releasing microorganisms' biofilm enables sustainable bioelectricity production as well as the simultaneous removal of

organic carbon from waste water. The MFC is a small reactor that uses the metabolic activity (anaerobic oxidation) of microorganisms to produce energy on demand from biomass. MFCs are discovered to be viable tools for recovering bioenergy from wastewater while simultaneously cleaning the wastewater, lowering operating expenses associated with treating wastewater using traditional techniques. A proton exchange membrane separates the anode in the anodic chamber from the cathode in the cathodic chamber in a basic MFC setup (PEM). MFC works on the basis that biocatalysts oxidise organic substrates in the anodic chamber, liberating protons and electrons as well as creating CO2 in the process. Protons are simultaneously moved from the anode chamber to the cathode chamber through PEM as the anode collects electrons and transmits them to the cathode via an external circuit. Water is created at the cathode when protons and oxygen are combined with electrons.

The electricity produced from wastewater using MFCs is pure and usable right away. In contrast to the hydrogen and methane generated by the anaerobic digestion process, which cannot be utilised directly, it does not need additional purification, separation, or other conversion procedures. Because MFC technology can run under normal settings and generates electricity without causing pollution, it is ecologically friendly. Even though CO2 is one of the byproducts of microbial oxidation, the process is regarded as carbon-neutral since the substrates use CO2 for photosynthesis throughout their life cycles. Depending on the strength of the effluent, MFC may create energy at a rate of 1.43 to 1.8 kWh/m3. Despite this, only uses 0.024kW, which is about an order of magnitude less than what the anaerobic digestion process uses (0.3kW). The numbers indicate that MFC has a larger potential for cost-effective wastewater treatment and the production of renewable energy since it uses around 10% less energy to operate than the activated sludge method. Even though MFC technology can effectively cleanse wastewater while simultaneously producing electricity, it still has difficulties moving outside of the lab for field applications or commercialization. MFC technology is being constrained in direct field applications by a variety of problems, including the expense of the electrode materials, the need for precious metal catalysts, limited performance, low power densities, and expensive PEMs. Even though these problems can be solved in the lab, pilot size studies are eventually required to evaluate the effectiveness and durability of materials on a wide scale, particularly when working with wastewater that doesn't have stable conditions over time (composition, temperature, etc.). Many research have been published in recent years, with the majority of them concentrating on how to make MFCs as efficient as possible while also lowering the cost of the electrode materials. The goal of this study is to discuss the most current developments in electrode materials and MFC design. 3. Improvements in MFC design: Rather than only the activity of the biocatalysts, the materials from which an MFC is constructed have a significant impact on its performance. In an MFC, electricity is generated by a number of steps, including microbial catabolism of organic matter, electron capture by the anode, reduction of the electron acceptors at the cathode, and simultaneous proton transfer from the anode to the cathode through PEM. All of these processes control the effectiveness and performance of an MFC, and many research have been documented that address the problems posed by these processes. In addition to this, reactor configuration also affects MFC performance overall.

Microbes are used in the anodic chamber to perform anaerobic oxidation of organic material, and the cathode chamber is used for oxygen reduction, which is the most frequent process. Basic reactors known as two chamber MFCs are often used to investigate electrode materials, microbial activity, and parameter tuning. This fundamental design cannot lower the energy costs associated with treating wastewater since it continuously sparges air into the cathode chamber, a process that uses energy. In MFCs, when reactor volume is increased, the surface area to volume ratio declines, which affects the reactions at the electrode surface. Therefore, the deployment of bench scale designs to large-scale field applications will necessarily lead to lower power densities and performance. It is commonly accepted that over potentials and energy losses with larger size are to blame for the lower power densities. As a result, improvements in reactor designs could facilitate the use of MFCs in waste water treatment. The configuration of a single chamber MFC was later based on Lui's research on hydrogen fuel cells, which suggested that direct cathode bonding to PEM would enable direct contact of airborne oxygen with the cathode. An anode chamber, a gas diffusion layer that separates the cathode from the anode chamber, and a pathway for passive oxygen delivery to the cathode make up a single chamber MFC. Unlike the two chambered MFC, this design does not need an energy-intensive aeration phase. Later, as illustrated in Figure 2, a single chambered tubular MFC was created for continuous flow operations using ferric cyanide as the catholyte and granular graphite as the anode material. Acetate and glucose produced maximum power outputs of 90W/m3 and 60W/m3 (net anodic compartment), respectively. With residential wastewater, the reactor was able to produce 48W/m3 (net anodic compartment) of power density and display 96% Coulombic efficiency. The findings suggested that the proposed design might be used to the treatment of waste streams containing high levels of volatile acids, such as the effluent from anaerobic digesters.

Setup geared for cathode fouling, particles removal, nutrient removal, COD, and BOD utilising household wastewater as anodic fuel. The reactor with 12 anodes and cathodes could produce 380mW/m2 of power density (at a loading rate of 0.19-0.66kg/m3/day) and remove 80% of pollutants (at 8h hydraulic retention time). Despite cathodic fouling brought on by the deposition of Ca and Na, metal doped MnO2 (Co-MnO2, Cu-MnO2) cathodes performed noticeably better than Platinum based cathodes employed spacers to minimise the size of the MFC reactor without compromising performance in an effort to construct the MFC more compactly. The company was able to create 97326mW/m2 with the use of 1.5mm plastic expanded spacers, which was comparable to the outcomes produced without the use of spacers. Additionally, it was claimed that the oxygen leak into the reactor caused power densities to decrease when the spacer's thickness was lowered to 1.3mm. The company has come to the conclusion that using spacers can maintain pressure-driven airflow while lowering the cost and reactor capacity. Ahn Y and Logan B E [19] have created a single chamber MFC with graphite fibre brush multi anodes and an air cathode in an effort to construct scalable MFCs. Separator Electrode Assembly, which combines the bioanode and air cathode with a separator on each side to further reduce the electrode gap (SEA). With the planned reactor and continuous flow conditions, the group was successful in attaining 0.200.04V within 8h of the hydraulic retention period and the greatest Columbic efficiency of 85%. Additionally, it was shown that this multi electrode design may

help the creation of consistent current and power while operating in continuous mode. Later, a hybrid MFC was created by fusing with a membrane bioreactor (MFC-MBR) for simultaneous wastewater treatment and ultrafiltration, producing clean water that is immediately usable.

The design's conductive ultrafiltration membrane served as both the cathode and the membrane. When 0.1 mm residential wastewater (prefiltered) was employed, the reactor could effectively remove 97% of COD, 91% of all bacteria, and 97% of Ammonia and nitrogen. The findings have led to the conclusion that wastewater treatment using the MFCMBR reactor is possible at the cost of little energy.A two stage MFC adjoined anaerobic fluidized bed membrane reactor (MFC-AFMBR) with lab scale capability was created in another work to solve the problem of producing high quality water from home wastewater with little energy input. Without performing any membrane back washings, the reactor system was operated continuously for 50 days with residential wastewater (total COD 21011mg/L) at room temperature. Total suspended particles were nearly entirely eliminated after 9 hours of hydraulic retention. The study's findings led the team to the conclusion that even in mild weather, the MFC-AFMBR combination system can create high-quality water from household primary effluents, which lowers the cost of the water treatment process. In order to reduce the time needed for the treatment of huge amounts of wastewater and maintain power production even when one or more of the cells failed to function, a multi chamber MFC with four anode chambers and a cathode chamber separated by a membrane was developed. The MFC was constructed such that the four anodes and cathode chamber operate as separate cells linked in parallel. Utilizing wastewater with a COD content of 8720 mg/L, a peak power density of 135.4 mW/m2 was attained utilising a graphite electrode and potassium ferricyanide catholyte (100 mM). Based on the findings, it was determined that the design may be scaled up to provide high power in a small area.

Electrode components

In an MFC, electrons are typically generated at the anode via the anaerobic oxidation of fuel by microorganisms. They are then carried through the external circuit to the cathode, where the oxidising agent is reduced. Electrons flow continuously as a result of the co-occurrence of the oxidation and reduction processes, creating electricity. It is thought that a number of elements, including membranes, mediators, electrode arrangement, electrode materials, and biocatalysts, affect how well an MFC works. Electrode materials have the most impact on performance out of all the above elements because they may restrict the amount of power that can be produced due to changes in electron transport between microorganisms and electrodes. The choice of electrode base materials is an important step that requires the greatest caution to avoid corrosion. Due to galvanic corrosion, copper, for instance, seems to provide better power densities when used as electrodes. On the other hand, it has been discovered that copper ions have antimicrobial properties. In a similar manner, the utilisation of stainless steel electrodes is dependent on the alloy's chromium content.

Different electrode properties, such as biocompatibility, active surface area, high conductivity, and electrode surface characteristics, among others, can influence an MFC's performance.

Although much study has been done to identify the materials for MFC, the need for less expensive electrode materials is preventing the MFC technology from being used outside of the lab. The goal of research has shifted in recent years from developing electrodes that can support the most bacteria to developing electron collectors. Metals that can transmit electrons over greater distances are preferred in the present situation. Platinum and other precious metal catalysts may enhance the production of electricity, but their use on a wide scale would incur unaffordable material costs. The development of affordable electrode materials (anode/cathode) for MFCs is the main topic of this study.

Performance of the anode, which serves as a hub for important bioelectrochemical processes and a mediator of electron transport from exoelectrogens to electrode, largely determines an MFC's efficiency. As a result, emphasis must be placed on the anode's construction and materials. The performance of MFC is significantly impacted by a number of anodic characteristics, including surface area, chemical resistance, lifespan, and electrical conductivity. Numerous experiments have been conducted using low cost basic electrode materials treated under various circumstances to enhance MFC performance while spending little money. Phosphate buffer and ammonia gas were applied to carbon cloth anodes in an effort to enhance MFC performance. By increasing solution conductivity and electrode surface charge, the treatment significantly improved performance. These two therapies together generated an increase in power.

Compared to MFC with an electrode without the treatment, production increased by 48%. This innovative method produced a power density improvement of 1970mW/m2 . Additionally, a cheap carbon mesh was investigated as an anode material. Compared to untreated mesh (893mW/m2) and costly ammonia gas-treated carbon cloth (988mW/m2), individually heated and high temperature ammonia gas-treated carbon mesh anodes achieved 922mW/m2 and 1015mW/m2 power densities, respectively. The improved N/C atomic ratio on the surface of the mesh electrodes was shown to be responsible for the gains in power densities. From the findings, it has been concluded that carbon mesh treated with ammonia gas may be a viable alternative to expensive carbon cloth and paper electrodes. Three alternative processes—heating, soaking in acid, and combining acid soaking and heating—were applied to carbon fibre brushes. The power output of carbon mesh anodes that have undergone both acid and heat treatment has risen by 34%, exceeding the respective gains of acid soaking and heat treatment of 25% and 7%. Anodes treated with this new combination of heat and acid soaking performed better than anodes treated with heat and ammonia gas. Contrarily, it has been determined that the suggested methodology would not be a method that can be used to build cost-effective MFC for real-world applications since it uses ammonia gas and heat treatment, both of which are energy-intensive. The improved performance of the MFCs is also a result of the anode materials' surface alteration. To create a bifunctional anode, a carbon fabric was altered by covering it with biologically reduced Palladium nanoparticles. MFC has shown enhanced power density and coulombic efficiency by 14% and 31%, respectively, using this surface-modified carbon cloth. The improved performance could be brought about by the biogenic palladium's lower charge transfer resistance. An anode made of vertically grown multiwalled carbon nanotubes and nickel silicide was created

by Mink J. E. *et al.* In a microsized MFC with a 1.25 L capacity, this manufactured electrode generated current densities of 197 mA/m2 and power densities of 392 mW/m3. The outcomes were far better than those obtained with a simple carbon cloth. The increased surface to volume ratio for microbial adherence, electron transfer, and low resistance by the nickel silicide contacting material, it was stated, were responsible for the improved performance. It has been shown that the related costs may be significantly decreased since the procedure does not need any costly materials to construct MFC. An MFC's anode was made of carbon paper that had been treated with polyethyleneimine and multiwalled carbon nanotubes using a layer-by-layer assembly method. Due to the modification's provision of a three-dimensional network structure for bacterial adhesion, interfacial charge transfer has been reduced.

In comparison to a carbon cloth electrode that had not been changed, this improved anode could generate 20% more power densities. As anode materials in MFC applications, stainless steel meshes covered with heat-treated and unheated goethites extracted from mine mud were employed. When compared to a traditional stainless steel mesh electrode, goethite catalyst-based electrode recorded higher powers and coulombic efficiencies. Reduced mass transfer losses and quicker electron transport between the biocatalysts and electrode were the causes of the increased performance. Due to its lower cost, goethite may provide MFC electrode materials that are both economically viable and have respectable power output. Even though it demonstrated greater performance, using carbon cloth or graphite granules as anodes in lab size MFC still had to overcome scaling-up difficulties. Due to its high cost, carbon cloth cannot be employed in large-scale systems. Similarly, it would be challenging to apply proven carbon cloth arrangements from lab settings to huge scales. Similar to graphite, poor porosity makes graphite granules susceptible to clogging. Since they will be in continual touch with the electron transfer, materials utilised in biofilm based bioreactors (MFCs) for wastewater treatment need to have a high structural strength and be free of clogging. In addition, in every operational circumstance—from open-flow to saturated flow systems—the material supports should still be intact with the biofilm. Therefore, it is not practicable to employ such support materials.

Materials for the cathode: In a multi-fuel cell (MFC), the cathode chamber is thought of as an electron sink where oxygen is converted to water. At the interface of the three phases of air, liquid, and solid in the cathode chamber, oxygen is reduced. An electrode support, catalyst, and air diffusion layer make up a typical MFC cathode. However, a possible cathode should have the qualities of high electric conductivity, high mechanical strength, and an efficient catalytic nature. Electrode materials utilised for anodes may also be employed for cathodes. The most typical operating conditions for MFCs are moderate operating temperatures and a neutral pH. Under these circumstances, the rate of oxygen reduction is very low, increasing over potentials and limiting the performance of an MFC as a result. The cathodic carbon based support materials need to be modified with additional catalysts for strong cathodic reactions in MFCs. Platinum is the most often used cathode catalyst because of how well it reduces oxygen. The practical uses of MFC technology are being restricted by the employment of pricy metal catalysts as cathode materials. Platinum catalysts are expensive and more prone to clogging when used with bad

water. Many research initiatives have been made to lower the cost of cathodic catalysts by identifying less expensive platinum substitutes without compromising performance. This review talks about the cathode materials that are economical and how they affect MFC performance. In one investigation, the polytetrafluoroethylene base layer was followed by several coatings of polytetrafluoroethylene as a diffusion layer, covering the carbon fabric on one side with a combination of carbon powder and polytetrafluoroethylene. Platinum catalyst was applied to the carbon cloth's water-facing side. In a single chamber MFC, carbon cloth that had been thusly created served as the cathode. When compared to carbon cloth with base layer alone, the MFC demonstrated improved power densities (42%) and coulombic efficiencies (200%) Four coats of polytetrafluoroethylene are said to have generated the highest power density because they can lower water losses via the cathode. The catalytic activity of metal porphyrine and pthalocyanine-based cathode catalysts toward oxygen reduction in MFCs has been investigated. At neutral PH, an iron pthalocyanin-based cathode has shown higher oxygen reduction rates than a platinum catalyst. Ketjenblack carbon has reported improved activity when compared to Vulcan XC carbon owing to the increased surface area of Ketjenblack carbon. Iron pthalocyanin-Ketjenblack carbon produced a maximum power density of 634 mW/m2 at neutral PH, exceeding the value of pricey Platinum catalyst (593 mW/m2) under like circumstances.

The research have shown that the macrocyclic catalysts based on transition metals are less expensive and may be utilised effectively in large-scale MFCs for commercialization of the technology. Activated carbon air cathode was created by Zhang F, *et al.* as a cost-effective alternative to platinum catalyst. Cold pressing polytetrafluoroethylene and activated carbon around a nickel mesh current collector produced the cathode. The company claims that the highest power density of the thusly manufactured cathode is 1220mW/m2, which is much greater than the maximum power density of the platinum catalyst (1060mW/m2). The group came to the conclusion that activated carbon-derived cathodes from metal mesh collectors may be employed as effective and financially viable air cathodes in MFCs. According to another company, increasing the activated carbon loading has improved the power densities of air cathodes made of polytetrafluoroethylene and activated carbon. This could be as a result of the activated carbon load's decreases in Warburg impedance, contact resistance, and charge transfer resistance. Although cathodes degraded following 1.5–5 months of use, they nevertheless showed power densities that were equivalent to those of platinum-based cathodes. Commercial activated carbons from various sources were ammonia gas treated in a separate research to enhance their functionality as oxygen reduction catalysts in MFCs at neutral PH. Because of the decrease in oxygen content and increase in nitrogen groups on the surface of the catalyst material, ammonia gas treated activated carbon catalysts have shown superior results than untreated activated carbon have created an activated carbon-iron ethylenediaminetetraacetic acid cathode with stainless steel mesh current collector to improve the cathode catalytic activity of activated carbon. The cathode that was thus constructed has a power density that is ten percent higher than plain activated carbon and on par with platinum catalyst-based cathodes (158080mW/m2). The electrode is reportedly more resilient than platinum cathodes. The active pyridine, quaternary nitrogen, and iron groups were discovered to be responsible for the improved performance of the carbon-Iron

ethylenediaminetetraacetic acid cathode. According to the research, pyrolyzing activated carbon with iron ethylenediaminetetraacetic acid would increase the catalytic activity of commercially feasible activated carbon cathodes. Later, to improve the performance and lifespan of activated carbon cathodes, combined activated carbon with carbon black. After five months of continuous operation, it was observed that the maximum power density had decreased by 7% for the carbon black-based cathode but by 61% for the platinum catalyst. By varying the configurations the power densities of carbon cloth. When a single carbon cloth tube was first used, it produced a lower power density than a flat carbon cloth electrode. It was observed that when the number of concentric tubes rose, the cathode surface area also increased, increasing the power density. The business used two carbon cloth tubes as the cathode and was able to achieve a power density of 83mW/m3. It was also claimed that the power density rose to 128mW/m3 when the cathode was wrapped around the anode like a tubular MFC.

Finally, it has been determined from the data analysis that the power density is unaffected by the cathode geometry, whether it is tube-shaped or flat. Instead of geometry, the cathode surface area is what determines the power density. Catalysts and catalyst binders are often attributed with the majority of the expenses of MFC cathodes. Four carbon-based materials—granular activated carbon, granular semi-coke, carbon felt cubes, and granular graphite—were used to create packed bed air cathodes, and costly binders and catalysts were omitted from the manufacturing process. The maximum power density was recorded by a packed bed air cathode made of granular activated carbon, which measured 676 93 mW/m2, and the lowest by a cathode made of carbon felt, which was 60 43 mW/m2. In an effort to expand the surface area, it was found that power output dramatically decreased when the quantity of granular activated carbon and semi-coke was increased. The oxygen reduction rate was decreased by using carbon materials with thicker layers because oxygen transport was constrained. According to the findings, packed bed air cathodes made of granular semi-coke and activated carbon may be employed as prospective, affordable replacements for platinum air cathodes. As a cathode material, mesoporous nitrogen-rich carbon produced at various temperatures was employed to solve the problem of pricey platinum catalyst. Mesoporous nitrogen-rich carbon cathodes produced 14% less power than platinum cathodes, although they only had a 7% drop in power after one month of operation, compared to 11% for platinum cathodes. To increase the rate of oxygen reduction, experimented with nitrogen doped ionothermal carbon aerogel as the air cathode. Due to its extremely porous nature, high surface area, and huge pore volume, this aerogel-based electrode produced a 1.7 times greater power density than typical platinum electrode as well as the majority of oxygen reduction catalytic air cathodes. According to the company, the suggested aerogel electrode might be a useful, affordable air cathode catalyst for MFC applications. Massive research has been done in recent years to improve MFC performance via design and electrode material improvement. The technique is still having trouble becoming commercially viable, however. In conclusion, careful study is required to produce the hardware and commercially viable materials for MFCs that might permanently address the scaling up concerns as well as the production of maximum power.

This study outlines the many reactor designs and cathode and anode materials that have been created to raise the efficiency and lower the price of MFCs. Reactor design is inferred to have a major impact on MFC performance. In terms of power output and endurance, carbon-based electrode materials may compete with costly catalysts. From a cost viewpoint, expanding the uses of MFCs will need more research into new materials or advancements in current electrode materials. Additionally, MFC coupled bioreactor systems may provide economically feasible outcomes in wastewater treatment. From bioelectrochemical systems to microbial fuel cells, MFCs are by far the most investigated and reported BESs, as was previously indicated (Fig. 1a and b). The fundamental reason for developing this technology is the possibility to replace the current expensive wastewater treatment systems with one that can be self-sustaining or even produce a net amount of energy that is positive while removing contaminants. a displays a broad schematic representation of the microbial fuel cell. Parallel to this, a number of additional intriguing bioelectrochemical systems have been created.

The Microbial Electrolysis Cell (MEC), among them, is one of the most intriguing and thoroughly researched. It was initially presented in 2005. With more than 4000 citations, related papers on MEC rose with time, reaching 141 in 2016. Here, a picture of a microbial electrolysis cell is shown. Since the majority of the energy originates from the chemical energy derived from substrates oxidised at the anode, MEC only needs a little amount of external energy to conduct electrolysis to create hydrogen at the cathode. Therefore, using bioelectrocatalysis in conjunction with other low energy power sources, hydrogen may be created with a low energy usage. Since hydrogen is a valuable gas generated and is essential for the developing hydrogen-energy economy, MEC is of special significance. A number of advancements and improvements in cell design, membrane removal, use of microbial catalysts, or Pt-free catalysts, have successfully been proved to boost hydrogen generation. Recently, a large-scale application including the hydrogen synthesis from vineyard effluent has also been shown. Three cases from recent times dealt with MECs that were quite large size (order of magnitude of one litre and higher). This made it evident that scientists intended to scale up MEC systems for use in practical applications while researching the drawbacks of expanding reactor sizes. Other BESs have most recently been created with trigenerative and cogenerative goals in mind. The development of a microbial desalination cell (MDC), which has the intriguing potential to clean wastewater, produce energy, and desalinate water all at once, is one of them.

The various EA biofilm substrates

Heterotrophic bacteria may oxidise a broad range of organic molecules (substrates) in order to provide energy that is necessary for their growth and metabolic maintenance. Then, the substrate acts as a source of carbon-energy for the bacteria. Any type of organic material, including simple molecules (glucose, acetate, carbohydrates, etc.) and complex compounds (cellulose, molasses, etc.), as well as organic matter found in wastewater treatment plants, agricultural wastes (dairies, manure, etc.), domestic wastes, and any type of substrate that can be fermented, can be used as a substrate by EA biofilms. The employed substrates are described in two reviews. The

composition, properties, and concentration all affect how well the organic substrate is converted into energy via bioelectrochemistry. The composition of bacterial populations that develop inside EA biofilms is influenced by the nature of the substrate(s), as is the bioelectrochemical performance, such as the current density or the coulombic efficiency of bioanodes. Acetate is often used as the primary substrate for the generation of electrons at the bioanode in BES research. For several metabolic routes based on the oxidation of complicated carbon sources, such as acetic fermentation from ethanol (vinegar synthesis), the Entner Doudoroff route from glucose, and acidogenesis from complex organic matter, acetate is a simple substrate and the end product of fermentation (protein, saccharides, lipids, etc.). When compared to butyrate, propionate, or glucose, acetate has reportedly been shown to be a superior substrate for the production of electricity. In experiments involving electro-microbial systems, glucose or lactate are two often utilised substrates. However, the original EcoBot, which was fed glucose, also utilised the following substrates: arabitol, cysteine, ethanol, propionate, fumarate, starch, fake wastewater, etc. The study is focused on using actual raw effluent at the bioanode, but, in other more practical research, so that it may be more closely related to industrial uses or processes.

In many ways, microbial fuel cells are still regarded as a scientific wonder from where they first emerged. Researchers very recently discovered that this actually is a platform technology, which basically implies that MFCs may be used in a variety of applications. MFCs are appropriate for distant area access through robots or remote power generation since they are the only technology that can create energy from waste without the input of external or extra energy. The MFC is one of the very few technologies that can directly capture the microbial reaction and metabolism and generate this as an analogue electrical signal. Microbes are incredibly sensitive, specific, and accurate "sensors" of their own environment. As a result, the device has built-in sensing capabilities that work in any environment and are friendly to the desired bacteria. It has been shown that the catholyte, a liquid found within the cathode compartment, which possesses antiseptic qualities, is produced or synthesised when ceramic materials are used as chassis and ion exchange membranes. Pathogens are eliminated only by being exposed to the MFC environment due to the formed biofilm's antagonistic relationship with any non-electroactive pathogenic organism. The last two examples may both enhance cleanliness, which is particularly important for developing world nations and areas. For many people, and undoubtedly from the standpoint of funding and development, MFCs are still in their infancy, but it is through ongoing research into such technologies that we may find solutions to our environmental problems on a global scale, and MFCs will play a part in the future for the planet and the following generations. We must take action to make it happen.

With the need to reduce and manage carbon emissions expanding, sustainable energy sources and more effective energy usage are becoming more important. The demand for energy is rising quickly across the globe. In order to meet this problem, significant effort is being made in hydrogen fuel production and renewable energy-based technologies. As a result, technologies are being developed that, for instance, employ wind or solar energy to electrolyze hydrogen. Hydrogen may also be created via solar thermochemical processes. The question of whether this

is a practical way to store energy (as hydrogen) vs whether the new battery technology is more suitable is still up for dispute. Alternative techniques for creating hydrogen (or methane) from plants and biomass include fermentation, photobiological processes, and the utilisation of algae [7]. None of these methods can currently match the price of producing hydrogen from fossil fuels. It is doubtful that any one method can completely meet the prospective needs for hydrogen (or electrical) energy. Many of these processes, including the conversion of carbohydrates to hydrogen, have efficiency constraints. Therefore, in order to flourish and function alongside other energy supply lines, more effective alternative techniques must be found.

Due to the promise of fuel cells to provide a constant supply of clean and effective electricity from hydrogen, research and technology development (R&TD) in the field of fuel cells has increased significantly concurrently with that of hydrogen production. Although highly valuable, this research and development falls short of meeting the expanding need for sustainable energy production since fuel cells mostly consume hydrogen supplied from hydrocarbon sources. But there are plenty of'renewable' carbon-based potential fuels on the planet, both naturally existing and created via industrial processes in the form of wastes or by-products. Although research is being done on how fuel cells may be used inadvertently to benefit from some of these possible fuel sources, for instance, via the purification (and reformation) of biogas, many carbon sources are not yet suitable fuels for fuel cells. Currently, the majority of these carbon-based compounds are disposed of as garbage. In contrast, biofuel cells (BioFC) have the ability to cheaply and directly use a variety of carbon sources, such as urea, trash, and sludge.

The ability of biofuel cells to convert easily accessible fuels (fuel types) from sustainable sources into hydrogen or electrical energy gives a chance to significantly reduce the world's energy needs.

CHAPTER 5

MICROBIAL FUEL CELLS' ELECTRON ACCEPTORS

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Microbial fuel cells (MFC) have lately gained increasing attention due to their outstanding potential for safe and effective wastewater treatment and contaminant removal. Pollutants may generally be removed either as electron donors through microbially catalyzed oxidation at the anode or as electron acceptors through reduction at the cathode. Some contaminants can also serve as electron mediators at the anode or cathode. Despite past studies exhaustively assessing them, cathodic electron acceptors or mediators have not been as well described as electron donors. Because it is readily available and has a high oxidation potential, oxygen is widely used as an electron acceptor.

A bioelectrochemical device called a microbial fuel cell (MFC) uses electrons produced by the anaerobic oxidation of substrates to produce energy. A proton exchange membrane separates the two components of the MFC, which are called the cathode and the anode (PEM). In the anode compartment, organic compounds such as acetate, glucose, lactate, and ethanol undergo anaerobic oxidation, which results in the release of protons, electrons, and carbon dioxide. In this instance, the PEM and an external circuit are used to transmit protons and electrons from the anode chamber to the cathode chamber. An electrical current is created by this electron transport from the anode to the cathode. Oxygen serves as the terminal electron acceptor in the MFC structure. A PEM membrane separates the two chambers of a conventional MFC, which include an anode and a cathode. By way of an external circuit, electrons are moved from the anode compartment to the cathode compartment, where they react with protons or oxygen to generate water.

Nitrogen Species

One of the frequent forms of nitrogen that is extensively present in waterways is nitrate, which is responsible for several harmful environmental and health issues that endanger both human and animal health. In this regard, the US and Europe have nitrate in drinking water limits of 44.43 mg/L and 50 mg/L, respectively. Nitrate may now be used in MFCs for denitrification and energy production thanks to the use of biocatalysts. The viability of using nitrate as an MFC cathodic electron acceptor. In this work, microorganisms carried out denitrification in a tubular reactor without the need for energy.

Manganese Oxide

According to studies, manganese dioxide makes an effective cathode and catalyzer for battery or alkaline fuel cells. Electrons can be moved from the cathode to an electron acceptor via the

MnO2/Mn2+ redox pair. Because using oxygen directly can be challenging (because of its limited solubility), using electron mediators between the cathode and oxygen is a more effective alternative.

Bio-Cathode Classifications

Many materials, including oxygen, transition metal complexes, inorganic salts, or carbon dioxide, are now employed as the biological cathode's ultimate electron acceptor. Numerous studies have shown that microorganisms are crucial to the bio-cathode electron-transfer process, even though the mechanism behind biological electron transfer is not entirely understood.

Oxygen as the Terminal Electron Acceptor

Oxygen is the most often used terminal electron acceptor due to its high redox potential (+1.229 V) but also inexpensive supply cost. Microorganisms either directly transport electrons from the anode to the oxygen or help oxidize transition metal compounds to do so.

Oxygen as A Direct Electron Acceptor

Coastal sediment MFCs, which are primarily used for providing analysis and monitoring equipment, is one of the first MFC uses. The performance of the cells was affected by marine life, according to 1997 research on seawater batteries. Slimes made of bacteria that grow on the cathode surface accelerate the reduction of oxygen. The on-load cell voltage rises from generally 1.2 to 1.6 V as a result of the cathode's increased catalytic activity. Different kinds of sludge and silt were combined in the MFCs system to create a cathodic inoculum with adequate microbial diversity. An MFC turns energy present in a substrate that can be bio-converted into electricity. By switching from a soluble acceptor, like oxygen or nitrate, to an insoluble one, like an MFC anode, bacteria may.

Simple Enhancement

Numerous elements, such as the cathode material, electrode spacing, electrode surface, design, buffer solution characteristics, and substrate concentration, have been shown to affect the power output of MFCs. To accomplish the cathodic reaction rate in abiotic cathode MFCs, for example, expanding the cathode surface area while preserving a relatively modest area of the anode has been frequently advocated. To boost the production efficiency of bio-cathode MFCs, individuals can use more strategies and simpler techniques. Increasing the cathode surface area in bio-cathode MFCs would presumably allow more catalyst bacteria to reside there, which would lead to a decrease in electrode over potential and an increase in cathode potential and power production. Additionally, the initial adhesion or subsequent colonization processes are significantly influenced by the features of the surface functional group or surface roughness.

Applications

Due to the obvious potential for cost reductions, waste elimination, and operational sustainability, bio-cathodes are a welcome development in the effort to deploy MFCs for

practical applications, including wastewater treatment or sediment MFCs. Numerous studies on the use of abiotic MFCs in the production of electricity, hydrogen, and the removal of organics from wastewater have been conducted. According to research thus far, the primary uses of bio-cathode MFCs are for nitrification and simultaneous electricity production.

Authenticities of the Material

Electrode characteristics including surface roughness, porosity, conductivity, surface area, or hydrophobicity are crucial factors in determining the development of biofilms and, subsequently, the effectiveness of biocathodes.

Roughness of the Surface and Biocompatibility

The degree of surface roughness may have a significant impact on the heterogeneity of biofilms on surfaces. The ability of biofilms to grow and endure on biocathode surfaces directly affects the process of electron transfer. In addition to the mass transfer of the substrate and product, structural heterogeneity and microbial characteristics might affect the biofilm's activities. Graphite has a rougher surface than stainless steel, which encourages the formation of biofilm, while the stainless steel electrode had a far greater current density. Due to the biofilm structure, solitary cells and small local colonies generate current at a greater density than dense colonies.

Microbial Characterization Methods

Several methods, including flow cytometry, microscopic analysis, transcriptomic or metagenomic analysis, proteomic or metaproteomic analysis, metabolomics and meta-metabolomics, and genomic as well as metagenomic analysis, are used to investigate microbial interactions and metabolism in microorganisms. Combinatorial metagenomic or metaproteomic research may be highly beneficial for learning more about the functions of the elements of biofilms. The information obtained by metaproteomics may be utilised to study the electron transport processes in biofilms formed by electrode-grown cells.

Biofilm Characterization

Numerous techniques, such as microscopic, biological, or chemical ones, may be used to describe biofilm on electrode surfaces and study its population. Each strategy has a different degree of taxonomical precision and a distinct application objective. Identification, appraisal, and characterisation of microorganisms based on functional genes or microbial activity and community composition are the three degrees of detection for each approach.

Finding Microorganisms

To examine microbial communities, one may use either independent research or approach depending on culture. Different methods are used to separate certain populations. After cultures are serially diluted or plated on a solid medium, single colonies are then further grown as a pure

culture. Methods that are not reliant on culture are also necessary for the complete detection of microbial populations. Another efficient method for counting and identifying the bacteria in a biofilm is fluorescence in situ hybridization.

Control of Microbial Activity and Functional Genes

In addition to the makeup and structure of a biofilm, it is also important to fully understand the roles that these microorganisms play in the environment as well as their activities. To do this, methods based on the metatranscriptome or metaproteome are used. Metatranscriptomics allows for the high-throughput assessment of the levels of gene expression in diverse microbial populations. It is believed that combining metaproteomic or metagenomic studies might assist uncover the elements and their activities in a biofilm.

BESs are a growing technology that are still in their infancy. A key component that affects the development and use of the technology is the cathode. In BESs, abiotic and biological cathodes have both been studied. Employing biocathodes provides a number of advantages over abiotic cathodes. A crucial element of the design that affects efficient electron transport and, therefore, power production is the biocathode. To progress the technology, one must have a thorough understanding of how microbiological factors and material characteristics affect electron transport. Investigations into electron transport channels have been explored using a variety of chemical and biological methods.

Fuel cells and biological fuel cells,

Standard Fuel Cells

The fundamental chemical energy in fuels may be directly converted into electrical energy using fuel cells, which are electrochemical devices. William Grove made the first fuel cell demonstration in 1839 by electrochemically producing hydrogen and oxygen in an acid electrolyte using platinum electrodes. The resulting hydrogen and oxygen were then utilised to create a little current (and voltage).

Saying that the fuel is being burned in a straightforward reaction without the production of heat is one straightforward approach to think about how a fuel cell operates. Fuel cells are not constrained by the thermodynamic constraints of traditional heat engines, specified by the Carnot efficiency, since the intermediary processes of creating heat and mechanical effort, characteristic of most conventional power generation systems, are eliminated. As a result, fuel cells promise to generate electricity with great efficiency and little harm to the environment. Additionally, fuel cells create electricity with less pollution since combustion is avoided. However, unlike batteries, fuel cells need continual replenishment of the reductant (hydrogen) and oxidant (oxygen) to enable continuous functioning. The fuel tank's storage capacity is the sole factor limiting the fuel cell's ability to operate for a long period of time. Figure 1 shows a schematic illustration of a traditional H2/O2 fuel cell. Although a broad range of fuels and oxidants may be processed by fuel cells in theory, the most frequent fuels now of relevance are hydrogen and air as the oxidant.

An oxidant (typically oxygen in air) and fuel are continually delivered to the cathode and anode, respectively, in a conventional fuel cell (positive electrode). The electrodes undergo electrochemical processes that result in an electric current that flows through the electrolyte and drives a complementary current that works on the load. The hydrogen gas ionises at the anode of, let's say, an acid electrolyte fuel cell utilising hydrogen fuel, releasing electrons and producing H+ ions (protons), which releases energy.

$$2\ H_2 \rightarrow 4\ H\text{þ}\ \text{þ}\ 4\ e\text{−}\ E0\ ¼\ 0V\ ½1]$$

Water is created at the fuel cell's cathode when oxygen interacts with protons that have internally moved from the anode to the cathode and electrons sent from the anode through the external electrical circuit.

$$O_2 = 4\ H = 4\ e = 2\ H2O\ E = 1\ 4\ 1{:}229\ V = 1\ 22]$$

The H+ ions must travel through the electrolyte and the electrons generated at the anode must pass via an external circuit in order for the reaction to continue continually. Since an acid is a liquid that contains free protons, it works well as an electrolyte for proton transfer. Solid electrolytes like polymers and ceramics may also be used to provide proton conductivity. The electrolyte must strictly prohibit electron transport and only permit proton transfer. Without it, the electrons wouldn't be able to go around the external circuit, "short-circuiting" the cell and rendering the fuel cell useless.

Theoretically, any material (the reductant) that is capable of chemical oxidation and can be continually supplied may be burnt "galvanically" as fuel at the anode of a fuel cell. The same is true for the oxidant, which may be any liquid that can be reduced quickly enough. The most frequent oxidant is gaseous oxygen, which is easily accessible from air, for practical reasons. Furthermore, fuels with simple molecules like hydrogen, methane, and methanol are frequently employed due to kinetic restrictions in catalysts for fuel oxidation. Because biological fuel cells may use a larger variety of fuel feedstocks, they have attracted more attention than traditional chemical fuel cells due to their kinetic limitations.

Biological Fuel Cells

Biocatalysts are used in biological fuel cells to transform chemical energy into electrical energy. Although normally the fuel is a hydrocarbon molecule, biological fuel cells function in a similar manner to chemical fuel cells in that there is a continuous supply of fuel going into the anode and a constant supply of oxidant going into the cathode. A fuel, such as glucose, is oxidised at the anode.And at the cathode the oxidant, for instance, oxygen, is reduced.

A current is created as a flow of electrons via the external electrical circuit as a consequence of the electrochemical reaction, and protons are generated internally in the cell by the oxidation of the fuel. As can be observed in reactions and , the theoretical cell potentials for such reactions, which are cited , are comparable to those of ordinary fuel cells. The utilisation of biocatalysts is the distinctive characteristic that makes a biological fuel cell unique.

Depending on the kind of biocatalysts utilised, there are two types of biological fuel cells: "microbial" fuel cells and "enzymatic" fuel cells. Enzymatic biofuel cells and microbial fuel cells both employ isolated and purified enzymes as specialised catalysts.

Using biocatalysts, biofuel cells may operate in one of two ways. The biocatalyst produces the fuel substrate for the cell via a biocatalytic transformation or metabolic process.

The energy generating process in this kind of fuel cell is really handled by a normal fuel cell, not the biocatalysts. For instance, utilising a multienzyme system and hydrogen-producing bacteria to ferment carbohydrates into hydrogen, link the bioreactor to a typical H2/O2 fuel cell using metal catalysts, such as Pt , and utilise the biohydrogen to power the fuel cell. Enzymes are not directly engaged in energy creation in this sort of enzyme fuel cell; instead, a conversional fuel cell produces the energy. Enzymes use a biocatalytic transformation or metabolic process to produce the fuel substrate for fuel cells. For traditional hydrogen-oxygen fuel cells, hydrogenase has been used successfully in a number of investigations to create hydrogen from glucose. Enzymatic fuel cells less often use this kind of biofuel cell. The biocatalyst actively takes part in the processes that transport electrons from the fuel to the anode.

Biocatalysts have a direct role in the bioreactions that generate energy in this sort of biofuel cell. Similar to conventional fuel cells, microorganisms or enzymes at the anode oxidise organic material and create electrons, while at the cathode, living things (microbes) or enzymes catalyse oxidant reduction and take electrons. The activity of the biocatalyst plays a key role in this sort of biofuel cell's performance.

Biological fuel cells are said to be more "environmentally friendly" than conventional chemical fuel cells. Biological fuel cells, as opposed to conventional fuel cells, use organic materials produced by metabolic processes or organic electron donors used in the growth processes as fuels for power production. Conventional fuel cells, on the other hand, typically use hydrogen as fuel and typically call for extreme conditions of pH or high temperature. The operating conditions for biological fuel cells are neutral pH and room temperature. The substrate oxidation of many fuels may be catalysed to a higher degree by microorganisms than by enzymes, and they can be less prone to poisoning and loss of activity under typical working circumstances.

Enzymatic Fuel Cells

The extremely specialised catalytic activity of enzymes in bioreactions are well recognised. The development in implanted medical devices for use in healthcare over the last ten years has sparked interest in creating enzyme-based bioelectronics, such as fuel cells and sensors. With in vivo biofuel cells as the power source for these implanted devices, several applications of the technology are suggested as biosensors for monitoring the changes in physiological chemicals, such as glucose sensing for diabetes patients. A biofuel cell operating in a blood artery is schematically shown in Figure 5.1.Utilizing glucose and dissolved oxygen as the cell's respective fuel and oxidant. The most popular commercial biosensors for point-of-care and home usage are

electrochemical glucose sensors because of their ease of use, adaptability, and cheap cost of electrochemical transduction equipment. In order to monitor certain particular contaminants, environmental sensors have also been utilised with enzymes. There are new applications for portable electronic gadgets including computers, cell phones, and MP3 players.

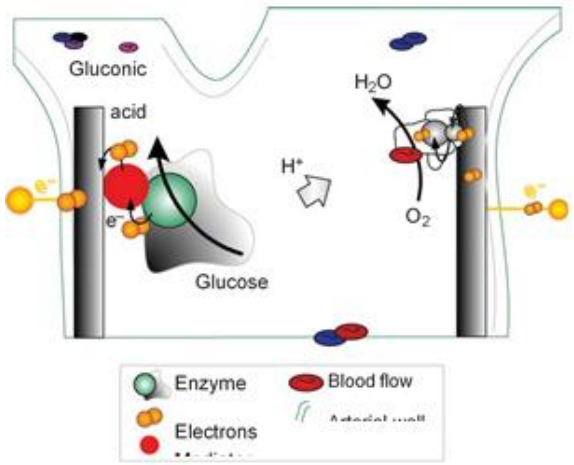

Figure 5.1: Illustrates graphically a biofuel cell in a blood vessel that is in operation.

Enzymatic biofuel cells, for instance, Sony has created a biofuel cell that powers a Walkman using sugar as the fuel and enzymes as catalysts. Since the 1960s, enzyme-based fuel cells have been documented. However, compared to traditional fuel cells, the development of enzymatic biofuel cells is still in its infancy because of the poor stability and low power outputs realised. The secret to improving the efficiency of biofuel cells is biocatalytically enzyme-modified electrodes. In recent years, there has been a lot of research done on the creation of enzyme electrodes for biofuel cell and biosensor applications. In order to enhance the performance of enzyme electrodes, research has been done on understanding the reaction mechanisms of enzyme catalytic reactions developing new biomaterials and studying enzyme modificationenzyme immobilisation techniques as well as enzyme electrode structures.

Different Biofuel Cell and Enzyme Types

Based on the mechanisms used for electron transport Based on the location of the enzyme active centres and techniques for creating electron transfer between enzymes and electrodes, redox enzymes may be categorised into three classes.

Enzymes that include poorly bound nicotinamide adenine dinucleotide (NADH/NAD+) or nicotinamide adenine dinucleotide phosphate (NADPH/NADP+) redox centres. This category includes alcohol dehydrogenases and glucose dehydrogenases (GDH). Enzymes, such as peroxidases, laccase, and other multicopper enzymes, that conveniently have at least a portion of the redox centre at, or close to, the protein shell come into this group. In enzyme reactions and

immunoassays, peroxidases such cytochrome c and horseradish peroxidases are often utilised. Enzymes that are tightly linked to their protein or glycoprotein shells and have a redox centre. The enzyme that has been explored the most, in particular for use in glucose sensors and biofuel cells, is glucose oxidase. The first two groups are capable of performing direct electron transfer (DET) between the electrode surface and the active sites of the enzymes. The primary variable influencing the enzyme's activity in the second group is the orientation of the enzyme on the electrode surface.

Due to the significant distance, >21 A°', between the active centres of the third set of enzymes and the electrode surface, DET between the active centres and electrodes is not possible. In this instance, electron transfer mediators may be used to create direct electrical connection with electrodes for enzymes whose active core is firmly embedded within the protein shell. Many redox enzymes may take these synthetic oxidants or reductants in lieu of their natural oxidants or reductants (in the case of reductive or oxidative enzymes, respectively). These enzymes have a wide variety of structural variations, which in turn affect their characteristics, notably their redox potentials. The operation of mediated electron transfer (MET) in enzymatic biofuel cells is shown in Figure 4. It is obvious that the characteristics and functions of both the mediator and enzyme molecules have a significant role in how well an enzymatic biofuel cell performs.

The diffusional process is the foundation of the mediators that serve as the electron transmission relay. The distance between the enzyme active centre and electrode may be shortened by diffusional penetration of the oxidised or reduced relay into the protein [55]. One of the most used mediators for glucose oxidase is ferrocene derivatives. The first "wired" enzymes were created by Degani and Heller [56], which had a mediator molecule covalently bound to the enzyme to initiate electron transport. As mediators for glucose oxidase, benzoquinone, hydroquinone, and pyrroloquinoline quinone (PQQ) have also been described.

Electrodes for enzymes

The chemical and physical characteristics of the immobilised enzyme layer are essential for the correct operation of an enzyme-based electrode. Physical procedures and chemical approaches may be used to immobilise enzymes. Gel entrapment - In this case, the enzymes were trapped in a gel matrix made of gelatin and polyacrylamide as well as dialysis tubing.

Adsorption

Because there is only a weak interaction between the enzyme and electrode surface, adsorption of the enzyme to the electrode surface is straightforward and doesn't need any extra chemicals. By adsorbing enzymes on a graphite surface, created enzyme electrodes for a biofuel cell that used Ni-Fe hydrogenase and laccase. Carbon monoxide had no impact whatsoever on the hydrogenase's quick electrocatalytic oxidation of hydrogen. Oxygen only partly prevented the reaction. The primary techniques for creating enzyme electrodes for biofuel cell applications are chemical ones. The techniques involve immobilising enzymes in polymer matrices and covalent immobilisation.

Multilayer Structure Enzyme Electrodes

The most stable and irreversible immobilisation method is covalent immobilisation, which is most often used to noble metals and carbon. With the enzymes immobilised on the electrode surface either in self-assembled monolayers (SAMs) or in layer-by-layer structures binding mediators to transfer electrons from the site of fuel oxidation at the enzyme to the electrode surface, the enzyme electrodes typically have a layered structure based on covalent bindings.

By reconstituting the enzyme using nitrospiropyran- and 2-aminoethyl-modified flavin adenine dinucleotide (FAD) cofactors, Katz and Willner established a way to create DET between the active core of glucose oxidase and the electrode surface along a predetermined structural route they used enzymes on the cathode and anode of a fuel cell, with gold serving as the electrode substrate. The cathodic reaction was the reduction of hydrogen peroxide by microperoxidase-11 (MP-11), and the anodic reactions, termed as were glucose oxidation employing reconstituted glucose oxidase connecting with a monolayer of PQQ as the mediator.

For the electroenzymatic oxidation of lactate, tgroups were used to bind SAMs with biospecific affinity for lactate dehydrogenase to the surface of gold electrodes. Covalent immobilisation of redox proteins, enzymes, and phospholipids to the SAMs of 3-mercaptopropionic acid on a gold electrode surface. Cyclic voltammetry was used to study the electrochemical properties of self-assembled octadecanethiol monolayers on polycrystalline gold electrodes. The monolayer transient total capacitance as well as the differential capacitance changes during the CV scan were measured in the presence of various redox probes placed in the majority of the supporting electrolyte. The findings demonstrated that the capacitance measurements are very sensitive to changes in a monolayer's structure during a redox reaction.

For molecular identification and electrical signal creation, multilayer enzyme electrodes have been researched. These electrodes use layer-by-layer supramolecular structures made of alternating layers of negatively charged enzymes and cationic redox polyelectrolyte. In structured structures with great spatial resolution, glucose oxidase (GOx), lactate oxidase (LOx), and soybean peroxidase (SBP) have been covalently linked to poly(allylamine) and electrically connected to the underlying electrode. The quantity of deposited enzyme determined by quartz crystal microbalance is 100 times less than the concentration of redox mediator integrated into the multilayers, which is determined from the voltammetric charge and a calculation of the layer thickness. By alternately depositing layers of poly(allylamine) (PAA) and periodate-oxidized glucose oxidase (GOx), an electrode. Electrochemical impedance spectroscopy was used to track and validate the covalent attachment process (EIS). The oxidation of glucose using ferrocenemethanol as the mediator produced outstanding electrocatalytic response from the gold electrodes modified with the GOx/PAA multilayers. The coverage of active enzyme on the electrode surface exhibited a linear connection with the number of GOx/PAA bilayers, according to the examination of the voltammetric signals, indicating that the analytical performance may be adjusted by varying the number of attached bilayers.

Polymer matrix-enhanced enzyme electrodes

There are several restrictions even though stacked enzyme electrodes have shown effective electron transport in a number of applications. First, a monolayer covalent binding strategy on the electrode surface limits the number of enzymes that may be immobilised on the electrode. Second, when additional molecule layers get immobilised on the electrode surface, more electric resistance is created, which in turn affects the electrode's ability to respond electronically. The direction in which the mediator and enzyme molecules are oriented will also have an impact on electrode activity. Redox polymer conductivity may be a way to get around these restrictions. In order to encapsulate enzymes and create enzyme electrodes, conducting polymers like polypyrrole (PPy) and polyaniline (PANI) are often utilised. One of the most often utilised conducting polymers in the development of bioanalytical sensors is PPy, which also has certain special characteristics that help some redox enzymes move electrons more easily and avoid some unfavourable electrochemical interactions [80]. By electropolymerization, enzyme electrodes containing PPy are created, and during the polymerization process, enzymes are entrapped in the polymer as a dopant. In order to create an electron relay, cationic pendant groups, such as the tris(bipyridyl)ruthenium (II) complex, may also be added to polymer films to functionalize PPy. Cosnier created a two-step procedure that included electropolymerizing the adsorbed monomers after an aqueous amphiphilic pyrrole monomer-enzyme combination was adsorbable on an electrode surface [88]. The electrooxidation of a newly synthesised biotin pyrrole facilitated the production of biotinylated conducting. This biotin pyrrole is functionalized by an electropolymerizable pyrrole group.

PPy films in an organic electrolyte showed a particular avidin-biotin interaction at the polymer solution interface. This offered a straightforward electrochemical method to enable the immobilisation of enzymes on electrode surfaces without the need for reagents [89, 90]. Figure 5 displays pictures of the PPy film entrapped with ferrocene-modified glucose oxidase obtained using scanning electron microscopy (SEM) and an atomic force microscope (AFM) [38].

Another popular polymer for immobilising enzymes is PANI. Cooper and Hall [72] developed an enzyme-mediator-conducting polymer model utilising the benzoquinone (Q)-PANI system, which may result in increased current densities [91]. PANI/poly(acrylic acid) films and redox enzymes were combined by Raitman *et al.* to examine the bioelectrocatalyzed oxidation of glucose or lactate [92]. In situ electropolymerized PANI-polyacrylonitrile composite film was used to improve the selectivity and stability of a glucose biosensor [93]. The surface reconstruction of the apo-enzyme on a PQQ-modified PANI is an unique technique developed by Willner's group to produce an integrated electrically connected GDH electrode [94]. Using poly (aniline-aniline boronic acid) wires made on ds-DNA templates, the same group also created an integrated enzyme-electrode in which glucose oxidase demonstrates direct electrical contact with the electrode [95].

Polymer mediators have been created and applied to the enzyme electrodes in order to establish electron transport between the enzyme active centres and the electrode surface and give the

structure for enzyme immobilisation. The most researched polymer is one based on osmium. The enzyme electrodes used in currently available continuous glucose sensors are made from osmium-based polymers. Wide redox potential windows from diverse derivatives for different redox processes, a quick electron transfer rate, and strong chemical stability are some of these polymers' benefits.

A redox epoxy was created in 1991 by Heller's group. It was intended for use in enzyme electrodes and was created by reacting two water-soluble materials (a poly(vinylpyridine) complex of Os(bpy)2Cl and a diepoxide) in a manner that was almost physiological. The binding simultaneously electrically links the electrode and immobilises the enzyme glucose oxidase. In this instance, the whole film is covered by the catalytic "reaction layer" [106, 107]. Since then, they have created a number of Os polymer derivatives for enzymatic oxidation and oxygen reduction processes in addition to biofuel cells that utilise these enzyme electrodes [21, 96-105]. In order to immobilise glucose oxidase, micro enzyme electrodes with 7 m diameter carbon fibres were created utilising redox hydrogels based on poly(vinylpyridine) Os(bipyridine)2Cl derivatives [108]. This design of carbon fibre electrodes was created for a miniature biofuel cell [109]. The power density of this device was five times larger than that of the finest biofuel cells previously available, which at 37 °C produced a power output of 600 nW, sufficient to operate tiny microelectronics.

Due to their toxicity, Os compounds may eventually seep out of implanted applications, raising concerns. Another concern with implanted devices is biocompatibility. The development of biopolymers based on phospholipid polymers that mimic cell membranes resulted in the reduction of blood coagulation, which could impair the operation of a device when it comes into contact with blood [33, 35, 110]. These polymers also have good biocompatibility and inhibit the adhesion and activation of blood cells. Investigations into the viability of adding redox characteristics to phospholipid polymers revealed that the biopolymers might be used to create enzyme electrodes for implantable applications by changing the side chain of the polymer [111]. In order to create amperometric biosensors, a hydrophilic copolymer called poly (vinylferrocene-co-2-hydroxyethyl methacrylate, or poly(VFc-co-HEMA)) was developed. This biopolymer also serves as a polymeric, electron transfer mediator. The poly(VFc-co-HEMA) membrane serves as an electron transfer mediator and an enzyme-immobilizing carrier matrix for the fabrication of glucose sensors.

Enzymatic biofuel cells' efficiency

A PQQ monolayer-functionalized Au electrode served as the anode and a microperoxidase-11 (MP-11)-modified Au electrode served as the cathode in one of the first enzymatic biofuel cells described by Willner and Katz [64]. H2O2 served as the cathodic oxidizer in this system, while 1,4-dihydronicotinamide adenine dinucleotide, or NADH, serves as the anodic fuel substrate. The biofuel cell produces a short-circuit current density of 30 A cm2 and an open-circuit voltage of 320 mV. At a 3 kilowatt external load, the cell's highest electrical output was 8 W.

A unique glucose/O2 biofuel cell without compartmentalization between the anode and cathode was another biofuel cell created by Willner and Katz. The reconstituted cytochrome c/cytochrome oxidase pair served as the cathode, while the anode was a surface-reconstituted glucose oxidase monolayer. The creation of stacked bioelectro catalyst electrodes was used to create the biofuel cell. A DET was created between the enzyme and the mediator as well as the mediator and the electrode surface. To create apoenzyme, the cofactor, the active core of the enzyme, was first eliminated. Before reconstituting the enzyme with the artificial active centre, the mediator attached to the electrode surface was covalently linked to the artificial active centre to create an electron transfer channel. Peak power output of 4 W and an open-circuit cell voltage of 0.11 V were attained. This technique opens the door to designing biofuel cells that can be implanted to produce electricity.

Katz and Willner created an electroswitchable and tunable biofuel cell based on the biocatalyzed oxidation of glucose by using the feature of conductivity change for the oxidation and reduction state of Cu-poly(acrylic acid) polymer. The biofuel cell performance is reversibly switched between "ON" and "OFF" states, respectively, by the cyclic electrochemical reduction and oxidation of the polymer films connected to the anode and cathode between the Cu-0-poly(acrylic acid) and Cu2+poly(acrylic acid) states. The cell's short-circuit current density reached 550 A cm2, while the open-circuit voltage was 120 mV. With a 1 k external load resistance, the cell's highest extracted power was 4.3 W. The Cu2+ polymer films' gradual decrease enables control over the amount of conductive domains present in the films and fine-tuning of the biofuel cell's output power.

Recently, a pH-switchable oxygen electrode and an enzyme-based biofuel cell with enzyme logic operations processing in situ biochemical input signals were produced. Two enzyme-based Boolean logic gates (AND/OR) were constructed to interpret biological signals and logically translate them into changes in the pH of the solution. By changing the pH of the fluid, the modified electrode's electrochemical activity may be switched. At pH values more than 5.5, the electrode was electrochemically silent, while pH values lower than 4.5 activated it for bioelectrocatalytic oxygen reduction. The external enzymatic systems acting as logic switches in the system employed the abrupt change between the inactive and active states to regulate the electrode activity. When the biofuel cell was turned on (by turning on the biocatalytic cathodic process), 380 mV of open-circuit voltage and 3 A cm2 of short-circuit current density were measured. There was a 700 nW cm2 maximum power density.

Using Os derivative polymer mediators for glucose and bilirubin oxidase on the anode and cathode, respectively, Mano et almost .'s recent research for a tiny, membraneless glucose-O2 biofuel cell was reported with a power density of In a physiological buffer including phosphate buffer saline at p. 7.0 at 37.5 °C, 4.8 W mm2 were generated at a voltage of 0.60 V.

Fruit juices have all been researched as possible fuels for a membraneless biofuel cell, including orange juice, grape juice, and banana juice. By employing 1,1′-dicarboxyferrocene as mediators on both the anode and the cathode, the cell was constructed using glucose oxidase and laccase as

the anodic and cathodic catalysts, respectively. This study showed that portable electronics might be powered by readily available fruit juice [10]. The Voc (0.191 V) and Isc (60 A, current density 146.3 A cm2) for grape juice and Voc (0.202 V) and Isc (72 A, current density 175.6 A cm2) for banana juice were attained by using them as fuels in the biofuel cell instead of glucose. These values are comparable to those for glucose. When employing orange juice as fuel, a fuel cell's Voc and Isc are respectively around two and three times greater than those of glucose. Orange juice was used to obtain the highest power density of 11.66 W (power density 28.4 W cm2) at 0.216 V [10].

Nontoxic mediators for enzyme electrodes are needed for implanted medical devices. At Kyoto University, a biofuel cell was created that uses poly-L-lysine that has been modified with vitamin K3 (PLL-VK3) as an electron transfer medium during the anode's catalytic oxidation of NADH by diaphorase (Dp). Co-immobilized PLL-VK3 and Dp were then covered in NAD(+)-dependent GDH on an electrode. When a swirled, a 2 mA cm2 oxidation current was seen in the electrochemical cell.

Microbial Fuel Cells

MFC development

A significant quantity of energy may be destroyed by bacteria in a variety of waste streams. Potter. has attempted to manufacture electricity using E. coli around the beginning of the nineteenth century, was the first to come up with the concept of employing microbial cells to produce electricity. Cohen developed many series-connected microbial half fuel cells in 1931. By employing hydrogen produced from the anode-based fermentation of glucose by Clostridium butyricum, DelDuca *et al.* successfully operate a hydrogen and air fuel cell. A biological fuel cell was in which C. butyricum was also employed to produce hydrogen via the fermentation of glucose. Yao *et al.* demonstrated in 1969 that glucose could be burned as fuel when a platinum-black anode was present. Bennetto conducted additional in-depth research on MFCs towards the beginning of the 1980s and created a fuel cell as a potential technology for developing nations to generate power. Bennetto demonstrated how mediators might speed up reactions and improve electron transfer efficiency. Since then, a significant amount of research has looked at a variety of MFC-related topics, including materials, bioelectrochemistry, and microorganisms.

Tanisho *et al* investigation used an MFC containing Enterobacter aerogenes and a platinum-plated stainless-steel net anode. Hydrogen, which the bacteria biochemically created from glucose, served as the primary anode reactant for Tanisho. Existing transition metal-catalyzed fuel cells cannot be utilised to successfully produce electric power from carbohydrates [12]; however, biofuel cells, in which complete cells or isolated redox enzymes catalyse the oxidation of the sugar, have been produced.

MFC's electricity generation system

It is still unclear exactly how power may be generated in an MFC directly from the oxidation of organic materials. When organic matter is oxidised by heterotrophic bacteria, a process known as

catabolism, energy is released. To transform chemical energy into electricity, MFCs utilise the catabolic activity of living organisms, specifically bacteria (biocatalysts). The respiratory enzymes that are utilised to store energy in the form of adenosine triphosphate (ATP) inside the cell receive the electrons that are captured when bacteria oxidise a chemical. Then, electrons are delivered to an electron acceptor like oxygen, iron, nitrate, or sulphate. Recently, it was shown that the same bacteria that respire using iron can also transport electrons to an anode, as shown in Figure 5.2.

Microorganisms engage in an oxidative metabolism when they consume a substrate (such as glucose) in the presence of oxygen, which is described in reaction.

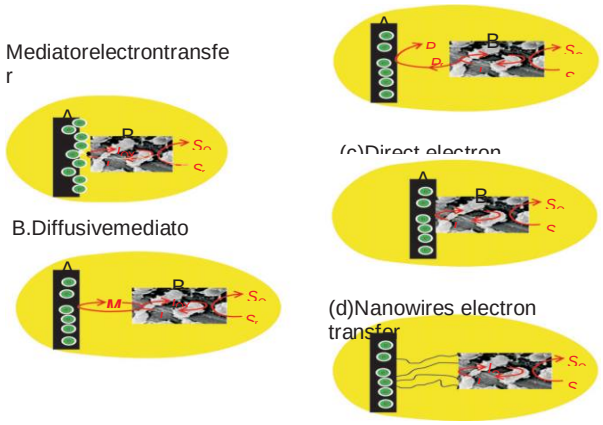

Figure 5.2: Mode of electron transfer mechanisms in an MFC.

M, mediator; A, anode; B, bacteria; C, cytochromes; D, nanowires; Sox, substrate oxidation; Sred, substrate reduction; Iox, intermediate oxidation; Ired, intermediate reduction; Pox, production oxidation; Pred, production reduction; and e−, electron.

MFC's Operational Principles

The MFC's main mode of operation A proton exchange membrane separates two chambers with an anode and cathode in aqueous solutions (PEM). Microorganisms transmit electrons from a reduced electron donor to an electron acceptor at a greater electrochemical potential, which results in the creation of current. One proton is created for every one electron when organic matter is oxidised by bacteria in an anode biofilm. Depending on the fuel source, carbon dioxide may also be produced as a byproduct of the oxidation process. Protons are transmitted via the membrane and electrons are delivered to the cathode through the external circuit, powering an external electrical load. On the cathode, electrons and protons interact, reducing the oxidant (often oxygen) to water and producing electricity.

Microbes are unable to transmit electrons directly to the anode unless the species in the anode chamber are anodophiles, which are bacteria with the capacity to decrease inert electron acceptor. As a result, electron mediators may be utilised in MFCs to speed up the electron

transfer in order to increase the device's power output. By taking up electrons, mediators are reduced from their oxidised form. The mediators are oxidised once again in the bulk solution in the anode chamber as a result of the release of these electrons to the anode. This cyclical process has the potential to speed up electron transmission and increase MFC power production. However, using mediators poses a number of issues for practical devices, therefore technological development is concentrated on mediatorless MFCs, or cells that don't utilise external chemical mediators.

MFC without a mediator

Recent research shown that wastewater-fed MFCs with diverse microbial populations generate soluble redox mediators such pyocyanin. It has been shown that some metal-reducing bacteria, mostly from the family Geobacteraceae, are capable of directly transferring electrons to electrodes through electrochemically active redox enzymes found on their outer membrane, such as cytochromes. Additionally, membrane proteins or nanowires or beyond cell surfaces are reported to be used by Geobacter sulfurreducens to transmit electrons to electrodes. It has been claimed that soluble chemicals in the culture transport electrons between the electrode and E. coli cells. Due to the excretion of hydroquinone derivatives via an extremely permeable outer membrane, E. coli cells that have developed under electrochemical stress in an MFC exhibit direct electrochemical behaviour. In addition to these species, Pseudomonas species also generate compounds that allow Gram-positive bacteria to accomplish extracellular electron transfer. Recent study has also revealed a number of other anodophilic bacteria, which are thoroughly discussed. These EAB-containing MFCs are referred to as mediatorless MFCs since they don't need intermediaries for the transmission of electrons to electrodes.

Elimination of Organic Material in MFC

Although the amount of power obtained, as of now, is not great, MFCs may employ a broader variety of fuel sources than other fuel cells, such as enzymatic biofuel cells (for example, complex organic materials in wastewater). The anode containing G. sulfurreducens is estimated to have the highest power per unit volume of 2.15 kW m3. Different power output and bacterial enrichment on the anode imply that substrate composition affects the anode's ability to produce current efficiently. Selecting appropriate organic and inorganic materials that may be utilised as energy sources has been the main focus of much research on creating dependable MFCs. Today, it seems that any biodegradable substance may be used to create power from simple mixes and wastes like glucose, acetate, butyrate, cysteine, proteins, and lignocellulose to pure substances like acetate and glucose. Food processing brewery residential chemical starch wastewaters, swine manure slurry manure waste landfill leachate and meatpacking wastewater have all been used as direct sources of energy by MFCs.

The treatment of wastewater is one of the most promising uses for MFCs, according to several research. Chemical oxygen demand (COD) reduction has been reported in the literature to range from 60% to 90% depending on the operating circumstances and the kind of reactor utilised. According to reports, the majority of MFC designs can effectively remove COD from

wastewater with an efficiency of 80% to 95%, proving the effectiveness of MFC as a wastewater treatment system. The upflow anaerobic sludge blanket (UASB) reactor is one commonly used anaerobic technology that this efficiency is similar to. When compared to genuine wastewaters, synthetic wastewater often has better Coulombic efficiency (CE) and organic matter removal. The CE of the MFC is defined as the percentage of total theoretical Coulombs that can be produced from the provided substrate divided by the quantity of Coulombs that the MFC actually harvests. Lower CE while treating genuine wastewaters is caused by the more complicated composition of the organic materials there than in synthetic wastewater, where researchers typically employ a single carbon source.

It is intriguing that MFCs may be utilised to treat wastewater containing cellulose in order to produce energy in addition to treating soluble organic materials. In contrast to the common soluble substrates that have been utilised in MFC as electron donors, cellulose is special because it needs a microbial consortia that can metabolise both an electron acceptor (cellulose) and an insoluble electron donor (sugar) (electrode). A specified coculture of *Clostridium cellulolyticum* and *G. sulfurreducens* was used to generate successful power from cellulose-fed MFC. One gramme of lone carboxymethyl cellulose and one gramme of MN301 cellulose were used in the coculture to produce maximum power densities of 143 (anode area) and 59.2 mW m2, respectively. These substrates could not be used to generate any power by a pure culture alone.

Because there is less time for substrate to be lost due to conflicting physical and biological processes, coulombic efficiencies for MFCs generally rise with power density. The maximal power density generated seems to be correlated with the substrate's "complexity" (i.e., a single compound versus many compounds). According to tests employing the same system, power output was only 146 mW m2 when household wastewater was used compared to 494 mW m2 when glucose was used. In a flat-plate MFC, Min and Logan discovered that the power output was 86% lower when dextran was utilised as the feed in place of glucose. The maximum power output may thus be decreased by the presence of numerous substrates or polymers in an organic solution. Additionally, fermentable substrates like glucose, dextran, and starch only generated Coulombic efficiencies of 14–21% whereas nonfermentable substrates like acetate and butyrate produced Coulombic efficiencies of 50–65%.

MFC Material Aspects and Operating Conditions

The inoculum, or the source of bacterial culture and bacterial strain(s) used at the anode, the fuel substrate and concentration, pH, conductivity, temperature, and operating conditions of the MFC, including hydraulic loading rate, as well as reactor design and cell materials for the anode, cathode, and anode-to-cathode separator, are some of the variables that affect MFC performance.

Temperature of operation

One of the most crucial factors in anaerobic digestion is temperature, which has a significant impact on methane generation. The properties of this process have been extensively researched and recorded, and the majority of anaerobic digesters operate in the mesophilic range. As

temperature dropped, the majority of studies found that methane production significantly reduced. Mesophilic bacteria thrive best at temperatures between 35 and 40 °C. The mesophilic bacterial consortia go through a protracted selection and adaption process when the reactor temperature is lower, which causes their activity to substantially slow down and leads to the development of a group of mesophilic psychrotrophic bacteria. Additionally, there is a class of bacteria known as psychrophilic bacteria that natively favour low-temperature settings these organisms have more recently attracted research attention.

The temperature has an impact on the functioning of the anode in an MFC, much like other biological wastewater treatment methods. In contrast, raising the temperature also enhances the kinetics of oxygen reduction and lowers cell internal resistance, which may result in higher current densities and CE; for instance, 43% at 30 °C compared to 8% at 22 °C. The pace of biochemical reactions may accelerate with rising temperature, which also accelerates substrate usage and accelerates biomass growth. A greater rate of growth would also hasten the microbial adhesion to the electrode.

35 °C was said to be the ideal working temperature albeit this is obviously dependent on the bacterial strain being employed. With a drop in temperature from 30 °C to 20/22 °C, reductions in power density (70-43 mW m2), CE, and COD removal efficiency were found. On the other hand, it was reported that thermophilic operation of an MFC at 55 °C for more than 100 days produced a power density of 37 mW m2 at a CE of 89%. Cell operation at 60 °C was found to create 10 times more power than operation at 22 °C in MFC producing energy from marine sediments. MFCs operating at 50 °C have also reported producing current successfully.

MFCs are commonly operated at pH levels of neutral or slightly higher in the cathode chamber and between 6 and 8 in the anode chamber (Figure 10). This is so because the anodic microbial process functions best at a pH that is close to neutral, and microbial activity declines at pH values that are higher or lower. For example, an anodic chamber pH between 7 and 8 is said to yield the highest CE and current. Such pH levels are often intrinsic to the feed/waste stream being treated; nevertheless, aand higher than pages 9.0, decreases in current and CE were found. Data reported so far indicate that, while operation of MFC at feed pH up to 10 is achievable, a pH between 6 and 7 may offer the MFC its best power output. A larger pH difference between the anode and cathode may increase the power output of the MFC owing to improvements in the kinetics of oxygen reduction, whereas a higher pH in the anode chamber supports better COD removal but lowers power.

Hydraulic retention time and organic loading rates

The performance of MFCs is typically influenced by organic loading rates (OLRs) and retention duration (residence time), which is especially reliant on the substrate being utilised as a fuel. In comparison to fermentable substrates like glucose, non-fermentable substrates like acetate provide better power densities and energy conversion efficiencies. Because acetate has a simpler metabolism than glucose and xylose when humic acid (HA) is used as a mediator in two-chamber MFCs, acetate produces more power. Due to HA's mediating function, the power for

glucose and xylose increased by 84% and 30%, respectively, in the presence of HA. The addition of HA had no discernible effects on acetate. The power production during the breakdown of fermentable substrate rises when an external mediator is added, showing that the bacteria that have grown within the cell have limited capacity to transmit electrons.

The architecture of the MFC being utilised and the wastewater being treated determine the optimal range of OLR to produce the highest COD removal efficiency and power. Comparable to those used in activated sludge processes are the OLRs utilised in MFCs. These, however, are significantly smaller than the OLRs utilised in the case of treating industrial wastewater in UASB reactors and only comparable with the OLRs employed for sewage treatment in high-rate anaerobic processes, such as anaerobic filters and UASB reactors. Both the power yield and the rate of substrate deterioration in the MFC will be significantly impacted by the applied OLRs. Researchers often employ OLRs between 0.05 and 2.0 kg COD m3 day 1 to get the most power out of the MFC. The MFC's geometrical design, size, electrode placement with regard to the membrane, and influent and effluent arrangement for optimum substrate distribution to the anode chamber are only a few of the aspects that can significantly affect MFC performance.

The natural oxidant of choice for MFCs is oxygen found in air. Although other substances like permanganate, hexacyanoferrate and hydrogen peroxide may also be used as cathodic electron acceptors and provide greater power densities, they are not regarded as sustainable since they still need to be replaced on a regular basis. Ferric ions, which can be converted to ferrous ions (Fe2+) at the cathode, may be used instead of oxygen. Using a bipolar membrane to separate the anode and cathode, MFC with ferric iron reduction at the cathode and concomitant biological ferrous iron oxidation of the catholyte was shown. In order to achieve an MFC power output of 1.2 W m2 and a current of the immobilised microbe Acidithiobacillus ferrooxidans oxidised ferrous iron to ferric iron at a rate that was high enough.

In general, selecting the right catalyst material is essential to employing air as an oxidant in MFCs. For excellent oxygen reduction kinetics, a pH approaching neutral is not the optimal setting [129]. Air cathodes are widely employed to improve the efficiency of MFCs since the cell voltage and power capacities of aqueous catholyte solutions with dissolved oxygen are limited. An effective arrangement that does not need active aeration or the addition of chemicals for the cathodic reaction is an air cathode MFC. Using protons to create hydrogen gas is an alternative to oxygen reduction in MFCs. Then, other power devices can use this hydrogen as a fuel to produce energy. However, this kind of microbial electrolysis doesn't generate electricity along with hydrogen; instead, it needs a power input (of a few hundred millivolts) to achieve acceptable production rates. This is brought on by the 1.2 V standard potential difference between oxygen reduction and proton reduction. To enhance the chance for direct microbial growth on the anode surface, the optimal material used for the cell electrodes should have a greater surface area per unit volume. For usage as the electrodes, felt, graphite granules, and carbon fibres or brushes may be appropriate substitutes. The generation of dead pockets (areas not utilised for direct microbial growth) and a decrease in the MFC's power output may result from an electrode material with a very high surface area and extremely small pore size. In

comparison to other carbon forms, graphite fibre brush anodes with large surface areas and porous structures may provide high power densities (1430 mW m2, 2.3 W m3). For an MFC utilising a carbon brush anode, a power density of up to 2.01 W m2 has been observed. In order to optimise power generation at the lowest possible cost for MFC construction, it is also necessary to investigate the potential of covering the electrode surface with a non-noble metal catalyst.

Proton transfer is often made possible by the electrolyte function of the membrane employed in MFCs to separate the anode from the cathode. However, the use of MFC for wastewater treatment may be limited by the usage of membrane. Because of membrane fouling anticipated as a result of suspended particles and soluble pollutants in large-scale wastewater treatment, proton transport across the membrane may be a limiting issue. The high manufacturing costs of MFCs will further restrict their use for large-scale wastewater treatment since membranes are pricey. Therefore, a low-cost alternative to the usage of membranes or an evaluation of the cell's design to do away with the necessity for a membrane is required for MFC to be economically viable. The membraneless design has the drawback of preventing large amounts of oxygen from diffusing toward the anode chamber, which would lower the CE. In mediatorless and membraneless MFC utilising plastic sieves rather than polymer electrolyte membrane (PEM), a greater power density of 346 mW m2 was found. Another illustration is a membraneless MFC, which was reported to have a maximum power of 49 W m3. Instead of PEM, the use of porous materials like J-Cloth was examined as a cathode-anode separator. Two layers of J-Cloth significantly reduced oxygen diffusion, which led to over a 100% increase in CE compared to cells without J-Cloth, as evidenced by power densities of 627 W m3 in fed-batch mode and 1010 W m3 in continuous-flow mode the effectiveness of a low-cost MFC made from an earthen pot with a 400 ml volume without the use of expensive commercially available membranes. This earthen pot MFC, with a total cost of production of less than $1 US, treated synthetic wastewater and produced a maximum power output of 16.8 W m3. It also outperformed MFCs using polymer membranes and pricey cathode catalysts.

MFC inoculum

The first and one of the most crucial phases in the bioconversion of organic waste is the hydrolysis of complicated polymers by hydrolytic organisms (the transformation of complex polymers into substances that may be efficiently biodegraded by microbial consortia. Despite the fact that many anaerobic bacteria are capable of hydrolyzing materials by the production of exocellular enzymes or attachment to solid substrates, this process is thought to be the most yield- and rate-limiting in biological conversion processes [196]. In reality, the rigidly organised, slowly biodegradable substances (such plant waste lignocelluloses) in mixed waste streams are the cause of anaerobic digestion's lower efficiency. High-quality biomass, such as cellulolytic crops or carbohydrate-rich wastewaters from the food sector, may achieve efficiencies of over 80%. Pure cultures have often been used as the MFC's inoculum. In the anode chamber of an MFC, facultative anaerobic bacteria Aeromonas hydrophila and Enterobacter aerogenes were shown to be electrochemically active. The facultative anaerobe may develop quickly in aerobic

environments by oxidising organic substrates with oxygen and in anaerobic environments by converting substrates into hydrogen and a residue. They are excellent candidates for an MFC because of their ability to produce H2 and consume O2. Acetate, lactate, propionate, butyrate, glucose, sucrose, cellulose, glycerol, and ethanol have all been used as substrates by *Ochrobactrum anthropi* YZ-1 to create current. In addition to converting starch and glucose into electricity, *Klebsiella pneumoniae* strain L17 biofilm cells demonstrated DET from fuels to electrodes.

One of the most common groups of bacteria utilised in MFCs is the species *Shewanella*, which is well recognised for the variety of terminal electron acceptors it can reduce. *Shewanella oneidensis*, also known as *Shewanella putrefaciens*, is a non-fermenting, motile (capable of moving by itself under the right conditions), facultative anaerobic bacteria that is found in suboxic sediments. The wild-type strain of *Shewanella oneidensis* is *S. oneidensis* MR-1, while *S. oneidensis* DSP10 is a more recent spontaneous rifampin-resistant mutant employed in MFCs. In contrast to strictly anaerobic circumstances, where the removal of oxygen should normally enhance the fuel cell efficiency and increase power production, *S. oneidensis* cultivated with glucose produces greater power. The fact that the power of *S. oneidensis* grows with oxygen exposure suggests that this organism may efficiently use complex carbon sources as electron donors in MFCs.

According to reports, *G. sulfurreducens* produces more power when utilised as an inoculum than mixed anaerobic sludge does. *Rhodopseudomonas palustris* DX-1, a phototrophic purple nonsulfur bacterium, is another helpful culture for high power generation (2720 mW m2) compared to mixed culture MFCs because it can effectively generate electricity by DET in MFCs using a variety of substrates (volatile acids, yeast extract, and thiosulfate). When glucose is oxidised, *Acidiphilium sp.* strain 3.2 Sup 5 cells have been shown to generate large currents of up to 3 A m2 even when the solution is saturated with air and has a very low pH. The discovery of such strains will aid in the development of MFC without membranes and the creation of greater current densities in MFCs since they are unaffected by oxygen and assist in resolving the issue of O2 diffusion from the cathode.

In the anaerobic environment with sugars, metal-reducing bacteria *Rhodoferax ferrireducens* have been demonstrated to play a significant role. Microbial electricity was ascribed to the electrochemical and biologically active cells linked to the electrode. Planktonic cells, or cells that are grown in liquid suspension as opposed to being connected to the electrode surface, have a restricted or nonexistent capacity to catalyse the production of electricity.

Because diverse types of organic matter found in actual wastewaters are widely accepted as a substrate, the utilisation of mixed cultures may result in increased current in MFCs. Recent research has shown that the MFC may produce current densities that are equivalent to certain pure cultures when it is injected with mixed anaerobic sludge. Additionally, household wastewater may be employed as an inoculum. The inoculum may be pretreated with heat in order to increase power output in MFC, according to reports. The performance reported was 2.5 times

greater than that achieved without any pretreatment of the mixed anaerobic sludge, and mild ultrasonication pretreatment of the mixed anaerobic sludge, utilised as inoculum, is also shown to be useful in enhancing MFC performance [208].

Sludge pretreatment is crucial for controlling the methanogens, the group of bacteria that produce methane when present in a mixed anaerobic culture. When nonfermentable substrate is employed, electrogenic bacteria have the potential to outcompete methanogens. However, methane generation is generally recorded in the MFC over longer operating durations [209, 210] when a fermentable substrate is utilised in MFCs, i.e. in the case of actual wastewater treatment. Therefore, a plan is required to suppress the methanogens both during inoculation and sometimes while the reactor is operating.

Microbial Electrolysis

The creation of hydrogen by "microbial electrolysis cells" (MEC), a significant offshoot of MFC research, is especially intriguing in light of the significant worldwide effort being made to utilise hydrogen as an energy carrier.

In the same way that an MFC is a biological equivalent to a chemical fuel cell, microbial electrolysis is functionally a biological counterpart to chemical electrolysis. An organic substrate is oxidised by microorganisms in MECs to produce protons, which are then transferred to the cathode and reduced to hydrogen gas. The evolution of hydrogen at the cathode occurs in a MEC in a manner similar to that of conventional water electrolysis. Overall, the advantages of MECs include their capacity to completely mineralize substrates to carbon dioxide, in contrast to most chemical electrolysis analogues, and their very low energy needs to create hydrogen for the majority of substrates. Furthermore, if hydrogen rather than electrical energy is needed, choosing MECs over MFCs may be justified, although there are other technical factors that are as strong. Compared to oxygen reduction, which requires overcoming the mass transport limits in gas diffusion electrodes as well as kinetic and catalytic restrictions, designing cells for gas evolution is easier and thus less expensive.

First, employing simple separators to isolate anode and cathode solutions, and second, a membraneless cell utilised to release hydrogen from a gas electrode, are two distinct functional MECs that have been documented. Utilizing a substrate like acetate is appealing since it has significant use in waste treatment and provides a cheaper cost of operation in terms of energy. However, alternative, more energetic substrates could be able to produce more hydrogen at a faster pace for a cheaper capital cost per cubic metre of hydrogen. The energy cost of conventional electrolysis is determined by cell voltages of around 1.8 V and large current densities, which are orders of magnitude greater than in MECs. MECs must operate at low cell voltages with inexpensive cell design and materials in order to compete with water electrolysis. This implies that capital costs must be significantly reduced in addition to operational expenses. Due to the utilisation of expensive components like Pt catalysts, gas diffusion electrodes, and Nafion membranes, the great majority of MEC research that has proven the idea is not feasible.

Over the last ten years, enzymatic biofuel cells have developed quickly. The majority of the study has been devoted to developing effective electronic interactions and communications between enzyme and electrode utilising diverse strategies. Enzymatic biofuel cells have demonstrated particular advantages over conventional energy devices due to the specific activity made available by available from enzymes and the capability of miniaturisation when it comes to the demands for dependable power for medical devices for implantable applications. Enzymatic biofuel cells may employ renewable fuels with excellent energy density and safety for microelectronics in addition to medicinal uses.

Further technological advancements face significant obstacles, nevertheless. The long-term durability of the enzyme electrodes, effective electron transport between enzymes and electrode surfaces, and better enzyme biocatalytic activity are the most important challenges to achieving greater power production from biofuel cells. The next-generation enzyme's primary goals are those.

CHAPTER 6

MICROBIAL FUEL CELLS FOR THE GENERATION OF ELECTRICITY

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Scientists and researchers began exploring for solutions to capture energy from renewable natural resources in this era of growing energy demand coupled with ongoing fossil fuel depletion and well-known environmental repercussions. On a human scale, these sources are often limitless, sustainable, and provide a suitable substitute for the usage of fossil fuels. Today, there are additional issues outside only meeting the world's energy demands, such as global warming. Due to the greenhouse effect, burning fossil fuels releases greenhouse gases, mostly carbon dioxide, into the atmosphere, which raises the earth's temperature. Massive amounts of energy are stored in fossil fuels, and it takes millions of years for them to regenerate after being used. Finding alternative green solutions is essential before they are all gone. Another current need is ensuring that future generations will have access to clean water. Nowadays, there is a lot of research interest in wastewater treatment methods and technology. Scientists today recognise the value of wastewater treatment for a variety of purposes, including the provision of clean water fit for human consumption, agricultural applications like irrigation, and most crucially, the assurance of environmentally safe waste water disposal. These technologies, however, demand a substantial expense for a procedure that also needs energy. Microbial Fuel Cells (MFCs) are a major and intriguing answer to the aforementioned environmental and planetary challenges. MFCs offer a completely novel technology in which bacteria is employed to oxidise organic materials and create current, therefore power. MFCs are useful in a wide range of situations and have various advantages. MFCs technology requires expertise in a variety of disciplines, including electrochemistry, microbiology, physics, and environmental engineering. However, the principles governing how an MFC functions may be clearly seen in what is known as cellular respiration. Energy is derived from organic substrates by humans and all other living things, which is very similar to what bacteria do when they break down organic waste.

Cell electrodes for biofuel. Fong areas are crucial for advancing the technology for real-world applications and commercialization in order to solve the hurdles and accomplish these goals: Creating native enzyme molecules with desired characteristics for particular uses via protein engineering. New biomaterials and immobilisation techniques to increase enzyme stability. The addition of nanomaterials to the enzyme electrode structure to enhance electron transport and catalytic activity. New fuel cell design options that increase power production and cell voltage.

With the requirement for significant gains in power output from accessible substrates, MFC research is still in its infancy. Thus, low molecular weight fuel is still required for the majority of MFCs. In addition to energy crops like maize and other cereals, waste biomass from agricultural,

municipal, and industrial sources also contains a lot of energy that is mostly stored as carbohydrates. There may be 24 electrons available if a glucose molecule were to entirely convert to CO2, but there is no straightforward chemical procedure to collect them. Utilizing microorganisms that have a variety of enzymes to aid in this transition is crucial.

A promising technology for producing renewable energy, MFCs are most likely to be used in the near future to produce electricity while simultaneously treating wastewater. In other specialised uses, such as as power sources for environmental sensors and environmental bioremediation, they will also be helpful. With some adjustments, MFC technology may be used for anything from producing hydrogen to creating biomass-based renewable energy. A treatment system based on MFCs provide an opportunity to improve the technology since the substrate is "free" and wastewater has to be treated. Approximately 2 billion people globally lack access to proper sanitation.

The successful advancement of biofuel cell technology depends on the combined efforts of numerous academic fields: biology to comprehend biomolecules; chemistry to gain insight into biochemical reactions and electron transfer mechanisms; material science to create novel materials with high biocompatibility and maintain activity from biomoleculars; and chemical engineering to design and establish the system. This ground-breaking technology will promote the use of renewable energy sources and have significant positive effects on medical research, clinical practise, and health care administration. The cost of the building materials for MFCs will be a crucial element in the technology's effective implementation on a big scale.

Problematic

Morocco has recently shown a growing interest in large-scale renewable energy projects, particularly those using wind and solar power. Due to the scarcity of fossil fuel supplies, the nation is dependent on imports to meet its energy demands. Morocco wants to generate 52% of its power from renewable natural sources by 2030. The 160 MW "Noor solar complex" in Ouarzazate, which was started in 2016 and uses CSP (concentrated solar power) technology, is the largest project the nation has so far undertaken in terms of energy output. However, Morocco is also concerned about water shortages, not to mention the terrible techniques of dumping sewage into the ocean and untreated lands. An approximate estimate of the total amount of sewage released annually is 800 million m3, and that figure is rising steadily. Additionally, the amount of organic contaminants in wastewater is about 135,000 tonnes. In agriculture, wastewater is recycled for irrigation with little to no treatment. For all of these reasons, additional study is required in the fields of wastewater treatment and renewable energy. In particular, MFCs provide a possible remedy for both problems by producing energy while treating wastewater.

Although MFCs' potential to generate electricity has been widely explored over the last 70 years, utilising them to effectively deliver enough energy to power a device is still novel and under investigation. The primary goal of this research is to construct a microbial fuel cell using readily available components in order to evaluate its capacity to generate electricity by calculating the

current and, consequently, the maximum power generated by the cell using a multimeter along with other useful parameters. The primary reaction occurs in the anodic chamber of an MFC, where wastewater or sludge is employed as a substrate and a source of electrons. Water will be gathered from an Ifrane sewage system where waste is currently being dumped into the environment.

The developed fuel cell will be compared to ones that already exist, and specific areas for improvement will be covered. Finally, this study will discuss the potential for scaling up and commercialising an MFC. The MFC's ability to generate power will be the primary area of attention.

Sweet Analysis A Steeple

An overview of the project's many effects is provided via analysis. Studying the project's effects on many other fields is crucial, but it's as critical to keep your eyes on the goal at hand. It may also be used strategically in organisations to evaluate the many factors to take into account before to beginning any project. The following STEEPLE analysis is suitable for an MFC implementation and focuses on Morocco.

Social Consequences

An MFC's socio-environmental impact is its primary societal effect. Human welfare depends on the health of their environment and natural surroundings. MFCs take the wastewater that is released into the environment and utilise it for a variety of purposes, including the production of energy. The present vision in Morocco emphasises renewable energy sources, which also apply to rural regions.

These areas have unequal energy distribution and are often ignored. Ifrane, for instance, has a different climate than other parts of Morocco, thus the locals there use more energy to heat their homes. The majority of the countryside surrounds the city, and although while electric heaters are the most effective, they are also the most expensive option. As a result, the locals heat their homes by burning wood. MFCs may thereby address societal problems that are directly tied to a lack of power.

Technological Consequences

MFC are a novel technology that are currently being scrutinised. Research that combines knowledge from several domains and has produced excellent and encouraging outcomes is still ongoing. MFCs support continuous research into renewable energy sources. It's also critical to recognise that because an MFC's operating principle is entirely natural, MFCs pave the way for other discoveries drawn from nature to advance technology.

Financial Implications

MFCs have a significant economic impact. First, energy must be used in water purification facilities via an extremely expensive procedure. For instance, the cost of treating wastewater in

the USA is over $30 billion a year and is rising. The energy required to operate and supply water treatment stations may be recovered using MFCs, making the four stations self-sufficient. The MFC then filters the wastewater to eliminate the environmental contaminants while generating power.

Environmental Consequences

MFCs directly benefit the environment in a beneficial way. They provide an alternative to using fossil fuels as a source of energy and lower greenhouse gas and carbon dioxide emissions while simultaneously generating power. In the field of water treatment, MFCs also contribute significantly to the solution of the hygienic and water shortage issues.

Political Consequences

Sustainability is a topic that is being explored more and more throughout the globe today, not just in Morocco. Because it affects the future of the next generations, it also plays a role in important political choices. Morocco in particular depends on imports to meet its energy demands, and given the world fossil fuel reserves' apparent decline, the nation is politically motivated to provide substantial resources to the area of renewable energies. MFCs are a relatively new technology, yet they support the nation's green mission.

Legal Consequences Legally, the Moroccan government and the rest of the globe are anticipated to accept MFCs. Research in several disciplines related to renewable energies is also encouraged by the ministry of energy and the environment. Implications for Ethics It is our duty to practise environmentally friendly conduct. Small acts that resemble great ones need to be commended and welcomed. The primary driving force behind this study is the persistent rise in environmental concerns brought on by industrialization and technological growth, which are two sides of the same coin. Despite the fact that human actions are essential to ensuring our life, it is critical to be conscious of the effects we are having on the environment. MFCs are morally acceptable and appropriate. Additionally, they stand for the advancement of sustainability and the battle against climate change.

CHAPTER 7

MICROBIAL FUEL CELLS: NOVEL BIOTECHNOLOGY FOR ENERGY GENERATION

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Microbial fuel cells (MFCs) provide fresh possibilities for the environmentally friendly synthesis of energy from reduced, biodegradable materials. MFCs work with a variety of carbohydrates as well as complex substrates found in wastewater. Since few electron transfer processes have been clearly characterised, little is known about the energy metabolism and makeup of the bacteria that use the anode as an electron acceptor. This information is crucial for optimising and developing MFC energy output. Different metabolic pathways are utilised by the bacteria depending on the MFC's operating settings. This affects the choice and functionality of certain organisms. Here, we examine how bacteria produce electrical output and how they employ an anode as an electron acceptor. The MFC technology is compared to the other energy producing options.

The idea of utilising microbes as catalysts in fuel cells has been studied since the 1970s and microbial fuel cells for the treatment of household wastewater were introduc 1991. However, microbial fuel cells with an increased power output have just lately been produced, opening up potential options for real-world applications. A MFC turns energy present in a substrate that may be bio-converted into electricity. By switching from a soluble acceptor, like oxygen or nitrate, to an insoluble one, like the MFC anode, bacteria may do this. Either membrane-associated components or soluble electron shuttles may carry out this transfer. The electron acceptor is decreased when they go through a resistor and arrive at a cathode. A MFC produces electricity and an off-gas that is mostly carbon dioxide, in contrast to anaerobic digestion. In comparison to the methods already in use for producing energy from organic matter, MFCs provide operational and functional benefits. First, high conversion efficiency is made possible by the direct conversion of substrate energy to electricity. Second, unlike all present bio-energy processes, MFCs function well at ambient and even low temperatures. Third, an MFC does not need gas treatment since its off-gases are often devoid of usable energy content and are carbon dioxide-enriched. Fourth, as long as the cathode is passively aerated, MFCs do not need energy input for aeration. Fifth, MFCs have the potential to be widely used in areas without electricity infrastructures and to increase the variety of fuels we employ to meet our energy needs.

In microbial fuel cells, metabolism

The metabolic pathways controlling microbial electron and proton fluxes must be identified in order to evaluate bacterial electricity production. The potential of the anode, in addition to the substrate's impact, will also affect how the bacteria use their energy. The anode's potential will

drop as MFC current increases, requiring the bacteria to transport electrons via increasingly reduced complexes. The final bacterial electron shuttle's redox potential and, therefore, the metabolism, are determined by the anode's potential. Based on the anode potential, it is possible to discriminate between high redox oxidative metabolism, medium to low redox oxidative metabolism, and fermentation. As a result, the organisms found in MFCs to date range from stringent anaerobes to aerobes to facultative anaerobes. Bacteria may utilise the respiratory chain in an oxidative metabolism at high anodic potentials. Protons and electrons may both be carried via NADH dehydrogenase, ubiquinone, coenzyme Q, or cytochromes. They discovered that certain respiratory chain inhibitors prevented an MFC from producing electrical current. They did not employ site 2 of the electron transport chain or the terminal oxidase, but instead utilised NADH dehydrogenase, Fe/S (iron/sulphur) proteins, and quinones as electron carriers in their MFC. In MFCs, oxidative phosphorylation-based processes have often been seen, producing high energy efficiencies of up to 65%. *Pseudomonas aeruginosa*, *Enterococcus faecium*, and *Rhodoferax ferrireducens* consortia are a few examples.

This metabolic variance, together with the information on measured redox potentials, provide light on the "electrodynamics" of microbes. A MFC will initially create little current while accumulating biomass when it is run at low external resistance; as a result, it has a high anode potential (low MFC cell potential). As a consequence, facultative aerobes and anaerobes are preferred. The metabolic turnover rate and hence the current will climb as the culture expands. Lower redox facultative anaerobes will thrive in the now-moderate anode potential. However, the redox potential in the anode compartment and maybe even the potential entry of oxygen through the membrane will continue to pose challenges for stringent anaerobes. Even at low current levels, a high resistance will result in a low anode potential. This will restrict the options for bacterial selection by favouring stringent anaerobes and low redox facultative anaerobes. Mechanisms of anodic electron transport in MFC A physical transport mechanism is required for extracellular electron transfer of the electrons that must be directed towards the electrode. This may happen either by using membrane-bound electron shuttling chemicals or soluble electron shuttles. The chemicals that are a part of the respiratory chain are assumed to be the source of the oxidative, membrane-associated electron transport. Examples of bacteria that are known to utilise this route include *Rhodoferax ferrireducens. Aeromonas hydrophila*and *Geobacter metallireducens*. The steric accessibility of a component seems to be the primary prerequisite for it to function as an electron gateway. (Physical contact between electron donor and acceptor). Whether the gateway is really utilised depends on how potential the gateway is in respect to the anode (an electron will not be transferred to a more reduced electrode).

Hydrogenases are present in a large number of the fermentative species found in MFCs, including *Enterococcus faecium* and *Clostridium butyricum*. Hydrogenases were capable of participating directly in the transfer of electrons, but only in conjunction with a mobile redox shuttle. They demonstrated how hydrogenases contribute to neutral red reduction at the bacterial surface. The electron may be physically transferred by bacteria using soluble components from an oxidised (intra)cellular substance to the electrode surface. Redox mediators were often

introduced to the reactor in investigations, including neutral red, thionin, and methyl viologen. These mediators' insertion often seemed to be necessary. The creation of organic, reversibly reducible molecules (secondary metabolites) and the formation of oxidizable metabolites are the two methods in which bacteria might manufacture redox mediators for themselves (primary metabolites). For numerous bacteria, including *Shewanella putrefaciens* and *Pseudomonas aeruginosa*, the first approach was recommended. Recent research has shown that these microbial mediators affect an MFC's function or, more broadly, interfere with extracellular electron transport. A *Pseudomonas aeruginosa* MFC isolate's current generation was 20 times smaller when the mediator-producing genes were inactivated. Other bacterial species may employ the redox mediators created by one bacterium to get to the electrode. Metabolites like H_2 and H_2S are used as mediators in the second method that bacteria may make redox mediators, which is via primary metabolites. E. coli K12 was utilised by Schroeder and colleagues to generate hydrogen gas, which was then re-oxidized at a platinum-catalyzed electrode immersed in the bioreactor. They were able to achieve current densities of up to 1.5 mA/cm2 (A, Ampere) that had never been done before. Similar to this, Straub and Schink discussed how *Sulfurospirillum deleyianum* reduced sulphur to sulphide, which was then reoxidized by iron to produce additional oxidised intermediates. Parameters that determine how well MFCs function both biological and electrochemical processes are necessary for a microbial fuel cell to produce any electricity. The rate of substrate conversion The rate of biomass organic loading (g substrate per g biomass present per day), the effectiveness of the proton exchange membrane for transporting protons, the potential over the MFC, and the number of bacterial cells all play a role in this. Bacterial kinetics (mmax, the maximum specific growth rate of the bacteria, and Ks, the bacterial affinity constant for the substrate) and bacterial kinetics also play a role.

The anode has overpotentials.

The open circuit potential (OCP) of MFCs is often measured to range from 750 mV to a reported high of 798 mV. The electrode surface, the electrode's electrochemical properties, its potential, and its kinetics, together with the MFC's current and electron transfer mechanism, are all factors affecting the over potentials.

Cathode over potentials

The cathode shows substantial potential losses, much like the losses seen at the anode. Many scientists have utilised hexacyanoferrate solutions to correct this. Hexacyanoferrate should be regarded as an electron acceptor rather than a mediator since airborne oxygen does not fully reoxidize it. MFC cathodes should ideally be open-air cathodes in order to be sustainable. Performance of a proton exchange membrane. The vast majority of MFC experiments to far used Nafione proton exchange membranes (PEMs). Nafione membranes, however, are susceptible to (bio)fouling, such as ammonium. The Ultrex cation exchange membrane without the membrane and pressed carbon paper as the separator produced the greatest results. The MFC internal resistance was greatly reduced as a result of this omission [4], but this form of separation also caused growth at the cathode based on anolyte components and allowed the cathode catalyst to

be poisoned [41]. Regarding the stability of these carbon paper-cathode systems for time periods greater than a few days, there are currently no data available [4]. The MFC's internal resistance This is influenced by the membrane resistance as well as the resistance of the electrolyte between the electrodes. Anode and cathode should be as near together as feasible for best performance. Additionally, proton migration has a major impact on losses due to resistance. These losses might be reduced with sufficient mixing. data on performance Results presented in power per anode surface and power per unit of MFC reactor volume clearly differ from one another. the most significant MFC outcomes that have been published so far. The majority of research calculated power output from descriptions of traditional catalytic fuel cells and represented it as mA/m2 and mW/m2 of electrode surface, respectively. The latter may be enough for chemical fuel cells, but MFCs are different because the catalysts (bacteria) have distinct needs and occupy a different amount of space in the reactor, which reduces the amount of free space and pore size. Each research makes reference to a particular set of parameters, including the anode surface, catholyte, organic loading rate, and reactor volume with proton-exchange membrane. It is currently impossible to compare this facts. From a technical standpoint, it is helpful to utilise Watts/m3 of anode compartment volume (liquid) as a baseline for expressing the performance of the reactors. This unit facilitates comparisons between all tested reactors and other bioconversion technologies in addition to within the context of the current investigations. The coulombic and energetic efficiency of reactors differ significantly. The ratio of the number of electrons transported to those theoretically provided by the substrate is used to compute the coulombic efficiency. Energy-related efficiency also refers to the energy of the transported electrons, which includes voltage and current. The link between MFC current and power is not always clear-cut, as seen in Table 2. The electron transfer rate at a certain potential and fine-tuning of the operational parameters, such as the resistance, need to be stressed.

In light of this parameter problem, it is necessary to decide if the final objective is maximum coulombic efficiency (for wastewater treatment, for example) or energy efficiency (for small-scale batteries, for example). The current range of measured power outputs for electrode surfaces ranges from mW/m2 to several W/m2. The choice of appropriate bacterial consortia and the adaptation of the bacteria to the optimal reactor conditions are implied by biological optimization. The pace of enrichment will be substantially determined by the choice of the bacterial inoculum, although the structural output of this method is not affected by this. After three months of microbial adaptation and selection, it was possible to see seven-fold increases in bacterial substrate to power conversion rates utilising glucose as feed and a mixed anaerobic-aerobic sludge inoculum. When there were bigger anode surfaces accessible for bacterial growth, increases were seen much more quickly. The concentration of organisms that can make soluble redox mediators is made possible by batch systems. Continuous systems favour biofilm-forming species that may either grow directly on the electrode to utilise it as an electrode or move electrons across the biofilm matrix via mobile shuttling molecules. A batch anode may be technologically optimised by the use of soluble redox mediators: redox mediators have been applied to MFCs and reliably increased electron transport. A low mediator potential, in the range of K300 mV or morereduced, was typically considered to be favourable in the empirical

selection of these mediators so far. Depending on whether high coulombic or high energy efficiency is the goal, redox mediators with the ability to allow bacteria to have a suitably high turnover rate in respect to the electrode should be chosen. By incorporating chemical catalysts into their anode materials, some researchers have created better anode materials. Power outputs up to 788 mW/m2 were produced using manganese-modified kaolin electrodes by Park and Zeikus.

A greater biofilm surface and a lower current density (which in turn reduces the activation overpotential) are made possible by increasing the specific surface of the anode. There is a clear limit to this however since germs may quickly block microscopic holes. Bacteria that have been separated from the food supply may decompose, reducing the electrode's active surface before lysis. The internal resistance and activation overpotentials will have the biggest an impact on power output.

Microbial fuel cells are developing into a straightforward, reliable technology. Certainly, middle-term use at market-value rates is possible in the wastewater treatment industry. However, additional technical advancements are required to raise the power output toward a steady 1kW per m3 of reactor. This technique may become a new fundamental method for converting carbohydrates into power in the future, provided that biological knowledge grows, electrochemical technology develops, and overall electrode costs fall.

Running Physicochemical Elements How a Microbial Fuel Cell Performs

Using the metabolic processes of live microorganisms, microbial fuel cells (MFCs), a family of developing technologies, have the rare capacity to simultaneously convert chemical energy into electrical energy. In addition to producing energy, this method has the potential to provide a number of applications or value-added goods, including biosensors, the synthesis of volatile fatty acids, the removal of heavy metals, bio-hydrogen, wastewater treatment, and water desalination. Despite these benefits, MFCs still have technical issues with low power and current density, which restricts their usage to only small-scale devices.

Elecrode-microbe interaction

The method that microorganisms cling to the terminal surface and the following electron transport is one of the most crucial features of MFC innovation. The fundamental requirement is that electrons be moved from the interior of the living microbial cell film to the external layer—either through the actual exchange of diminished compounds or by electron bobbing across the film using film-bound redox proteins—because terminals are surfaces that cannot enter bacterial cells.

Regardless of the technique, the extracellular electron transfer must result in a redox-active species that can link the bacterial cell to the electrode electrically. This species might be a soluble redox shuttle, a reduced primary metabolite, or an outer membrane redox protein. There have been a number of hypotheses up to this point about extracellular electron transfer (EET) activities between active microorganisms and electrodes.

Electrode Materials' Impact on Biofilm Formation

Because it provides the scaffold on which the biofilm develops and exchanges electrons to carry out anodic respiration, the electrode material is crucial in the creation of biofilms. The material employed determines the majority of the surface characteristics of bioelectrodes, including morphological structures, porosity, hydrophobicity, conductivity, charge, and bio-compatibility. These characteristics have a direct bearing on the process of microbial attachment and cell survival. The porosity of the electrode adds to an increase in surface area, which provides additional surface area for colonisation and attachment by live microorganisms. It has been shown that hydrophilic and positively charged electrode surfaces promote the development of conductive biofilms by electroactive bacteria. Positively charged electrodes are favoured because they provide a favourable electrostatic surface for negatively charged bacteria to cling to. Since many bacteria contain a net negative charge, this is the cause.

Challenges Mediated-MFCS

Some bacteria need soluble chemical redox mediators because they lack the electrochemical activity to transport their electrons to the electrode surfaces. There have been several efforts to break through the kinetic and thermodynamic obstacles to accelerate electron transit. In the microbial respiration cycle between the bacteria and electrode, mediated electron transfer is the process of transferring electrons by substituting synthetic molecules for oxygen to receive the electrons.

Hydrophilic mediators may interact with proteins in the periplasm but are unable to penetrate the cell membrane, which enhances the efficacy of microbial fuel cells. This is due to their high water solubility or high diffusion coefficient in aquatic environments. Menadione is one example of a lipophilic mediator that may diffuse outside of the cell and interact with the mitochondrial and cytoplasmic redox centres there to move electrons to the electrode surface. They cannot, however, cross through the cell membrane.

By allowing intracellular redox processes, expressing intracellular cell metabolic activities of target cells, or allowing high current signal intensity, a double-mediator system made up of a lipophilic and a hydrophilic mediator will compensate for each other's shortcomings and solve the aforementioned issue. The potassium ferricyanide-menadione double-mediator technique is often used to examine the redox activity of human cells and yeast cells of the *S. cerevisiae* species.

MFCs are a novel, ecologically friendly method for oxidising a variety of substrates to produce energy; nevertheless, substantial problems need to be fixed before the technology can be used commercially. The main difficulty is active biofilm generation, which is crucial to MFC's efficiency. Understanding electroactive biofilms, electron transfer channels, and the factors affecting biofilm growth are thus critical. The need of a practical architecture for scaling up MFC is also highlighted in this review study. High internal resistance, electrode spacing, or anolyte and catholyte exchange across PEM for scaling-up or long-term operation are common

drawbacks in most designs. Another challenge is finding reasonably priced PEM and electrode materials for MFCs. In the past, several conventional anode materials were developed, but they were unable to meet the demands of the time. Increased electron transfer rates and great MFC performance would be produced by a good microbe-electrode interface and the fundamental conductive properties of the ideal anode. The best materials for meeting current needs while minimising other drawbacks are natural resources and their composites. The choice of electroactive bacteria poses an additional issue since the kind of material to be utilised in the MFC system depends on how the charges on the electrode surfaces interact with those on the microbial surface. Before MFCs may be used on an industrial scale, further work has to be done to solve these challenges and enhance their overall performance.

On the other hand, oxygen reduction electrocatalysis is essential for a number of applications, such as microbial fuel cells. The cathodic oxygen reduction process demands the development of effective, high-performance electrocatalysts in order to permit power output in MFCs. The actual use of these technologies requires the replacement of expensive noble metal-based electrocatalysts with highly efficient and cost-effective nanomaterials for ORRs.

Diagnosis Tools for MFC Evaluation

Microbial fuel cells (MFCs) are a clean, renewable technology that might generate electricity right from biodegradable waste. Typically, this conversion is carried out by electrogenic bacteria, which may degrade both organic and inorganic substrates via their metabolisms and transfer the freed electrons to a solid electron acceptor (the electrodes). An electrolyte, an anode, a cathode, a separator, and electrical circuits are among the components that make up a conventional MFC. Each of these components is essential in demonstrating the overall efficacy of MFCs. MFCs presently have insufficient power outputs, which restricts their usefulness. To eliminate the bottlenecks, a deeper understanding of each component's capabilities and constraints is required

Over time, a lot of advancements have been made in the development of fresh analytical techniques to evaluate the performance of MFCs. The MFC technology as a whole incorporates elements from electrochemistry, molecular biology, microbiology, material science, and other related disciplines. Therefore, selecting the appropriate diagnostic tool requires a full comprehension of all diagnostic processes and the underlying concepts. The majority of techniques used to study chemical fuel cells, such as polarisation, voltammetry, and others, are often also used to study MFCs. These variations must be taken into account while doing the analysis or interpreting the data since the operating conditions and performances of each of these systems vary substantially.

Using electrochemical impedance spectroscopy for analysis (EIS)

The EIS is a crucial electrochemical instrument that the researchers use, specifically to measure the internal resistance of the cells. An MFC encounters three distinct types of significant polarisation losses, as was previously mentioned. EIS may provide information on the relative

contributions of each loss as well as an estimate of the cost of these losses under various operating scenarios. Because of its simplicity, non-destructive nature, and ability to gather a range of informative and helpful information about the cells, it is commonly employed by numerous studies to investigate MFC function. The properties of biofilms, the development and analysis of several novel electrodes, the comparison of different system components, as well as the analysis of various system losses, have all been accomplished using EIS. The fact that the cell under consideration is exposed to a known AC (alternating current) current makes this technique superior to others that use DC.

A potentiostat is the essential piece of gear required to conduct this experiment. Depending on what is being studied, the method may be used in either a two-electrode mode or a three-electrode mode. In the two-electrode technique, the working electrode is the anode/cathode (basically the reaction centre or the component that has to be investigated), and the counter electrode is the other electrode. When the reference electrode for the cell is connected to the reference potentiostat as well, similar connections are made in the three-electrode mode. A small electrical AC perturbation is applied to the system, and the resulting impedance and phase shift are monitored over time.

During an EIS scan inside a certain frequency range, a small AC sinusoidal signal is sent to the system, and the outcomes are shown as a Nyquist plot and a Bode plot. The Nyquist plot, where each point represents the impedance value at a certain frequency, is a simple example of the real and complex components of impedance.

The graph mostly shows a semicircle, however in rare occasions a straight line may be shown after the semicircle. While the straight line depicts the cell's capacitance, the semi-diameter circles show the cell's charge transfer resistance (Rp) or polarisation resistance. The Bode plot gives additional information about the frequency, phase angles, and impedance. However, in practical implementations, the impedance graphs are complex concoctions of capacitances or resistances that vary from system to system.

Electronic Transmission Microscopy (TEM)

By accelerating an electron beam that is passing through the object, TEM creates a picture. A thin specimen (less than 200 nm) is projected with electrons that have been accelerated to 100 KeV or more (up to 1 MeV), where they are either undeflected or deflected by the condenser lens system. The ability to gather image and diffraction data from a single sample as well as the extensive magnification range of 50 to 106 are two of the TEM's key benefits. TEM analysis is widely used to quantify the size, distribution, and shape of nanoparticles utilised as catalysts on the electrode surfaces of MFCs. TEM provides helpful information on the spatial organisation and cellular ultrastructure of the biofilm in addition to material qualities. Therefore, internal cross-sectional detail of each bacterium as well as the complete biofilm can be seen using TEM. The cost and amount of time needed for sample preparation are the main downsides of electron microscopy.

Additional Molecular Methods

Other molecular techniques for locating and describing microbial biofilms and communities include fluorescence in-situ hybridization, flow cytometry, and CLSM (confocal laser scanning microscopy).

Waste and Wastewater Analyses

The amount of waste eliminated by bacteria is assessed by MFCs using conventional laboratory analytical procedures or test protocols developed by the APHA (American Public Health Association). These techniques use a range of diagnostic tools, including measurements of hardness (mg L1 as CaCO3) by spectrophotometer, volatile residue by Muffle Furnace, inorganic anions by ion chromatography, alkalinity by colorimetry, total Kjeldahl nitrogen, biochemical oxygen demand (BOD), chemical oxygen demand (COD), and others. There are more sources where you may get the theories and guiding principles of these procedures.

This chapter describes a variety of approaches that have been used in the literature to look at and evaluate both the general performance of the MFC system and its individual parts. The fundamental ideas behind the techniques were covered in terms of methodology for material analysis, microbial community structure, wastewater analysis, electrochemical analysis, and MFC performance evaluation. To better understand the underlying electrochemical processes that take place in MFCs, novel electrochemical methodologies must be developed.

Microbial Fuel Cell Perks

Humans think that germs and bacteria do more damage than good, therefore the realisation that we can employ them to our advantage greatly pleases us. As the world becomes more advanced, finding alternative affordable clean, renewable, and conveniently available sources of energy is essential. Nature was the first microbial fuel cell since it has been converting organic substrates into energy (Adenosine triphosphate, or ATP) since the beginning of time. The technology of microbial fuel cells, which is still under development, uses hydrogen produced by bacteria' metabolic activities to produce electricity. Using MFCs is very environmentally friendly since it decreases pollution and sharply lowers the cost of water filtration. MFCs may desalinate water and provide stable power sources to isolated people in addition to being a source of energy.

Most microbial fuel cells could seem to be a new development. During his research, Potter believed that the possibility of harnessing the electricity produced by bacteria for human purpose would be revealed. Potter understood the idea of a microbial fuel cell but had no idea how microbes worked.

Future uses for microbiological fuel cells

Microbial fuel cells are an emerging technology that uses bacteria to create hydrogen and energy from waste, and the fact that they are now in use demonstrates this. By using this hitherto untapped source of power, clean, sustainable energy may be produced at a low cost. It is feasible to remediate wastewater using microbial fuel cells. Microorganisms connected to sewage may be

eliminated using MFCs. It has been shown that MFC may cut the amount of microorganisms in wastewater by 80%. During pre-treatment, toxins and other non-biodegradable pollutants are first removed from the wastewater. This process is considered challenging since it requires extensive treatment of noxious and dangerous sewage water before MFC can be used to clean the water. High operational sustainability or low material costs are necessary for efficient wastewater treatment.

Seawater Escalation

Microbial fuel cells can create energy, but only to the extent that large amounts of water can have the salt removed. It is possible, nevertheless, to carry out such a method, enabling the desalination of saltwater without the need of external power sources. To assess the desalination process, researchers employed a microbial fuel cell. This fuel cell is notably different from the ones previously mentioned in that, as seen in figure 7.1, there is a third chamber for the saltwater in between the two electrodes.

The negative and positive ions are attracted to the prospective electrodes as the proton-electron membrane removes the salt from the water, leaving it fresh. According to experts, up to 90% of the salt may be eliminated, but more effective removal processes are needed to produce grade A water.

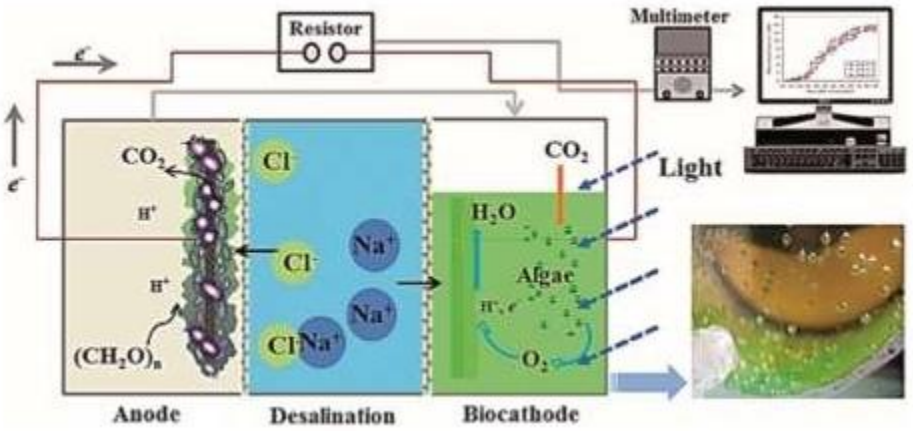

Figure 7.1: Illustrate a water, efficient removal procedures are required.

With the aid of a microbial fuel cell, hydrogen might be produced. This procedure converts the bacteria into hydrogen and carbon dioxide gas, however it does need an external power source (JD Coates).

Protons are released during the anodic reaction and travel through the PEM and toward the cathode, where they combine with oxygen to form water. The temperature of the hydrogen created by the microorganisms metabolising in an MFC from the electrons and protons produced may be dangerous.

Energy Source

Microbial fuel cell technologies were developed and advanced primarily with the goal of bringing reliable, inexpensive power to remote areas of the world, such as African villages where the bulk of the population lacks access to modern amenities like electricity. The use of homemade microbial fuel cells in Africa is still relatively recent. Studies are using manure and soil, which will be useful for those who reside in rural areas where the technology may be made as cost-effective as feasible. The electrical current produced by a simple DIY MFC may be used to recharge a mobile phone, which is a crucial communication and lighting equipment for rural African communities. The goal is to create MFCs that can be assembled from easily accessible, basic components. Dirt, dung, buckets, copper wire, and graphite fabric must be used to construct a simple MFC.

A bright future is now possible for both humanity and the planet we call home thanks to microbial fuel cells. The work that has been done so far and the applications that we already have will help us lessen our reliance on fossil fuels and allow us to produce energy from waste materials, bacteria-infested water, and previously salted water. These developments will also greatly benefit third-world nations as well as areas that are experiencing drought. Additionally, it will enable us to utilise previously salty water for farming, drinking, and other purposes. Additionally, it will provide energy to areas that now lack it. Although MFC technology cannot instantly alter the world, researchers are looking at methods to contribute to a shift over time. Microbial fuel cells have the potential to lessen our dependency on coal-fired power plants while also making the world a safer place to live, even if they may never be able to generate enough energy to do so.

Future challenges for microbial fuel cells

The coming energy crisis caused by the depletion of fossil fuels and the planet's warming as a consequence of greenhouse gas emissions justifies the urgent need for environmentally friendly energy sources. Fossil fuels kill animals as a result of the CO2 emissions they cause in the atmosphere. Therefore, owing to its severe repercussions, such as climate change and environmental pollution, the usage of energy sources based on fossil fuels has seriously endangered human survival.

Microbes and the Electrode Surface Interaction

Microbial interaction with the electrode surface has a substantial impact on the MFC's performance. The electrochemical behaviour of MFC depends on how many exoelectrogenic bacteria are in contact with the electrode or anode surface. In this instance, a fundamental requirement is that the bacterial biofilm be sufficiently anchored to the anode surface. This bacterial adhesion to the electrode may be explained by the concept of surface charges. Since most naturally occurring microbes are negatively charged, they are drawn to surfaces that are positively charged. Therefore, a wide variety of surface modification methods have been used to

facilitate this charge attraction process. For instance, researchers have been successful in treating the electrode surface with NH3, which makes it easier for negatively charged bacteria to cling to the electrode (anode) surface. Furthermore, when using NH3-pretreated electrode material, the power output of the MFC showed enhanced performance compared to untreated electrode material. However, the cost of this process has grown due to the high temperature required for this NH3 pretreatment, rendering it an economically unviable technology.

A MFC-Based Wastewater Treatment System

MFCs, also known as hybrid bio-electrochemical treatment (BET) systems, have recently acquired acceptance for applications outside energy generation, such wastewater treatment. Microbes use all the chemical energy present in the substrate/wastewater when oxygen is present, even though only a small portion of the chemical energy is available to the anodic microbial population in MFCs for their metabolic and growth activities because a significant amount is converted to electrical energy. The information above indicates that the redox potential of exoelectrogenic bacteria against SHE is around 0 V. Exoelectrogenic biocatalysts in MFC reduce the anode via the membrane surface using free energy produced by the substrate/wastewater oxidation. Because the bacteria only utilise a tiny percentage of the energy from substrate oxidation and the remaining energy is transferred to electrical energy, the yield in MFCs is nearly one-fifth that of an aerobic microbe. This illustrates how MFCs may significantly lower the expenses related to sludge disposal throughout the wastewater treatment process.

Future Prospects

We must talk about low power densities in MFC operation to prevent losses brought on by activation, ohmic, or concentration overpotentials. Targeting losses caused by unnecessary occurrences such as microbial metabolic reactions that do not progress the process or the direct oxidation of fuel by O2 diffusion into the anodic chamber is also necessary. On the other hand, it is essential to maximise internal energy savings while increasing system volumetric capacity. Stacking MFCs is a common solution to prevent catastrophic losses in this direction. There is also active research on tubular and other multilayer approaches. The population's exoelectrogenic microbial density must also be increased since it seems to be restricted for factors other than the availability of attachment locations on the electrode surface. Through bioaugmentation and potential field effects that may already present in the electrode owing to its favourable form and conductivity, this population density may be augmented. Ongoing efforts are made to establish better e-transfer paths between the electrode and the biocatalyst by changing electrode surfaces and coating them with active catalysts.

MFCs have a promising future as a bio-based reactor that transforms substrate chemical energy into electrical energy through a series of bio-catalytic reactions under anaerobic circumstances. Their primary use is in stationary power generation. MFC technology is the use of microorganisms to generate bioelectricity by oxidising organic substrates, which may range from synthetic substrates like acetate or glucose to a complicated mix of organic substrates made up of

food, dairy, distillery, animal, and domestic wastes [5]. The anodic biocatalyst will grow properly and form an electrogenic biofilm on the electrode surface at startup if the MFC is operated under ideal anodic circumstances, both of which will boost the fuel cell's efficiency over time.

Future Potential as well as Microbial Fuel Cell Challenges. Additionally, no external energy input is needed for open-air cathode or single-chamber MFCs. It could thus be practical for broad usage in areas lacking electrical infrastructure. This MFC technology hasn't technically matured yet because to obstacles to active mass and charge transfer as well as difficulties in persuading bacteria to work with permanent e-acceptors. Recent developments in material science and nanotechnology may provide specialised equipment for creating, transferring, and using the electrical energy collected by MFCs in the future for beneficial purposes.

Microbiological Fuel Cell Use as a Biosensor

Microbial fuel cells (MFCs) are being studied as a biosensor for a range of applications. It is crucial to utilise MFC as a sensor for the accurate and simple detection of various analytes since the power supplied by an MFC is now too low to power any useful devices. The following chapter covers a number of potential applications of MFC technology in the field of perception, with a particular emphasis on the bio-sensing of biochemical oxygen demand (BOD). The use of MFCs as BOD biosensors is one of the areas that has received the most attention, followed by the detection of volatile fatty acids or toxicity.

Microbiological sensors

An enzyme, microbe, or other biologically active substance acts as a bio-receptor in response to an analyte and a change in its concentration, and a transducer converts the response into a quantitative output. Additionally, an amplifier may be used to magnify the signal for covert reasons. The analyte and the biological sensing element are often taken into consideration when choosing a transducer. It is ideal to have biosensors that can offer speedy findings for the parameters that need to be examined while also being reproducible and portable.

Microorganisms are perfect for use in microbial biosensors in general because they can act independently, are easy to develop and maintain, and are also simple to adapt to and regenerate. In contrast to enzyme-based biosensors, which need a precise microenvironment to sustain the activity, microbial biosensors may work utilising a variety of substrates or habitats depending on the growing circumstances. Since enzymatic biosensors do not go through any kind of purifying procedure, employing microbes is far less costly than doing so. The same microorganisms may be utilised to detect several analytes depending on the transducer in use. Microbes also benefit specifically from the wide range of immobilisation strategies that may be utilised to entrap them while keeping them active. Furthermore, genetic engineering techniques have opened up new possibilities or applications for the field of microbial biosensors. Even though they may never be able to produce enough energy to achieve it, microbial fuel cells have the potential to reduce our reliance on coal-fired power plants and make the world a safer place to live.

The principle of the MFC as a biosensor

Electroactive microorganisms function as biocatalysts in the anaerobic anode chamber of an MFC by using the available nutrients. The concentration of the nutrient solution in the anode chamber is inversely proportional to the quantity of electrons produced by the metabolism of the nutrients contained in the medium. These electrons go to the cathode, where oxygen reduction occurs, through the external circuit. A proton exchange membrane that only permits protons to be delivered to oxygen reduction separates the cathode and anode chambers from one another. Baroreceptors, which are electroactive bacteria that are often attached to the transducer (anode), are able to precisely detect changes in the concentration of analytes. As indicated in Figure 7.2, the anode chamber's metabolism typically produces electrons that are tracked and utilised as the sensing signal.

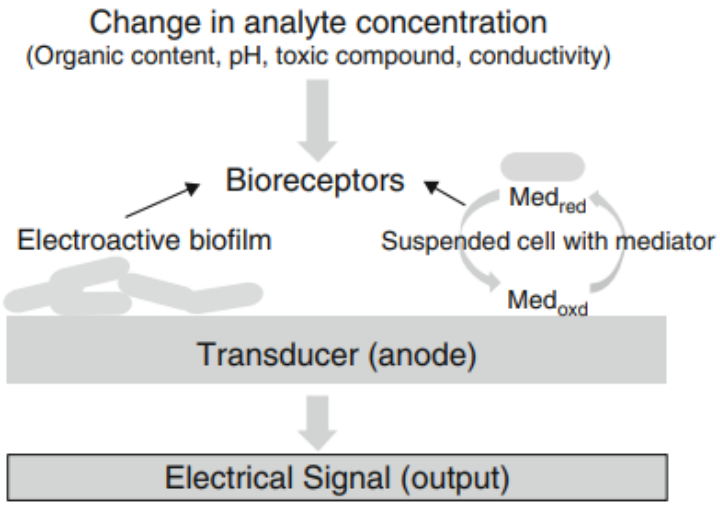

Figure 7.2: Illustrate the anode chamber's metabolism generates electrons, which are monitored and used as the sensing signal.

The Advantages of MFC as A Sensor

An MFC utilised as a biosensor eliminates the need for a separate transducer by acting as a biofilm anode. The ability of the bacteria to reproduce in the accessible volumes also contributes to the expansion of the microbial population, which gives rise to the possibility of self-regeneration. As a result, an MFC may be able to launch independently and work as a stand-alone biosensor. Depending on the size of the anode chamber and the bacteria used, the range of current produced by the cells is between mA and A. As a result, the output signal from the MFC, which is often represented by current or voltage, is simple to read and comprehend. The devices also don't need pricey probes, complicated construction methods, or labor-intensive maintenance throughout manufacture.

MFC'S Benefits as a Sensor

By serving as a biofilm anode, an MFC used as a biosensor reduces the need for a separate transducer. The capacity of the bacteria to multiply in inaccessible areas also adds to the expansion of the microbial population, which opens the door to self-regeneration. An MFC could thus be able to start up autonomously and function as a standalone biosensor. The range of current generated by the cells is between mA and A, depending on the bacteria utilised and the size of the anode chamber. As a consequence, it is easy to read and understand the output voltage from the MFC, which is often expressed by current or voltage.

One day, MFC could be developed into potential biosensor solutions that are marketed for use in practical applications. To fully use the system's built-in particular features, an interdisciplinary approach including the collaboration of researchers from other fields is necessary. The potential for MFC-based biosensors to be employed in practical applications is improved by the system's greater sensitivity and reproducibility. Miniaturization and multiplexing may also be used to swiftly and easily quantify a variety of pertinent analytes.

Microbiological Fuel Cell Application

Microbial fuel cells play a key role in the treatment of wastewater. Removing organic carbon waste may result in the production of electricity. Industries that produce wastewater with a high concentration of quickly degradable organic carbon are a suitable match for this purpose. Examples include the food industry, dairy farms, breweries, the bioproducts business, and the biofuels industry, which includes biorefineries.

Brewery management Wastewater may be handled by microbial fuel cells since the effluent from breweries and food producing facilities is a rich supply of organic molecules that bacteria may consume as food. Breweries are ideal locations for microbial fuel cells because the wastewater they consistently generate has a consistent composition that helps bacteria adapt and grow more efficiently. The power generated by cleaning the brewery wastewater is expected to pay for the original investment in the MFC in ten years.

Treatment of sewerage

It is also possible to employ microbial fuel cells to transform sewage effluent into a system that breaks down organic waste. Microorganisms are capable of both power production and wastewater degradation. MFCs are presently being taken seriously as instruments for producing energy while treating municipal, industrial, or agricultural wastewater.

A steady flow of electrical current is produced when bacteria oxidise organic compounds present in wastewater. If improvements can be made to the power production in these systems, MFCs could provide a novel approach to offset the operating expenses of wastewater treatment plants. If so, both industrialised and poor countries will be able to afford sophisticated wastewater treatment. Furthermore, compared to aerobic treatment, MFCs are known to create less additional sludge.

Desalination

Desalinating saltwater or brackish water for drinking has always been a challenge since it requires a lot of energy to get rid of the dissolved salts in the water. By deploying a modified microbial fuel cell, this operation may be carried out without the need for any external electrical energy input. Between the two electrodes of the standard MFC, a third chamber is inserted and filled with sea water. The cell filters the salt from the saltwater by using semi-permeable membranes and both positive and negative electrodes to attract the negative and positive salt ions in the water. Synthesis of Hydrogen A fascinating solution may be MFCs that generate hydrogen from organic waste. In such devices, the cathode chamber is maintained anaerobic and the cathode is given an additional 0.25 V of voltage. These conditions cause protons to be converted to hydrogen on the cathode. These modified MFCs are referred to as "bio-electrochemically assisted microbial reactors."

District Sensors

MFCs may be used to power low-power sensors that collect data from remote locations. a simple microbial fuel cell composed of a metal wire connecting the anode and cathode. The anode is placed in the anaerobic silt of a river or ocean, while the cathode is placed directly above the sediment in the aerobic water. This creates a current. Anaerobic bacteria may provide the little amount of current needed to charge a capacitor and store energy for the sensor since they naturally grow in silt. Microbial fuel cells (MFCs) have a much longer lifetime than ordinary batteries due to the fact that the bacteria proliferate; this is one significant advantage over using regular batteries in remote sensing. As a result, the sensor may be placed in a distant area unattended for a long period of time.

Remediating Contaminated Lakes and Rivers

Microbial fuel cells may also be used to bioremediate water containing organic pollutants like toluene and benzene, which are compounds generated from gasoline. In the MFC design, the fuel cell is adapted to float on top of polluted water. The anode is submerged in the water, feeding the bacteria that are fed by organic pollutants, while the cathode floats on top of the water. By converting the organic contaminants into carbon dioxide and water, the polluted lake or stream is cleaned up. The MFC may be left unattended in distant natural bodies of water, much as the remote sensor.

Generating Energy from Bio-Waste and Organic Materials

Direct use of organic materials and biowaste in the generation of power. This energy may be used by the waste treatment plant or sold to the energy market. In addition, the power generated may be used to create hydrogen gas. The energy might be temporarily stored as hydrogen as a buffer since waste flows are usually unexpected.

Off-gases from anaerobic activities generally include significant volumes of nitrogen gas, hydrogen sulphide, and carbon dioxide in addition to the intended hydrogen or methane gas.

However, MFC off gases often have little economic value since the anode received the majority of the substrate's initial energy. By moving the energy of the chemical in the form of electrons in the direction of the anode, the bacteria accomplished the separation. So long as there are no detectable concentrations of H2S or other odorous chemicals in the gas generated by the anode compartment and no aerosols carrying unfavourable microorganisms are discharged into the environment, the gas produced by the anode compartment may be released.

CHAPTER 8

DESIGN AND TESTING OF MICRO FUEL CELL

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Uses for Microbial Fuel Cells MFCs often serve two purposes, and this depends on the fuel source. The most extensively researched use is feeding different wastewater streams. The garbage in the water is digested by biofilms on the anode, resulting in cleaner water and the production of power from the ions produced. By choosing the right bacteria to inoculate the fuel cell, MFCs may be configured to treat a range of wastewater streams. These systems' development is the focus of many research groups, which will be further covered in the sections that follow. It is generally known that Ieropolous *et al.* used urine in MFC systems. One research employed a parallel stacking MFC system that was tested in 2015 at the Glastonbury music festival, where attendees used urinals that were expressly intended to collect urine and process it straight via the neighbouring MFC system. Over the course of three months, the electrical generation was observed using a light-emitting diode. The device was able to treat 2.5–5L of urine per day with an average power of 75 mW and 98% COD elimination during its peak performance.

There are further, less well-known uses for MFC. For a variety of applications, miniature MFCs have the potential to function as in-line real-time biosensors. An MFC will create a continuous steady voltage when fed continuously under circumstances that keep a biofilm stable. However, if the make-up of that feed changes, a shift in voltage will take place. This has uses in medicine and may be used to identify overall pollution in streams as well as track the amounts of particular chemicals in streams.

Producing fresh water may be problematic when there is biofouling in the desalination process, especially because it is tricky to identify biofouling. Being the primary food supply for fouling bacteria, absorbed organic carbon (AOC) levels in water are a crucial biofouling indicator. AOC may be found using time-consuming and consequently expensive techniques including flow-cytometric enumeration, biochemical oxygen demand BOD5, and bioluminescence. In order to offer inline real time monitoring of AOC, Quek *et al.* devised a two chamber MFC with graphite granular electrodes. This quickly reveals if input streams need pre-treatment before desalination. These compact systems provide the chance to power implanted medical devices (IMDs) in vivo as well. Current approaches employ lithium ion batteries, which need to be changed out often. Natural gut bacteria may be employed as the inoculation for the electrodes and intestinal fluid used by implanting an MFC into the large intestine. Use and Development of Microbial Fuel Cells. Additionally, their findings showed that these fuel cells had no discernible effects on the body, indicating that these systems may provide durable, secure power to gadgets.

Simple setup

Throughout their evolution, microbial fuel cells have taken on a variety of distinct shapes. Beyond the essentials, the anode has to be enclosed in a chamber to provide the anaerobic environment; the chamber may be made of glass, polycarbonate, or Plexiglas. The cathode compartment offers a little more flexibility since it may be either enclosed like the anode or left exposed to the environment as it is aerobic.

Maintaining anaerobic conditions at the anodes is the major challenge in MFC design, as shown in below figure 8.1. This is due to the fact that the compartment cannot be simply shut since carbon dioxide is produced as the organic substance breaks down. If the gas is not let out of the compartment, pressure will build up, harming the Nafion membrane and rupturing the fuel cell as a result. As a result, the anode compartment has to be built with a bleed for the carbon dioxide to go, and in order to prevent air from flowing back into the anode, the anode must bleed into a vessel that is filled with liquid.

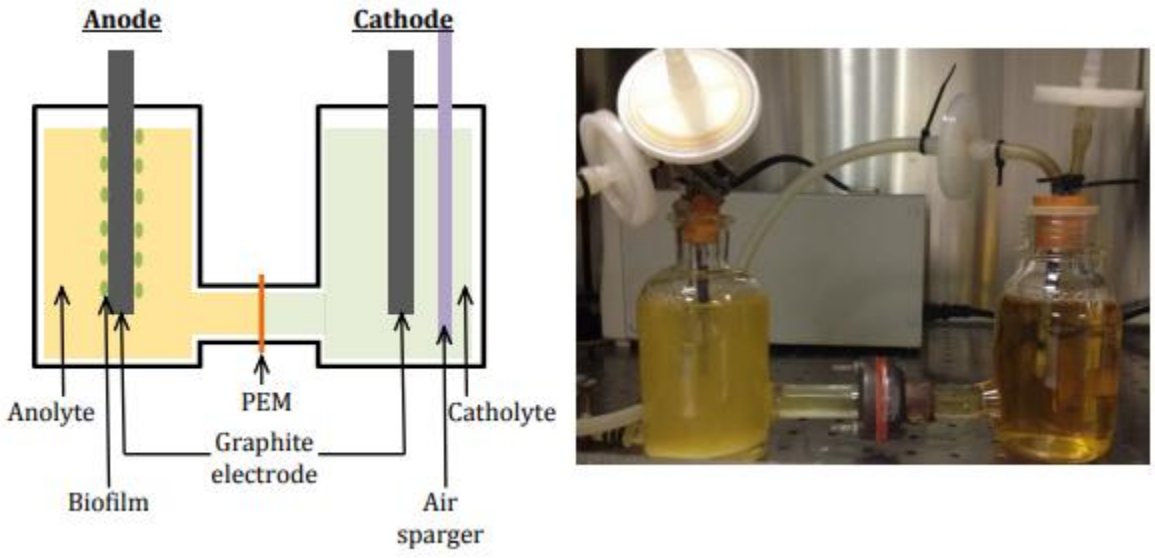

Figure 8.1: Illustrate the setup of Micro fuel Production.

Early research concentrated on the conventional H cell structure, which consists of two transparent chambers separated by a proton exchange membrane (PEM). Recent research has used a one chamber method, as seen in the figure above, which directly exposes the cathode to air or oxygen as opposed to in solution. Due to its simplicity and effectiveness for systems concentrating on the production of greater current, this design has gained popularity. The distance between the cathode and membrane is shortened when an air cathode is used since no room is needed for the solution. The cathode may therefore rest directly on the membrane in this situation. The overall advantage of this is a shorter distance between the two electrodes, which immediately lowers internal cell resistance and makes it easier to assist ion movement. The most popular lab-scale systems are often batch systems with a range of chamber shapes and layouts

linked by a proton exchange membrane. Batch systems, however, are not the most useful ones for treating industrial wastewater. The continuous system's primary downside is the need to pump wastewater through the fuel cell, which uses more energy than the fuel cell can provide. Eliminating its usage as a power source as a result

Electrode substance

To create an MFC with excellent performance, it is essential to consider the material that makes up the electrode surface. The electrode not only serves as an electron acceptor and donor, but it also gives the biofilm a place to develop. Both essential includes an air cathode MFC with a graphite brush anode and anolyte that has been infected with *Rhodopseudomonas palustris* DX-1 to give it a red hue. PEM Biofilm on Graphite Anodes Anolyte The electrical conductivity and mechanical robustness of the electrodes' graphite cathodes are essential for supporting bacterial growth and preventing the passage of potentially massive amounts of water while also easily transmitting ions. A little increase in internal electrode resistanceone or two ohmscan cause a significant reduction in power. In addition to these two essential characteristics, anodes in particular should ideally have a large surface area and porosity to support the quality and development of biofilms, as well as be inexpensive and easily accessible. The electrode material must also be biocompatible, non-toxic to species, and ideally have little fouling due to the biological nature of the system. The species being employed will influence whether the material is suitable since different species have different cell characteristics such as size, type, charge, usage of cellular appendages, and electron transport mechanisms. These systems now employ three basic materials: carbon-based, metal-based, and mixed carbon and metal-based.

Made of carbon

Although carbon-based electrodes may provide a reasonably inexpensive material with good electrical conductivity, their mechanical strength is compromised by their fragility, which is a challenge for long-term fuel cell applications. Despite this, carbon-based electrodes are favoured because they have undergone the most extensive research and testing in a broad range of configurations. The electrodes made of carbon paper, carbon cloth, and carbon felt are the most often studied. These materials don't, however, have the right properties for adhering to biofilms. The thin 2D structure, which allows for the placement of electrodes closer together and reduces the distance between the electrodesone of the primary causes of ohmic loss in an MFC setupis their principal benefit over graphite competitors. Reticulated vitreous carbon (RVC) and graphite, which are commonly utilised in the form of rods or brushes, have both been tested with varied degrees of success; brush clogging and high resistance from RVC have both been observed. The performance of the carbonaceous anode has been attempted to be enhanced. Some notable examples include chemical treatment, such as covering electrode surfaces with ferric oxide, and ammonia treatment, which witnessed a 48% increase in power generation. However, the utilisation of costly equipment, high temperature requirements, and extended treatment durations adds to the expense of these materials' alteration. These extra expenses have a detrimental effect on a system. There have also been created alternative innovative materials.

Commercial towels were carbonised at 1000°C under nitrogen before being combined with adhesive and placed on a carbon plate with a stainless steel wire running through it to serve as an electrical connection. During testing, the material produced a maximum current density of 0.80 0.06 mA cm2, which was greater than that of graphite felt, carbon cloth, and carbon mesh. By increasing the anode's specific surface area using additives like activated carbon, carbon black, and nanoparticles, the electrode and microorganisms may interact with each other more effectively.

Another very promising improvement for anodes is carbon nanotubes. They provide a large surface area, strong mechanical stability, high conductivity, and good mechanical characteristics. They are utilised to coat and alter electrodes rather than being used in bulk due to their high cost. A thicker biofilm forms on anodes with a homogenous carbon nanotube covering due to the increased surface roughness and stronger electrochemical contact that results. A macroscale porous textile comprised of randomly entwined polyester threads with widths of around 20 m was coated with 200 nm thick carbon nanotubes by Xie *et al.* to create a carbon nanotube modified electrode.

This material was chosen because it had an open internal pore structure that allowed for the formation of internal biofilms and allowed enough room for substrate movement. The greatest power density this system was capable of producing was 1098 mW m-2, which was 68% greater than the plain carbon cloth tested in the same system. Due to its enormous surface area, great mechanical strength, and significantly, high conductivity—especially when compared to other carbon materialsgraphene has recently been used in fuel cells. With MFCs, electrodes have been coated in graphene by first covering them in graphene oxide, which is subsequently reduced on the surface, leaving graphene. Studies on its usage, meanwhile, show conflicting results in terms of effective biofilm development. Some say that graphene has an antimicrobial effect that prevents growth, while others assert that growth is promoted.

Compared to carbon electrodes, metal-based electrodes have excellent conductivity and much greater mechanical strength. These electrodes suffer with biocompatibility, thus adjustments are required to encourage the formation of biofilm. Usually, this manifests as an increase in electrode surface roughness. Because they inhibit bacterial development, metals like copper and silver have long been employed in medicine. Recent research, however, suggests that this may not hold true for organisms that are electrochemically active. The electrodes used were sheets of the metal material with a geometric surface area of 1.5 cm2 that were rinsed with isopropanol to remove organic residue before being sonicated in DI water for 30 minutes[. Although proven to work, the reason why is not understood, but is suggested to be due to them having no toxic effects specifically towards bacteria. Baudler *et al.* successfully grew biofilms producing average current densities of 1.1 mA cm-2 on silver and 1.5 mA cm Membranes.The anolyte/anode and the catholyte/cathode must be physically separated in conventional fuel cell configurations. Although the electrodes and related liquids must be physically separated from one another, it is still necessary to promote ion flow in order for the reduction process to take place at the cathode. The optimal material for this function must be robust, selective to ionic conductivity,

Exoelectrogenic Microorganisms

Many species have been examined in an MFC setup; the capacity to provide or absorb electrons to or from an electrode surface is the most crucial aspect of these species. Numerous terms have been used to describe this, including electrode respiring, biocatalyst, and electrochemically active bacteria [50]. The role of the species within the cell is better described by other terminologies that are utilised. A species that can take electrons from a cathode is known as an electrotroph, while an exoelectrogen is an anode-donating species that transfers electrons outside of its cell wall. The word "exoelectrogen" will be used in this paper. Redox processes are used in microbial transport chains, like in many other electron transport chains, to move electrons from a donor with low redox potential to an acceptor with a higher redox potential. As the power density grows, the current density generated will be limited by the maximal rate of electron transfer by the bacteria, assuming there are no further limiting constraints. This is really incredibly difficult to do since the system's internal resistance is too strong. The advantages of utilising a highly exoelectrogenic bacterium are diminished until the problems with internal resistance can be resolved. Normally, the architecture of the cell, namely the distance between electrodes and the conductivity of the fuel cell, determines the maximum power density rather than the bacteria.

Production of electrons

Exoelectrogens produce electrons by stopping bacterial respiration, which is brought on by an anaerobic anode that prevents oxygen from completing the organism's electron transport chain. The oxidation of a substrate to decrease the enzyme nicotinamide adenine dinucleotide from NAD+ to NADH is made possible by proteins in the cell wall. The Krebs Cycle then uses the NADH to oxidise it, transferring the resultant electrons to adenosine triphosphate (ATP), the bacteria's energy source. Prior to ATP accepting the electrons, MFCs transfer the electrons to the anode electrode. The species must then move the electron from the cell's interior to the anode. Electrons may be carried to an electrode surface in a number of ways because to the large diversity of species that possess this capacity, and additional transport options are also produced by the process's diverse environmental settings[88]. Studies that try to better understand this phenomena employ pure cultures to determine how certain species are transferred. Due to the emergence of several electron transfer routes and pathways, this kind of research is challenging in MFCs with mixed cultures. Direct transfer and mediated transfer are the two currently recognised separate exoelectrogen transmission mechanisms.

Transferred electrons combined

Although there are two distinct methods of electron transfer, researchers believe that depending on where the bacteria are located within the biofilm, many species may be able to use both simultaneously. Even when utilising a clean culture, the species directly in touch with the electrode surface may use a different strategy than those merely in contact with other bacteria as layers of bacteria grow. Despite efforts to fully understand how species transfer electrons, it is frequently challenging to draw firm conclusions because of the variety of extracellular pathways

that species use, the chemical complexity of the feed, particularly when using real wastewater, and the difficulty of quantifying and identifying electron shuttles in biofilms due to their relative low concentration. Internal resistance restricts the pace of the biofilm, making it difficult to understand how well it functions. The impact of internal resistance will diminish as designs get better. The transmission of electrons would be the limiting factor in a perfect setup. The only situation in which the involvement of the bacterium may be completely explored in order to comprehend its transmission rate and mechanism is this one.

The purpose of the experiment is often taken into consideration when different research organisations utilise microorganisms in MFCs. Pure cultures often allow users to concentrate intently on a particular metabolic function of the bacteria, an electron transfer technique, or patterns in biofilm formation, and then optimise the system as necessary. Scale-up, industrial applications, power performance, and wastewater treatment features are often the main concerns for those who employ mixed cultures.

Researchers can learn more about the process from pure cultures, whereas mixed cultures typically yield more encouraging results. Researchers have found species that can handle particular contaminants to treat particular waste streams by concentrating on the bacteria within the biofilm. Utilizing Geobacter sulfurreducens to reduce uranium ions in nuclear wastewater streams is a prime example of this. This research may provide an innovative and practical way for treating wastewater contaminated with uranium from the nuclear industry.

Specific species identification

Despite the widespread usage of mixed cultures, especially those derived from wastewater treatment, little is known about the species included in such mixed cultures or how they transport electrons. Exoelectrogenic these mixed cultures, there are instances of research organisations utilising a variety of methodologies to identify the species, but there is little consistency across each studied biofilm. The most reliable method of identification makes use of 16s rDNA analysis, also known as 16s rRNA gene analysis, which matches species based on matching a sequence with a similarity score of 97% or higher, 95% to genus, and 80% to phylum [88]. With more biofilms being studied, it is anticipated that this number will increase. Using this technique, it has been determined that nearly 50 species from an MFC biofilm are exoelectrogenic. This approach aids in determining the effects different diets have on the dominant species in a mixed culture.

According to Phung et al., the feed was just as important in selecting which species will predominate inside the electrode biofilm as bacterial inoculation. injected with sediment from a river. either manmade waste water or river water were used to feed the fuel cells. When comparing the results of using potato wastewater and dairy wastewater as feed and inoculum for MFCs, 64.5% showed similar results. Those fed with river water contained a higher percentage of betaproteobacteria within its biofilm, comprising 46.2% of the total. However, those fed with artificial wastewater had a much lower proportion of betaproteobacteria, 21.1%, with the dominant class being alphaproteobacteria. A clear dominant species of *Geobacter sulfurreducens*

made up 37% of the biofilm in MFCs using potato wastewater. While Thauera and Clostridium strains co-dominated the biofilm in the dairy wastewater fed to MFCs, each accounting for 17% of the biofilm

Even though there are numerous ways to transfer electrons, not all species can accomplish this. Some, referred to as bioremediators, are helpful for breaking down organic materials because they reduce COD without releasing any electrons. Koch and colleagues are concentrating on creating a database that lists every species' exoelectrogenicity. This will facilitate tailoring of biofilms to increase the number of exoelectrogens and decrease the number of bioremediators within a biofilm, which will aid research in this area. Their most recent work highlights the difficulty in detecting electrogenicity since there are no established standards for methodically analysing a species, and there are no discernible patterns across electroactive species.

There are 94 species that have been recognised as being exoelectrogenic; however, only a small number have been properly studied in a fuel cell, and several are known to only operate exoelectrogenically in mixed cultures as opposed to pure cultures. Escherichia coli and Shewanella putrefaciens are the two organisms that have received the greatest attention and have been studied in pure cultures. The characteristics and research into exoelectrogenic bacteria are listed in the table below.

Design and Testing of MFC

The objective of this work is to create an MFC system that can be scaled up to treat wastewater industrially. The design had to adhere to a variety of requirements and criteria since an industrial sponsor was funding the project; nonetheless, it was meant to demonstrate an idea rather than create a finished system. This chapter will outline the system's development, including its prerequisites and desired goals. The development of the experimental process will be addressed in addition to the system's development.

Design of fuel cells

The first fuel cell design was created to test the idea of integrating MFC wastewater treatment with an adsorbent device. The ultimate goal is to build a system that can both generate some electricity and minimise COD. The industrial sponsor, BC Environmental, is working to find new applications for the Arvia process, which uses a graphite-intercalated substance called Nyex to remove colours and other contaminants from wastewater.

The usage of Nyex in an MFC

Nyex is a kind of intercalated graphite compound, which has molecules sandwiched between graphite sheets. In this instance, sulfuric acid-oxidized graphite flakes were used to introduce bisulfate groups to the molecules between the sheets. As part of the "Arvia" method, this substance has been employed as an absorbent to remove organic molecules from water, as shown in Figure 8.1.

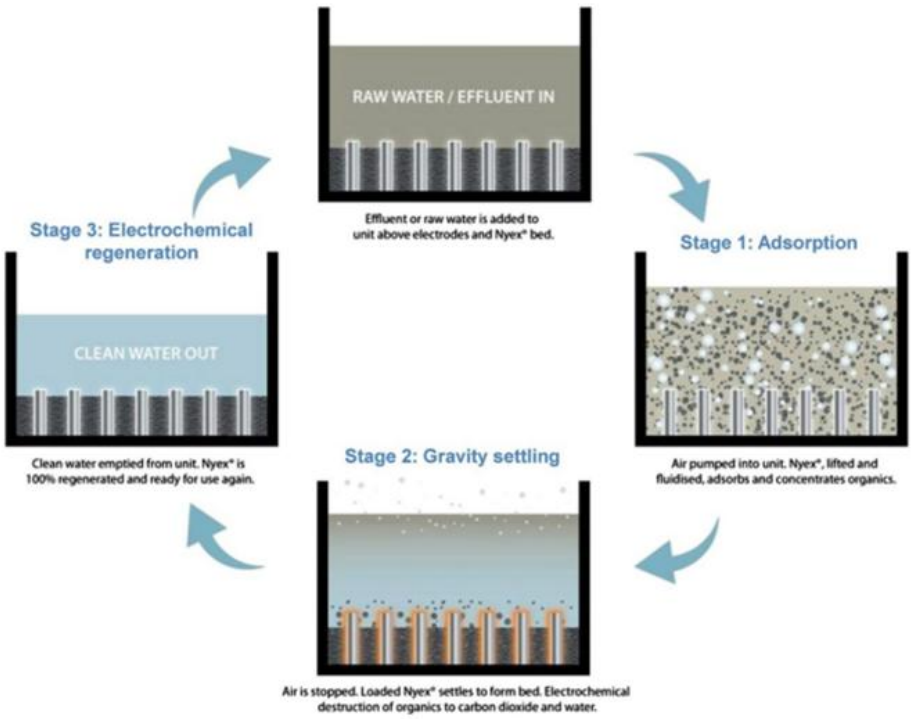

Figure 8.2: Illustrate the process of water purification

The Arvia method concentrates organic pollutants on the Nyex surface by fluidizing a bed of Nyex with an air flow. When the air flow is stopped, the bed settles and the water may be drained. The remaining Nyex is then electrochemically oxidised so that it may be renewed. Nyex, unlike other carbon compounds like granular activated carbon, has a reduced surface area (1 m2g-1) due to its non-porous structure (600- 1200 m2 g -1). The use of this non-porosity in biological applications has historically been discouraged because it may limit the development of biofilm. According to earlier research by Nkrumah-Amoako et al., this material has no interior surface area, making it considerably denser and improving its electrical conductivity and ease of regeneration. The major goal of this research is to determine if an MFC containing Nyex provides a useful support for the development of biofilms. The following are the primary distinctions between the MFC's usage of Nyex and the Arvia process: The bed won't be fluidized with air since anaerobic conditions must be maintained. Nyex will be employed as a support for bacterial growth and movement will be accomplished instead by movement of the feed. Electrons from the biofilm will be carried by Nyex to the terminal electron acceptor; normally, electrons are only transmitted for regeneration.bThe Nyex utilised throughout this project was Nyex N2114, which was given by Arvia Technology Ltd. To guarantee uniformity, all of the Nyex provided was made in the same batch.

Anaerobic waste water sludge from a United Utilities treatment facility's main clarifier was given by BC Environmental and used to inoculate the anode compartment. This mixed culture, which is easily found on wastewater treatment sites and has previously been modified for use in treating

waste water, was utilised to simulate how the cell would be employed in industry, as explained in Chapter. In terms of COD reduction and voltage production, the adoption of a mixed culture is considered as the most effective inoculation. The variety of metabolic pathways available to break down any waste in the meal is the cause of this.

Experimental technique

On days 1 and 2 of the first studies, inoculations were performed. For each inoculation, 50 cc of infected wastewater were added to each anode compartment. With a total estimated amount of 400ml, this makes up 12.5% of the anode's original capacity.

As previously stated, it is anticipated that the bacteria grown on the anode will break down the acetate in the synthetic wastewater into protons, electrons, its intermediate bicarbonate, and finally carbon dioxide[50], which will travel through the cell in various ways before reuniting at the cathode. In order to account for enrichment, which is the early stage of biofilm formation, the anode was drained and refilled every day for the first five days of operation. Feed replacement then took done every three days following that. As stated, the feed utilised was synthetic wastewater based on potassium acetate.

Both the identification of species inside a biofilm and the power outputs offered by the fuel cell depend critically on the components of artificial wastewater. Since it is challenging to obtain uniformity across research when utilising actual waste water, artificial waste water is often employed in MFC investigations. The preparation method developed by Lorenzo *et al.* is followed in the composition of the feed that is employed. The fuel source for the biofilm is acetate, which was chosen since it is the most abundant fatty acid in anaerobic habitats and is often utilised in MFC investigations.

The addition of additional nutrients will extend the log growth phase when the availability of nutrient supply is restricting growth. After the enrichment period, this may be seen as a constant maximum voltage. The cells enter the death phase after passing through the stationary phase, during which proliferation decreases. Here, the number of cells declines and they cannot be replaced by the culture.

A specific dying phase is not attained in these tests. This occurs because new feed is readily available and old feed is removed. The length of time it takes for death phase to arrive will depend on how the system is operated as a full batch system. This would be shown by a steady reduction in voltage until it reaches zero and stays there until a new inoculation is administered; this is not apparent from the data. The significance of understanding the culture's life cycle, especially in an MFC, is determining when the culture will want additional nutrients, which is an indicator of when the culture will have used up all of the available nutrients, i.e., the waste water has been treated. By preventing the dying phase, it also guarantees the biofilm's continued good health. The total voltage climbed gradually throughout the enrichment phase, reaching an average of 390mV despite the frequent voltage decreases. During the first phase, there was some fluctuation between the two fuel cells, with fuel cell 2 performing around 200 mV worse than

fuel cell. After this time, the two fuel cells, however, converge. The growth and development of biofilm on the electrodes is the sole difference between cells, suggesting that this is the reason for the temporarily different performance since one seems to have evolved more quickly than the other.

Further knowledge of the biofilm's composition would provide light on how feed is used and voltage produced by the bacteria if the topography and structure of the biofilm developed on the anode surface were better understood. This chapter will list each species that made up the biofilm formed after a mixed anaerobic wastewater sludge inoculation and assess the capacity of the dominating species to transmit electrons. Additionally, a thorough examination of biofilm variation and its impact on performance will be conducted. Diverse species will be present in the biofilm developed by mixed anaerobic wastewater sludge inoculation on the anode surface. The inoculation's composition and, thus, the biofilm's composition are both unknown. Knowing the species that make up the biofilm, especially the dominant species, can provide you more information on how well the biofilms can convert feed into voltage. Not all bacteria possess the capacity to transmit electrons; mixed consortia will also include a number of bioremediators that can break down feed but are unable to transport electrons. A high rate of COD removal will be seen in biofilms with a lot of bioremediators, but the voltage output will be reduced. Future biofilm customization will be possible by knowing the specific roles that different species play within a mixed biofilm. By adding or deleting species according to their capacity to both break down feed and transmit electrons, this has the potential to create a more effective system.

CHAPTER 9

APPLICATION OF MICRO FUEL CELL

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Sporomusa ovata, an anaerobic Gram-negative bacteria that ferments hydrogen and carbon dioxide into acetate, is one of the promising microorganisms that generates electric current. MFC considerably reduces the energy need of wastewater treatment facilities as compared to traditional approaches. To do this, MFC uses electroactive bacteria that can oxidise organic materials and transfers the liberated electrons to an electrode-anode, a solid electron acceptor.

The development of materials such as catalysts, electrodes, and membranes has assisted in lowering production costs and increasing the effectiveness of the technology. Biofilm dynamics (pure or mixed culture microbes, extracellular electron transfer, as well as interface characteristics) have also been better understood. Due to its potential to recover energy during wastewater treatment, the microbial fuel cell (MFC) technology has received a lot of interest recently. Utilizing an MFC in the industrial sector is a highly appealing notion since it can turn organic wastes into electricity, lowering energy expenditures and waste disposal expenses while improving corporate profits. Despite these encouraging promises, however, the challenges posed by MFCs' poor performance and expensive prices have prevented their widespread use from being successful to far. The primary uses of the MFC systems and its innovations, notably the improvements in configuration and operating circumstances, are intended to be covered in this study. The various modelling methodologies as well as the diagnostic methods for assessing MFC performance are explained. A cost study is also given in the section devoted to the MFC's debut to the market.

Microbiological Fuel Cells Produce Electricity

Energy is generated through electron donations to the anode by organisms that reduce electrons. In order to generate electricity, MFC makes use of a number of microorganisms that can catalyse electrochemical oxidations or oxidation/reduction processes. Different paths are used by bacteria using an electron transfer mechanism to produce energy. The following is a description of a few of these mechanisms:

A soluble mediator in the fluid around the anode transfers electrons to the cathode. Proteins on the bacterial outer membrane delivered electrons straight to the anode. Shewanella oneidensis, for instance, uses cytochrome c to transport electrons. Lactate must be converted to acetate in an anaerobic environment, however. • Bacterial pili, nanowires, or bacteria that create a thick biofilm on the cathode that transmit electrons to the anode. In this process, materials in the

cathode chamber are reduced by electrons from the cathode. In anaerobic settings, microorganisms convert carbon dioxide to methane or acetate, nitrate to nitrite, nitrogen or sulphate sulphur ions, or oxygen to water. We may use Geobacter sulfurreducens as an example, which uses electrons from the cathode to change fumarate into succinate.

Industrial Uses for Microbiological Fuel Cells

It has been noted that MFC is being used more often in industry, for instance, in the creation of biosensors, wastewater treatment, and robotics. Electrochemical batteries, such as lithium batteries, are often used in sensors and biosensors created using traditional methods. These batteries need to be periodically recharged or replaced since they have a finite lifespan. A reliable power source for remote monitoring sensors and biosensors is the self-renewable MFC. Additionally, this technique reduces environmental hazards and operational expenses. Analyzing the water's quality for consumption by people, animals, and plants is crucial. The water quality is influenced by a number of important factors. Since the presence of any harmful material will negatively influence the metabolic activity of microorganisms, MFC functions as an appropriate sensitive system (biosensor). But choosing a certain kind of electroactive bacterium makes it more sensitive to certain toxins. Therefore, it is essential to design biosensors with the necessary monitoring requirements in mind.

The levels of biochemical oxygen demand (BOD) are also determined using MFC. Controlling BOD is crucial for keeping track of the amount of contaminants and microbiological activity in groundwater. MFC-based BOD sensors provide a quick, painless, economical screening technique. Because many microorganisms can remove sulphides as needed in wastewater treatment, there is a high need for MFC in this application.

Cambrian Innovation is known for creating and selling MFC-based environmental products. It has created a cutting-edge bioelectrochemical wastewater treatment system that is used in a number of commercial and municipal buildings. It generates electricity by using exoelectrogens, or bacteria that are in direct touch with electrodes. The business has also created real-time, inexpensive, and durable bioelectrochemical nitrate sensors employing MFC for surface water monitoring, which is crucial in many different sectors, including precision farming.

A patented cell that houses electrically active, nitrate-reducing bacteria is the basis of Cambrian Innovation's denitrification device. By removing nitrates from discharge water, this device aids in addressing a critical issue that affects a variety of industrial and agricultural sectors. The National Aeronautics and Space Administration (NASA) provided funds to Cambrian Innovation so they could create crewed life support utilising MFC. It showed how to generate power from water for later use. There are several ways to use this energy. In order to lower the expense of carbon dioxide reduction, Cambrian Innovation has also created a better air revitalization system for life support on board the International Space Station.

Although MFCs have been investigated as an alternative energy source, their use is still restricted to a few specialised fields. Scaling up and using MFCs as a renewable energy source

would be viable with future advancements in design, cost effectiveness, and performance efficiency based on these short-term uses.

Treatment of waste water

Microorganisms have the capacity to both generate electricity and degrade effluents. MFCs are now being seriously considered as tools for generating electricity while treating industrial, agricultural, and municipal wastewater. Electrons are generated when microorganisms oxidise organic substances found in waste water, creating a consistent supply of electrical current. MFCs may provide a new way to offset operational costs of waste water treatment facilities if electricity production in these systems can be enhanced, making advanced waste water treatment more cheap in both developing and industrialised countries. Additionally, MFCs are reported to produce less extra sludge than the aerobic treatment method.

Powering underwater surveillance equipment

Although data from the natural environment may be useful for understanding and predicting ecosystem reactions, the functioning of the sensors placed throughout the environment needs electricity. Such devices may be powered by MFCs, especially in river and deep-water settings where it is difficult to regularly access the system to change batteries. In order to monitor natural systems including creeks, rivers, and seas, sediment fuel cells are being created. Sediment fuel cells have low organic matter concentrations and high inherent internal resistance, which together result in poor power densities. Energy storage systems that deliver data to central sensors in bursts may, however, compensate for the low power density.

Power source for distant sensors

The power used by electronic devices has significantly decreased as a result of advancements in microelectronics and related fields. Chemical sensors and telemetry systems are often powered by batteries; but, in certain applications, changing batteries on a regular basis may be expensive, time-consuming, and unfeasible. Utilizing self-renewable power sources, like MFCs, which can run for a long period using local resources, is one answer to this issue. The selection of acceptable organic and inorganic materials that might be employed as energy sources is the main focus of extensive study into the development of trustworthy MFCs in this regard.

BOD detection

Utilizing the MFC technology as a sensor for pollution measurement and in-situ process monitoring and control is another possible use for the technology. In water that is rich in organic waste, such as sewage, the biological oxygen demand (BOD) is the quantity of dissolved oxygen needed to fulfil the metabolic demands of aerobic organisms. MFCs can be used as BOD sensors due to the proportional relationship between the coulombic yield of MFCs and the concentration of assimilable organic contaminants in wastewater. The service life of an MFC-type BOD sensor is much longer than that of other types of BOD sensors reported in the literature, lasting over 5 years without additional maintenance.

Production of hydrogen

Using modified MFCs that produce hydrogen from organic waste could be a worthwhile alternative. In such devices, the cathode chamber is kept anaerobic, and an extra 0.25 V of voltage is provided to the cathode. On the cathode, protons are converted to hydrogen under these circumstances. Bio-electrochemically aided microbial reactors are what these modified MFCs are known as.

Despite the fact that this technology has great potential as a renewable energy source, it will be some time before large-scale, very efficient MFCs are used in commercial settings. Due to the urgent demand for alternative energy, the many research organisations operating throughout the globe will undoubtedly overcome the current challenges.

CHAPTER 10

ELECTRON ACCEPTORS IN MICROBIAL FUEL CELLS

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Because of its remarkable potential for environmentally friendly wastewater treatment and contamination removal, microbial fuel cells (MFC) have lately attracted more and more interest. In general, pollutants may be eliminated either as electron acceptors by reduction at the cathode or as electron donors through microbial catalysed oxidation at the anode. At the anode or cathode, certain pollutants may also act as electron mediators. Cathodic electron acceptors and mediators have not been as well characterised as electron donors, despite prior research thoroughly evaluating them. Due to its high oxidation potential and easy accessibility, oxygen is often utilised as an electron acceptor. However, due to the variety of redox potential, the need for alternative and more effective cathode reactions, and the expansion of MFC-based technologies in many fields, recent research have started to evaluate the use of various electron acceptors. This chapter is compiled to assess the effectiveness and application of different electron acceptors and mediators used in MFCs. Select electron acceptors and mediators were also examined for their related performance, benefits and drawbacks, and prospective future uses (such as nitrate, iron, copper, and perchlorate).

A bioelectrochemical device called a microbial fuel cell (MFC) uses electrons produced by the anaerobic oxidation of substrates to produce energy. A proton exchange membrane separates the two components of the MFC, which are called the anode and the cathode (PEM). In the anode compartment, organic compounds such acetate, glucose, lactate, and ethanol undergo anaerobic oxidation, which results in the release of protons, electrons, and carbon dioxide. In this instance, the PEM and an external circuit are used to transmit protons and electrons from the anode chamber to the cathode chamber. An electrical current is created as a result of the transport of electrons from the anode to the cathode. Since organic materials may readily be oxidised as fuel in the anode compartment, MFCs can be utilised for wastewater treatment. Several other applications, including the generation of hydrogen, seawater desalination, biosensors, and microbial electro synthesis, have been used MFC-based systems in recent years.

MFCs have not been able to expand beyond the pilot size despite having promising early findings for a variety of reasons. The kind of substrate, exoelectrogenic microorganisms, circuit resistance, electrode material, reactor arrangement, and electron acceptors are only a few of the variables that affect the MFC's power output. The efficiency of electricity generation is impacted by the physical and chemical differences between various electron acceptors (such as oxidation potential). Due of their enormous influence on electricity production, it has become more important in recent years to investigate the application of novel electron acceptors in MFCs.

Due to its high oxidation potential and the fact that it produces water following reduction, oxygen is the most popular electron acceptor employed in the cathode compartment. However, the majority of research indicate that oxygen delivery to the cathode compartment requires energy. The drawbacks of oxygen consumption include contact issues at the cathode-air surface and the necessity for costly catalysts, despite the fact that oxygen in the air may be utilised directly by utilising an air cathode.

Alternative electron acceptors may boost power output, lower operating costs, and broaden the range of applications for MFCs. Recently, it has been shown that several refractory substances may be used as an electron acceptor in the cathode. These results imply that environmental pollution can be controlled using MFCs. An example of a contaminant in wastewater streams is nitrate. Nitrate may be employed as an electron acceptor in the cathode compartment because the redox potentials of oxygen and nitrate are so similar. In this instance, the cathode compartment's denitrification mechanism converts the nitrate to nitrogen gas. Other than nitrate, other heavy metals, like copper, may also be reduced to less hazardous forms by acting as electron acceptors. As a result, both the production of power and the treatment of wastewater happen at once. The performance of MFC has recently been affected by the anode, cathode, and membrane components. Along with various cathodic processes like denitrification and iron reduction, a summary of the electrode materials employed in the anode and cathode compartments is also included.

Electron acceptors contribute significantly to the efficiency of the MFC since they take in electrons from the cathode. Although there are several research on various electron acceptors in the literature, there isn't a thorough analysis of this topic. This research methodically compiles and evaluates the numerous electron acceptors utilised in MFCs in terms of performance, benefits, and application areas in wastewater treatment. Finally, this essay offers potential directions for further improvement. Low but stable uranium concentrations exist in uranium processing regions' leachate, which may affect groundwater, water sources, and sediment. Metal-reducing bacteria are often utilised in conjunction with the acetate feed to remedy this issue on the spot. Adsorption, biological reduction, and membrane filtration are all methods for removing uranium, but cathodic U(VI) reduction also seems to be a potential approach. a reference electrode in uranium-contaminated aquifer sediment and a second electrode at the surface of an acetate-fed MFC system. During sulphate reduction and U(IV) removal, this low-cost, minimally intrusive device exhibited 10 mW/m2 power density. However, the U(IV) elimination mechanism is yet unknown. A non-acetate oxidising sulphate reducer's reductive immobilisation of U(IV) may be used to explain how the uranium was removed from the system.

Chloroethenes

Due to their poisonous and carcinogenic characteristics, chlorinated aliphatic hydrocarbons (CAHs), which are often employed as solvents and degreasers, might pose a serious concern. Some anaerobic bacteria may eliminate these contaminants by decomposing the chlorines in

CAHs using electrons from an external electron donor or externally provided voltage. Using insoluble electrodes to provide electrons to communities that are engaged in dechlorination is a different way to implement this strategy. Studies with two distinct communities (a mixed culture of dechlorinating bacteria and a pure culture of *Geobacter lovleyi*) demonstrated that TCE dechlorination under acetate-fed conditions was effectively accomplished in a mixed culture. On a molar basis, the dechlorination products that were produced were cis-DCE (83.9 8.0%), VC (3.5 2.0%), ethene and ethane (12.6 7.0%), and others. The ability to completely dechlorinate TCE with a mixed culture using polarised carbon paper electrode as the only electron source has been shown. External electron donors being supplied to the contaminated zone may cause certain undesirable reactions and lead to an accumulation of byproducts. Since bacterial oxidation takes place in the anode and no additional organic material is supplied to the site, MFC with a solid electrode has a significant benefit in this situation.

2-Chlorophenol

It has been noted that employing solid electrodes as the only electron donors is preferable than using soluble electron donors directly in the case of chloroethens. By using electrodes to supply the required electrons for pollutant reduction, metals and chlorinated pollutants may be bioremediated. One of the usual organisms utilised for this is *Geobacter*. Additionally, *Anaeromyxobacter dehalogenans* was able to dechlorinate 2-chlorophenol to yield phenol by transferring electrons to it. In their experiment, *Anaeromyxobacter dehalogenans* was first given acetate (10 mM) as a substrate, and 80 M 2-chlorophenol was utilised as an electron acceptor. The fastest dechlorination rates were 40 M Cl/d (in 200 mL), demonstrating the potential use of bioremediation of pollutants using electrodes that operate as an electron donor. Dechlorination of 2-chlorophenol, more recently. Under ideal dechlorination conditions, 150 micro molar 2-chlorophenol was eliminated by a crude laccase enzyme. Using solid electrodes as an electron source for chloroethens reduction has a number of benefits. First, microorganisms may be efficiently given electrons to help them reduce the pollution. Second, the electrode may be attached to the spot with ease as an electron donor. Thirdly, unintended reactions may be prevented if impurities and electrodes are directly responded. Finally, contaminating metals that collected on the electrode surface may be removed.

Future Prospects

The numerous cathodic electron acceptors that have been employed in MFCs are included in this review. In aquatic systems, several of these electron acceptors are also contaminants. So using MFC also makes a treatment procedure feasible. Table 1 provides an overview of the various cathodic electron acceptors employed in MFC and the power generated as a consequence. The list is by no means complete, however, since more electron acceptors could appear as cathodic catalysts, electrode materials, and solution chemistry advance. As a terminal electron acceptor in the cathode chamber, oxygen was often utilised in the early uses of MFC. However, in recent years, scientists have been investigating more unusual cathodic electron acceptors in an effort to increase MFC voltage potential while also treating specific wastes or recovering important

chemicals. In terms of producing bioenergy and lowering the price of specialised pollution treatment, the generation of electricity with the reduction of particular electron acceptors in the cathode offers great potential (e.g., nitrogen species, persulfate, mercury, copper, chromium and perchlorate) As a result, reduction in the cathode compartment may be able to remove pollutants with significant redox potential. By using certain electron acceptors, MFC might be made more effective. For high power output, ferricyanide or hydrogen peroxide might be employed, or iron could be used to liberate certain important components like phosphate from wastewaters.

The variety of cathodic electron acceptors employed in MFCs has increased. From initial high voltage output to energy generation, stubborn pollution treatment, or valuable chemical recovery, the goal of alternative electron acceptors investigation changed. Applying electrodes to the ground and externally supplying voltage to transmit electrons to microorganisms is a similar use of the MFC configuration in the cleanup of polluted sites as discussed in Section Chloroethenes and 2-Chlorophenol. The effective delivery of electrons might be made possible by this application. By doing this, electrodes may be placed on the site in accordance with the needs for cleanup. On the other hand, by precipitating on the electrode surface, decreased metals and other contaminants may be successfully removed from the site.

The presence of electricity is a sign of microbial activity in MFC. As a result, MFC may be used to construct biosensors that can identify chemicals that might negatively impact microbial activity (i.e., BOD or toxic compounds). The activity in the anode compartment is connected to this process, and MFCs may be designed as cathodic biosensors to track certain contaminants in the cathode compartment in accordance with various redox potentials.Microbes are living, evolving creatures that help create a variety of cutting-edge technology. Microbial fuel cells (MFC), which may generate power from organic waste and lower carbon footprint and environmental pollution, are one such emerging technology. This technique has been embraced by several sectors for a variety of uses, including biosensors, bioenergy generation, and wastewater treatment. An anaerobic Gram-negative bacteria that ferments hydrogen and carbon dioxide into acetate, is one of the promising microorganisms that generates electric current. MFC considerably reduces the energy need of wastewater treatment facilities as compared to traditional approaches. To do this, MFC uses electroactive bacteria that can oxidise organic materials and transfers the liberated electrons to an electrode-anode, a solid electron acceptor.

The development of materials such as catalysts, electrodes, and membranes has assisted in lowering production costs and increasing the effectiveness of the technology. Biofilm dynamics (pure or mixed culture microbes, extracellular electron transfer, and interface characteristics) have also been better understood. Energy is generated through electron donations to the anode by organisms that reduce electrons. In order to generate electricity, MFC makes use of a number of microorganisms that can catalyse electrochemical oxidations or oxidation/reduction processes. Different paths are used by bacteria using an electron transfer mechanism to produce energy. The following is a description of a few of these mechanisms: Through a soluble mediator in the solution around the anode, electrons are transported to the cathode.

Proteins on the bacterial outer membrane allowed for direct electron transport from the cathode to the anode. Shewanella oneidensis, for instance, uses cytochrome c to transport electrons. However, the conversion of lactate to acetate needs an anaerobic environment. Bacterial pili, nanowires, or the bacteria that coat the cathode in a thick biofilm send electrons to the anode. In this process, materials in the cathode chamber are reduced by electrons from the cathode. In anaerobic settings, microorganisms convert carbon dioxide to methane or acetate, nitrate to nitrite, nitrogen or sulphate sulphur ions, or oxygen to water. We may use *Geobacter sulfurreducens* as an example, which uses electrons from the cathode to change fumarate into succinate.

CHAPTER 11

APPLICATION OF MICROBIAL FUEL CELL (MFC) FOR PHARMACEUTICAL WASTEWATER TREATMENT

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Drugs are now extensively employed in many other sectors, including agriculture, poultry farming, fisheries, and human health, thanks to the pharmaceutical industry's recent fast growth. Around the world, aquatic environments are being contaminated by thousands of anthropogenic and natural trace organic pollutants, including pharmaceutically active compounds (PhACs), hormones, chemical products, biocides, polyaromatic hydrocarbons (PAH), illegal drugs, herbicides, pesticides, and surfactants. The structural rigidity and nonbiodegradability of medications, despite the fact that they offer numerous advantages for humans, have resulted in enormous environmental damage. Drugs have been discovered in the soil, surface rivers, and drinking water, among other environmental components. They are regarded as a developing contaminant that poses a risk to both human health and the environment. For instance, the proliferation of antibiotic-resistant bacteria (ARB) and genes associated with antimicrobial resistance has made antibiotics in the environment a significant cause of worry (ARGs). Diclofenac (DCF), a substance that has the highest level of toxicity among nonsteroidal anti-inflammatory drugs (NSAIDs), may have both acute and chronic toxic effects on fish liver, kidneys, and gills. Pharmaceutical effluents differ from conventional sewage in that they discharge organic contaminants and drug components, such as antibiotics, antiepileptics, vitamins, and cosmetic ingredients. These substances' adverse effects on the environment are poorly understood and are growing into a serious environmental issue. Microbial fuel cells (MFCs), which are powerful technology for handling pharmaceutical waste, are developing as a solution to this problem. The potential of MFC technology to generate power in an environmentally responsible way has led to a lot of research in this area over the last 10 years. Because MFCs generate energy during wastewater treatment via microbial metabolic processes rather than by consuming it, they are preferred to traditional techniques for treating wastewater. While MFCs produce energy by oxidising biodegradable trash in the anodic compartment, a standard two-chamber MFC consists of an anode chamber, a cathode configuration, and a separator or membrane, which isolates both anode and cathode. Even though the majority of MFCs are exclusively used in the lab, they have recently generated a lot of interest in cleaning dirty water. Various methods for treating pharmaceutical wastewater (PWW) are covered in the current review, with a focus on MFCs and their relationships.

MFCs are a bio-electrochemical system that uses the catalytic activity of microorganisms to transform the chemical energy found in organic substances into electrical energy. In MFCs, free

electrons and protons generated during the oxidation of organic molecules are collected from a cathode through an electric circuit and a selective proton exchange membrane, respectively, under anaerobic circumstances. Wastes of many kinds, including industrial, agricultural, and municipal wastewaters, may be treated by MFCs. As a result, carbon-based materials are often employed for anodes in order to promote the development of microorganisms. An oxygen reduction reaction (ORR) for a paired redox reaction is carried out in the cathode compartment on the cathode side with the creation of water. Different electrode materials with greater conductivities are used to enhance the cathode and MFCs' overall performance.

The main components of MFCs are as follows:

(i) Anode, where organic matter is oxidised by electroactive bacteria.
(ii) Cathode, where oxygen or carbon dioxide is reduced in the presence or absence of catalysts.
(iii) Ion exchange membrane, which facilitates the passage of protons from anode to cathode through simple diffusion.
(iv) Electroactive microorganisms, which are microorganisms with the ability to change their MFCs come in two different configurations: single-chamber and dual-chamber.

Single-chamber MFCs have an open cathode system where the cathode is exposed to air for the reduction reaction, whereas dual-chamber MFCs have separate compartments for the anode and cathode. The effectiveness of MFCs depends on the wastewater properties and operational factors, such as pH, temperature, flow, hydraulic retention time (HRT), etc., in addition to its fundamental parts and reactor architecture.

Conventional and Advanced Oxidation Processes for the Treatment of PWW

PWW typically has a complex composition with a high proportion of organic material, salt concentration, and microbial toxicity, all of which must be treated. Pharmaceutical businesses work in batch mode, producing a variety of pollutants from a variety of raw materials and production techniques. The main pollutant in PWW is organic matter, and the most economical and efficient method for getting rid of organic contaminants from PWW is biological treatment. The three kinds of biological treatment procedures often used for the treatment of PWW are aerobic, anaerobic, and combination anaerobic-aerobic processes. All three are claimed to lower the concentration of pollutants. Recent scientific research and technological developments have centred on improving PWW treatment employing physicochemical technology as the main approach, encompassing both traditional (such as coagulation, ozonation, adsorption, and flotation) and sophisticated oxidation processes.

Coagulation

Chemical agents are introduced to wastewater, quickly mixed in to distribute them, and then transformed into unstable and precipitable particles to transform pollutants. For improved PWW treatments, bound water must be compressed and removed from the area around a hydrophilic colloid. Therefore, a key factor in determining the coagulation action is the type of the

flocculent. Inorganic metal salts and polymers are often used as flocculants. This technique may be used to get rid of chromaticity and dangerous organic particles. Sedimentation is the next most common operation after coagulation. Gravity may be used to remove pollutants from wastewater since pollutants have a greater density. Coagulation and sedimentation have several advantages, including being simple to use and being tested methods, however it is challenging to remove dissolved organic material. In order to treat real PWW, a hybrid sequential treatment approach combining coagulation, E-beam irradiation, and biological treatment was investigated. At the same time, the effects of the combined treatment on the physicochemical characteristics, biodegradability, and toxicity of the treated wastewater were investigated. The PWW streams go through a sequential treatment pathway that includes biological degradation, coagulation, and electron-beam irradiation. This results in synergistic degradation and detoxification with improved COD and TOC degradation potential, with eliminations of 89% and 94% for high (HOSW) and low (LOSW) organic-strength wastewater, respectively. Cytotoxicity testing against the particular bacteria has shown that combined wastewater treatment effectively lowers toxicity.

Adsorption

Large specific surface area, multilayer porous structure, adsorption capacity, and stable chemical properties are all features of activated carbon. It is used to cleanse hazardous industrial effluents with stringent discharge requirements. There are two different forms of activated carbon adsorption: chemical and physical. Physical adsorption has no adsorbate selectivity and is reversible. Activated carbon that has been saturated with adsorbates may be easily desorbed. On the other hand, chemical adsorption is irreversible and difficult to desorb since it only adsorbs one or a few different adsorbates. Activated carbon may be rejuvenated to recover its capacity for cyclic adsorption. Due to its ability to be recycled, stronger therapeutic impact, and broad application, this technology is widely used for advanced treatment. Its application is limited, nevertheless, by a number of issues, including high relative costs, insufficient regeneration efficiency, and challenging operation.

Ozonation

Ozone has a long history of usage as a potent disinfectant and oxidant. In situations that are acidic, ozone acts primarily as an oxidant. However, in neutral and alkaline pH settings, it mostly depends on free radical processes. As a consequence, most organic molecules in water may be quickly oxidised and broken down by ozone, efficiently eliminating pollutants. Several chemical treatment methods have been used to remove refractory organic compounds from water and wastewater by oxidising hydroxyl radicals. It is believed that the use of catalysts in advanced catalytic ozonation would enhance molecular ozone breakdown, produce highly active free radicals, and aid in the mineralization and degradation of a range of organic materials. Hazardous organic pollutants in wastewater have been extensively degraded using the advanced catalytic ozonation process. Low ozone utilisation efficiency and poor ozone mineralization of organic contaminants may be fixed by advanced catalytic ozonation. Since direct mineralization is

challenging, a material that breaks down quickly in the body must be developed. Additionally, it is effective at simultaneously eliminating turbidity and pathogens from wastewater. When various wastewater treatment methods are combined with ozone, ozone-advanced oxidation technology is created. This method has a better capacity for oxidation but a lower selectivity for the reactants O3/H2O2, O3/UV, and so on.

In addition to sedimentation, flotation may also be used to remove suspended particles from secondary effluents. By adding air to wastewater, this technique creates a large number of small bubbles, resulting in floating floc that is less dense than the wastewater. Additionally, it may segregate wastewater by rising to the top. As a consequence, it is often used as an adsorbent or catalyst carrier to remove contaminants. Pharmaceutical micropollutants in aquatic environments are one of the biggest environmental problems. Constructed a sequencing batch reactor (SBR) using a powdered composite adsorbent (CA) and a sequencing batch reactor to treat synthetic wastewater. Ammonia was combined with ciprofloxacin, atenolol (ATN), and diazepam (DIA) to make synthetic wastewater (CIP). SBR was used by during biological treatment using an activated sludge system to eliminate ACT, CAF, and IBU in a concentration of 2 mg L1 through five distinct routes. Wastewaters containing the three target medications have been treated biologically using activated sludge and carbon adsorption. 35-day experiment revealed that the combined system reduced effluent COD levels by 32.5%, while PhCs boosted organic matter removal effectiveness by 46.2%. The combined system produced effluents in the target pharmaceuticals when it came to PhC removal, but biological treatment without activated carbon produced only moderate levels of CAF and IBU. In comparison to a standard activated sludge treatment, the combined technique suggested in this research produced effluent that was PhC-free and of higher quality. Authentic PWW from an erythromycin (ERY) production factory is treated with gamma irradiation. Raw wastewater samples containing antibiotics were linked to the location and concentration of the ARGs. In addition to ERY, tetracycline and sulfamethoxazole were found, however at amounts three orders of magnitude lower.

Additionally, it was shown that the drop in ARGs and antibiotics was much greater than the reduction in antibacterial activity and COD. With a 50 kGy absorbed dose, the clearance percentages of ARGs, ERY, antibacterial activity, and COD were 96.5%, 99.8%, 90%, 47.8%, and 10.3%, respectively. The ability of Trametes versicolor to extract PhACs from sterile synthetic and nonsterile hospital wastewater in a stirred tank bioreactor (STB) and a trickle-bed bioreactor (TBB). T. versicolor was used in this research to treat hospital and industrial wastewater in two different bioreactor designs. The STB retrieved 85.0% of the naturally occurring medicines found in hospital wastewater and 95.7% of the 16 pharmaceuticals that were introduced into synthetic wastewater. 89.8% of real wastewater and 88.6% of synthetic wastewater were eliminated by a TBB made from fungal biomass immobilised on rice bran. However, 73.3% of the reduction was due to the biomass of the bed being absorbed. According to toxicological studies, hospital wastewater becomes less harmful after being treated in a TBB. These findings suggest that the fungal fixed-bed reactors, which can completely remove PhACs

while simultaneously cleansing actual wastewater, may be a more effective method for removing pharmaceutical compounds from contaminated water than the stirred tank reactor.

Modern Oxidation Methods (AOPs)

Free radicals are used by AOPs to oxidise pollutants. A typical oxidising agent is unable to break down these pollutants. AOPs include electrochemical oxidation, moist air oxidation, photocatalytic oxidation, ultrasonic oxidation, and supercritical water oxidation. a thorough analysis of AOP-based PWW therapy. Supercritical water oxidation (SCWO) oxidises organic materials with a high oxidation efficiency while generating no secondary pollutants. However, this method has a number of drawbacks, including the necessity for very high operating temperatures and its high cost. Recently, materials based on metal-organic frameworks (MOFs) have shown promise as catalysts for sulphate radical-based AOP (SR-AOP) applications. Most crucially, the relevance of AOPs in treating pharmaceutical wastewater has drawn substantial attention to the application of MOF-based materials in SR-AOPs for wastewater treatment. Fenton's reagent is a specific kind of reagent used in the Fenton system that was initially largely utilised in organic synthesis. However, as people became more knowledgeable about the system, they began to use it more and more to treat the wastewater in industrial settings.

Without affecting the environment, the Fenton reaction may be run at standard pressure and temperature. With straightforward reaction conditions and great oxidation effectiveness, it is a sophisticated oxidation technique. A valuable AOP is called Fenton oxidation, which uses Fe as a catalyst to accelerate the creation of OH. With efficient mineralization, it has proven possible to degrade emerging contaminants (ECs) by more than 80% when utilising Fe-based catalysts such Fe oxide, zero-valent Fe, and multi-metallic composites. A more recent and less expensive method of Fenton oxidation is bio-electro-Fenton (BEF). The BEF is a bio-electrochemical system that combines electro-Fenton oxidation with bio-electrochemistry to digest wastewater that contains pharmaceuticals. Because electroactive microorganisms oxidise organic substrates at the anode, producing H+, electrons, and CO2, BEF is popular because it produces the H+ ions required at the cathode for the formation of H_2O_2. On the other hand, it is impossible to ignore the shortcomings of the Fenton reaction. One is oxidant loss brought on by free radical scavenging and H2O2 oxidation. AOPs are often viewed as a workable substitute for treating effluents with limited biodegradability.

Utilizing a mix of advanced Fenton oxidation and traditional biological treatment, the used an effluent from an organic chemical synthesis pharmaceutical company with about 1.4 g L1 of TOC and poor biodegradability. An easily degradable discharge was produced as a consequence of lowering the temperature from 120 to 70 C and using an H2O2 uptake that was 65% less than that used in the Fenton technique at the PWW treatment facility. TOC removal was also much higher as a result, at over 90%. A process that combines UV light with an oxidant is called UV photocatalytic oxidation, sometimes referred to as photochemical oxidation. In response to UV light stimulation, oxidants undergo oxidative breakdown, generating free radicals with increased oxidative capability that may oxidise more difficult organic contaminants using just oxidants.

Long used for disinfection, chlorinated cyanurates are created by adding hypochlorite to cyanuric acid in different ratios. Because of their structural similarity to chlorinated amides, which have a low reactivity to radicals, and because they are composed of numerous - Combining UV with chlorinated cyanurates (UV/Cl-cyanurates) may be an unique and efficient AOP since Cl may have high molar absorptivity at 254 nm owing to red-shifting absorption.

Membrane Discrimination

When a component of water preferentially passes through a permselective membrane, separating the medium across the membrane at a specified driving force, membrane separation is a practical technique. As a consequence, the target substance may be extracted from the mixture and separated, purified, and concentrated. Membrane separation techniques used in wastewater treatment include microfiltration, reverse osmosis, ultrafiltration, and electrodialysis. PWW cannot be treated with biological treatment techniques after secondary treatment methods because they are least biodegradable. We cannot, however, ignore the advantages of biological therapy, which include affordable costs and consistent therapeutic outcomes. They are a sophisticated therapeutic formulation that may be used.

The care of PWW with MFC

Although numerous conventional methods have been used to treat PWW, including coagulation, filtration, biological membranes, and advanced oxidations, each of these techniques has limits in terms of its application and effectiveness. The anaerobic breakdown of resistant organic pharmaceutical contaminants presents a challenge to existing water treatment technology because to their susceptibility to microbial respiration and their natural habitat. In the anode of an MFC, microorganisms may break down different organic compounds into CO2, H2O, and energy. Various mechanisms are used by microbes to interact with an electrode. The electrode functions as an electron sink during anaerobic respiration. Numerous studies have shown that using MFCs as a remediation strategy may hasten the degradation of pharmaceutical contaminants and shorten response times since cathode processes are so powerful.

MFC's advantages over conventional processes

Direct power generation, energy-efficient procedures, anaerobic treatment owing to low sludge output, centralised and decentralised applications, and a lack of pricey aeration are some of the benefits of MFCs. These benefits also include those related to the environment, economy, and operations, such as self-generation of microbes, high resilience to environmental stresses, and real-time monitoring. Environmental benefits include water reuse, low carbon emissions, and a relatively small carbon footprint. Economic benefits include money from energy, less expense, and the elimination of downstream methods. Other significant benefits of MFC technology include better energy conversion from substrates, less sludge volume, and recovery of higher-value products. MFCs do not need the separation, purification, or conversion of energy products; instead, they produce clean energy directly from organic matter in polluted water. However, anaerobic digestion may also produce hydrogen and methane, which must first be

separated and purified before being utilised. MFCs are eco-friendly technologies because they can generate clean energy on the spot and function at low temperatures, particularly at room temperature.

One of the most challenging problems in the commercialization of MFCs is the efficient and affordable design for scaled-up versions of laboratory-size reactors. Laboratory-size MFCs have shown excellent results in the generation of power and in the cleaning of surfaces. To replicate such findings on a wide scale, however, other factors must be taken into consideration, such as the separator's design, mechanical strength, electrode cost, method of oxidant supply, energy and space requirements, etc. The majority of microbial electrochemical reactions are based on oxidation-reduction reactions, which are influenced by a number of operational factors. The commercialization of MFCs is a serious problem for real-world applications. The low power output of MFCs is one of its main weaknesses, although it may be remedied in a number of ways. The performance of MFCs is significantly influenced by electrodes. An MFC may function better if its electrodes have been treated on the surface to encourage microbial affinity. Few research on the long-term performance of MFCs have been done, and there are still a number of issues that need to be cleared before MFCs may be employed in the real world. When it comes to eliminating pollutants, MFCs work quickly.

In order to properly deploy MFCs in the field, operational stability must be increased over the long term. Additionally, the bulk of current MFC research has been carried out in the lab and has to be expanded to real-world applications. When compared to the conventional treatment method, CW-MFCs have shown promising results in the difficult sewage treatment process and recovered green energy. A major issue with CW-MFCs, however, is the low practical power output for direct application. The operational mechanism of CW-MFC systems must thus be better understood in order to increase power output while maintaining financial viability. A techno-economic analysis of MFCs with dual cathode systems for wastewater treatment was carried out under various scenarios, taking into account the maximum power density of the cell with and without Pt cathodes as well as the pessimistic and optimistic scenarios. Results showed that MFCs are a more desirable and lucrative alternative to traditional methods in the majority of the circumstances. However, a number of hopeful scenarios based on techno-economic factors for hydrogen generation highlighted hydrogen as a potential major technical obstacle if it is not well controlled on the cathode side.

Environmental regulatory agencies have encouraged researchers to develop or provide innovative approaches and technology for the improvement of the environment and human well-being in response to the world's ever-growing population. PWW has a variety of features due to the complexity of pharmaceutical processes, which leads to the high concentration and constrained biodegradability of pollutants. In order to effectively cure PWW, appropriate therapies must be used, and the development and implementation of efficient, workable, and sustainable technologies is crucial. Although MFC technology offers enormous promise for PWW treatment and other wastewaters, one of the most difficult parts of the commercialization of MFCs is the proper and economical design of scaled-up versions of laboratory-size reactors.

Miniaturized power sources are a current study topic as a result of the development of research in distributed wireless sensor networks, wearable sensors, and implanted medical devices in recent years. Piezoelectric nano-generators triboelectric nano-generators fast charge-discharge batteries biofuelcells and high-power micro-super capacitors are just a few of the many miniaturised power sources that have been thoroughly studied. These devices are very tiny, have a high surface area to volume ratio, and charge quickly. Additionally, batch production and micro/nanofabrication are often advantageous for small power sources because they provide precisely regulated shape and cheap cost. The microbial fuel cell industry has likewise embraced the trend of downsizing power sources (MFC).

The chemical energy of organic components in biomass is directly converted into electrical energy by microbial fuel cells, which are bionic-based electrochemical fuel cells. Exoelectrogens, also known as Anode-Respiring Bacteria (ARB), are specialised microorganisms that function as catalysts in this process. Due to its direct and efficient power conversion efficience, the abundance of exoelectrogen or ARB in rivers and oceans, which negates the need to supply an external energy source; and electricity-producing bacteria that can metabolise and reproduce on their own, theoretically it can supply electricity to the sensor network in remote areas of rivers and oceans, MFCs are very suitable as a long-term, maintenance-free stable power supply. Miniaturized MFCs offer an advantage over large and medium sized MFCs in that they are smaller and have a higher surface area to volume ratio, which often leads to higher current and power densities. The current world records for the areal power density and volumetric power density of MFCs. However, compared to conventional lithium ion batteries, the current and power densities are still by an order of magnitude lower. It is essential to increase MFCs' power density. Further research must be done on the key elements impacting power density, such as MFC design, anode/cathode materials, and exoelectrogen or ARB.

The chemical energy of organic components in biomass is directly converted into electrical energy by microbial fuel cells, which are bionic-based electrochemical fuel cells. The anode chamber and the cathode chamber are typically the two chambers that make up MFCs. A proton exchange membrane or an ion exchange membrane separates the two chambers. The anode in the anode chamber produces exoelectrogen or ARB, which the microbe uses to catalyse the breakdown of organic materials like sodium acetate into water, carbon dioxide, and electrons within the bacterium. The extracellular electron transfer process moves the electrons from the anode surface to the cathode in the cathode chamber, where they are subsequently oxidised by an oxidant such oxygen. The microbe's produced electrons cannot be transmitted to the anode and oxidised at the cathode without extracellular electron transfer capacity. In comparison to big or medium sized MFCs, miniature MFCs have a lower chamber capacity, and they are often made using microfabrication methods.

Miniaturized MFCs have a high surface area to volume ratio, which increases current and power density. the first to report on miniaturised MFCs, which use Saccharomyces cerevisiae as an exoelectrogen to break down glucose to produce electricity. This MEMS-based MFC uses a flat

gold electrode with a 0.07 cm2 size as its electrode. It has a low power density of 5.95 nW/m2. The power density is 4 mW/m2 and 40 W/m3, respectively. A tiny MFC was suggested by Qian *et al.* in 2009 as a power source for lab on a chip. An areal power density of 1.5 mW/m2 and a volumetric power density of 15 W/m3 are reported using Shewanella oneidensis as an exoelectrogen and gold electrodes (Qian et al., 2009). Geobacter sulfurreducens was originally employed as the electricity-producing bacterium for MEMS MFCs by Parra and Lin in 2009. Geobacter sulfurreducens has a greater power density when compared to other exoelectrogens. The MFC produced an aerial power density of 0.12 W/m2 and a volumetric power density of 0.34 W/m3. A miniaturised MFC based on Geobacter sulfurreducens was described the anode was a flat gold electrode. The greatest volumetric power density of all MFCs known at that time, with an areal power density of 47 mW/m2 and a volumetric power density of 2,333 W/m3. Three-dimensional graphene foam was used as the anode and Shewanella oneidensis was used as the exoelectrogen.

A surface power density of 0.894 W/m2 and a high volumetric power density of 745 W/m3 were recorded. A flexible, elastic MFC with conductive and hydrophilic cloth was reported by Pang *et al.* in 2018. Exoelectrogen *Pseudomonas aeruginosa* PAO1 was used, and a maximum power density of 0.01 W/m2 was attained. A yarn-based MFC was described by Gao *et al.* in 2020. The exoelectrogen used was *Shewanella oneidensis* MR1, and the cathode used an Ag2O electron acceptor. A maximum current density of 315.45 A/m3 and a maximum power density of 22.12 W/m3 were recorded. In order to increase power density and minimise acidity in biofilms, Ren *et al.* built two- and three-dimensional graphene scaffolds in 2016. These improvements improved biofilm conductivity. This research achieved a volumetric power density of 11,220 W/m3, which is much greater than that of earlier investigations.

Miniaturized MFCs take use of the scale effect and have a greater surface area to volume ratio than big and medium-sized MFCs, which leads to a higher power density. Miniaturized MFCs have set a new record for current power density in the area of MFCs. The performance of tiny MFCs must be further improved in order to increase the power density of MFCs. The performance of MFCs also depends on the electrode materials. Numerous research have used electrode materials with a high surface area to volume ratio and high conductivity in an effort to increase the performance of MFCs. Due to their greater electrical conductivity, greater surface area to volume ratio, and mechanical and thermal durability as compared to conventional carbon-based materials, nano-structured carbon-based materials have been extensively used as two- or three-dimensional anodes in recent years. Nanostructured carbon materials, such carbon nanotubes and graphene, may grow on a planar electrode thanks to two-dimensional carbon-based electrodes, and a biofilm can also develop on the electrode's surface. The highest measured volumetric and areal power densities to date are 0.83 W/m2 and 3320 W/m3, respectively.

Three-dimensional electrodes are more desirable than two-dimensional electrodes because they permit the development of thicker electro-generating biofilms. The performance of microbial fuel cells is improved by the three-dimensional electrode, which can grow thicker biofilms due to the large electrode thickness, in contrast to previous studies on the extracellular electron transfer,

which claim that exoelectrogens located tens of micrometres away from the anode have difficulty transferring electrons to the anode due to extracellular electron transfer limitations. Microbial fuel cells with a variety of three-dimensional nanostructured carbon-based electrodes have been reported, including reduced graphene oxide on sponge, three-dimensional graphene on nickel sponge, carbon nanotube textiles made of conductive polypyrrole and reduced graphene oxide, carbon nanotube/polyaniline composite, reduced graphene oxide carbon fibre, and carbon nanotube/polyaniline textiles. Many investigations of miniaturised MFCs in recent years have used three dimensional electrodes. With 3D graphene scaffold, the greatest volumetric power density of all MFCs is currently possible. Adopting 3D electrodes as the anode and cathode is crucial to further increasing the current and power density of tiny MFCs.

Exoelectrogen, often known as ARB, is a significant performance-limiting element for microbial fuel cells. Numerous microbes that generate energy have been discovered. The two most significant are Shewanella oneidensis and Geobacter sulfurreducens. Geobacter sulfurreducens now holds the world record for microbial fuel cell power density. The power density of microbial fuel cells depends on the process and bottleneck of extracellular electron transfer of electricity-producing bacteria. Researchers from all over the globe are still unsure about the mechanism and bottleneck of Geobacter surfurreducens' extracellular electron transport. Not only is it a significant scientific finding, but understanding the mechanism and bottleneck of Geobacter sulfureducens' extracellular electron transfer also has a significant impact on microbial fuel cell and even microbial electrochemical technology performance. Regarding Geobacter surfurreducens' extracellular electron transport, there are two competing hypotheses:

(1) Electron hopping

(2) Microorganisms produce conductor-capable metallic nanowires.

There are typically four phases involved in the breakdown of organic materials facilitated by *Geobacter surfurreducens* and the transport of electrons to the electrode. Electrons are transferred from inside the outer cellular membrane of *Geobacter surfurreducens* to the outside of the cellular outer membrane through electron hopping or conductive nanowires, electrons from inside the outer cellular membrane of *Geobacter surfurreducens* transfer to the electrode, electrons are transferred from outside the cell to the vicinity of the electrode through electron hopping or conductive nanowires; and electrons are transferred from the electrode to the electrode. Researchers have now clarified the first and fourth phases' mechanisms, however the second and third steps' mechanisms remain a mystery. There are presently few reports on the second step how electrons are moved from the interior of the cell's outer membrane to the outside—and there is disagreement over the third step—whether electron hopping or microbial conductive nanowires are responsible for the electron transfer. The efficiency of microbial extracellular electron transport is crucial to the operation of these cells as fuel sources.

The reasons limiting the performance of microbial fuel cells may be identified by investigating the process of microbial extracellular electron transfer. By understanding this mechanism, researchers are able to address the bottleneck and increase the performance of microbial fuel

cells. Numerous investigations of miniature MFCs have used *Geobacter sulfurreducens* and *Shewanella oneidensis* recently, and good performance has been attained. Additionally, it is crucial to check for exoelectrogen capable of generating more current and power. To do direct and parallel comparisons of the microbial electrochemical activity of several exoelectrogens, Hou *et al.* presented 24 miniature MFC arrays. MFC arrays were provided by Tahernia *et al.* for direct and parallel comparisons of genetically modified exoelectrogens. Potential exoelectrogen with highly efficient EET capability may be discovered using large MFC array screening, increasing the current and power density of MFCs.

Additionally, batch manufacturing of series/parallel MFC stacks/arrays with similar dimensions is made possible by the benefit of microfabrication for miniaturised MFCs, which lessens the issue of current reversal in MFC stacks/arrays. It has been shown how to link miniature MFCs in parallel and series.

Exoelectrogens in each MFC in the stacks or arrays may effectively transmit electrons to the anode when the dimension of each MFC is tiny, which has the potential to increase current and power density. Miniaturized MFC arrays/stacks can deliver greater voltage output compared to single cells. In the future, it will be possible to increase not only the current and power density but also the output voltage to 10-100 s of volts by stacking huge arrays of identical-sized miniaturised MFCs produced in batches. The MFC stacks/arrays may be able to directly power devices without the need of DC-DC converters at such a high output voltage.Microbial fuel cells (MFC) have expanded their applications recently, and this presents a potential to enhance wastewater treatment. MFCs provide a sustainable alternative to treating wastewater that traditional treatment has been unable to do so efficiently. They do not, however, offer a magic solution.

A microbial fuel cell converts chemical energy into electricity by using microbes. Any kind of organic material that degrades may serve as the fuel source. MFC is perfect for wastewater treatment since the bacteria are simultaneously digesting organic matter and purifying the water. Extracellular electron transfer is a process carried out by naturally existing bacteria that releases electrons as part of the respiration process. These electrons are then caught to produce electricity, continually drawing energy away from the bacteria and speeding up their metabolism. In comparison to traditional systems, the system speeds up the treatment process by regulating the pace at which electrons are extracted.

MFC's issues and restrictions

MFCs were first investigated as a clean energy source, but once the inherent limits of electricity production were realised, the emphasis moved to wastewater treatment. MFC must be scaled up to accommodate large amounts of incoming wastewater in order to be a practical option for wastewater treatment. This has proven difficult for a number of reasons, including minimising the distance between anode and cathode to reduce electrical losses and being cost-competitive with other treatment technologies. Costly components are used, such as a catalyst to provide adequate power and membranes to separate the electrodes, which are prone to fouling.

New strategies

By solving the aforementioned design restrictions, Aquacycl created the first MFC for wastewater treatment that was commercially feasible. Instead of focusing on energy recovery, the system was tuned for efficient wastewater treatment. Aquacycl developed a modular solution, where each reactor is the size of a typical car battery and they are stacked together to maximise treatment quality and volume capacity, as opposed to scaling the system inside a single unit. Due to their tiny size, they avoid the mass transfer losses seen in bigger system designs. Additionally, the system doesn't employ a membrane, preventing biofouling and increasing cost- and efficiency-related factors. The treatment may potentially be done without the need of a sewer or electrical infrastructure due to the tiny, modular design and energy-neutral operation. The reactors are linked in hydraulic series since one reactor is unable to completely extract all of the organics from the effluent. The system features numerous treatment trains running in parallel to enhance the amount of wastewater treated, and it also has the additional advantage of being easily serviceable. As a result, the system may continue to function while maintaining one treatment train. The power generated by the MFC allows for remote monitoring, control, and troubleshooting in real-time as well as the earliest possible system fault identification.

Microbiological Electrolysis Cell and Microbiological Fuel Cell

Two METs with a lot of study are MFCs and MECs. The two different kinds of METs have a similar technological setup, but they have different responses, processes, and end products.

A proton exchange membrane separates the anodic and cathodic chambers in a conventional MFC (PEM). Anaerobic bacteria may develop as an electrogenic biofilm on the anode and generate electrons and protons via the oxidation of substrates. Through the use of an external circuit, the electrons are sent to the cathode, where they interact with the oxygen and protons to form water. A voltage may be produced by the movement of electrons.

By using the protons and electrons produced by the microbial biofilm to create biofuels like hydrogen gas and methane, MEC is a modified version of the MFC technology. The MEC's cathode chamber, in contrast to the MFC, is situated in an anaerobic environment. This indicates that hydrogen gas is created by reducing water and protons using electrons that are transmitted from the anode to the cathode. Due to the reaction's unfavourable thermodynamics, an extra 0.2–0.8 V must be applied to facilitate the electron transport. Instead of being released as in the MFC, the carbon dioxide generated at the anode may potentially interact with protons and electrons to make methane.

The effectiveness of METs is greatly influenced by the microbial ecology and the interactions between microbes and electrodes. Because of their low long-term stability and decreasing electroactivity over time as a consequence of the challenging and unstable environmental circumstances, many METs are now severely constrained. Hydrogels could provide a solution to this problem. The gel's porous structure provides areas for microbial colonisation, and its special swelling qualities maintain a stable interior environment, guaranteeing the bacteria' long-term

survivability. The gel also makes it simple for nutrients and waste to diffuse into and out of the matrix, giving the microorganisms the resources they need to survive. Microbe binding and electron transport may also benefit from matrix polymers' capacity to change their functional groups.

Hydrogels are now often employed for microbial immobilisation, which is essential for METs, during the last 10 years. The stiff matrix and extremely porous structure provide a stable habitat that shields bacteria from environmental changes. This peaceful setting may reduce outside noise, enhancing sense signals.

Five different strains of strontium-resistant bacteria were immobilised in an acrylamide hydrogel polymer, and it was discovered that the immobilised microorganisms were more effective in removing strontium from the environment.

Alginate, cellulose gelatine, and silica hydrogel have all been employed by several researchers to immobilise various bacterial species for a range of sensing applications. Due to its quick growth and simple manipulation, E. coli is often employed as the model organism in numerous investigations.

Other often employed bacterial species include Lactobacillus sp., G. oxidans, and S. oneidensis. Microbes often need to be cultivated just before usage since it has been shown that while kept in storage, they lose their biological activity. Even after 40 days of storage, E. coli that had been immobilised in poly (vinyl alcohol) (PVA) hydrogels still exhibited excellent biological activity.

Since the metabolism of electrogenic microorganisms can convert chemical energy to electrical energy, electrochemical sensors often use them in place of transducers. Although persistent biofilms made of the electrogenic bacteria are often found in consortiums, this may reduce the species' selectivity.

Single species are advantageous since organic consumption and voltage output are closely correlated, however single-species biofilms are uncommon in nature and often exhibit instability. Hydrogels may function as an artificial matrix that enables the embedding of a single species. S. oneidensis was implanted into a PVA hydrogel anode by Kaiser et al., who then compared the electrochemical performance to that of an anode that solely contained a natural biofilm. An enhanced voltage output was seen from the anode implanted in the hydrogel.

Hydrogels may also enhance immobilisation for highly electrogenic pure culture bacteria that lack the genes for biofilm formation. Hydrogel clearly offers enormous benefits for transporting and immobilising germs while improving their function.

Water-Based MFCs

For use in MFC research, hydrogels have been customised and modified to increase the anodic biofilm's metabolic activity and the effectiveness of electron transfer. Numerous research have reported on anodes, cathodes, separators/membranes, and electrolytes made of hydrogel.

Hydrogels for anodes

Hydrogels of Conducting Polymers

A family of materials known as conducting polymer hydrogels (CPHs) combines the hydrogel's porous structure with the strong electrocatalytic activity of conductive polymers. They may physically interact with cell membranes, assisting in the facilitation of electron transmission, which is why they are utilised in the anodes of MFCs. By encasing bacteria in a buffered environment and improving electrocatalytic activity, the hydrogel lowers the electron transfer barrier and encourages metabolic activity.

Applications and future of MFC

A centralised treatment facility is probably never going to be replaced by microbial fuel cells. The greatest uses now include industrial pretreatment of difficult-to-treat, low-volume wastewater, such as hazardous chemicals from hydrocarbon processing or high-concentration streams from food processing. These streams are currently being handled by transportation, incineration, or land application, which is expensive and unsustainable.

Future uses of the technology include widespread sanitation for rural populations or disaster zones and manure management as technology prices continue to drop. MFCs may work well as an alternative for certain waste streams if some of the major scaling issues can be overcome. As previously said, they are not a panacea and probably won't cover the majority of the market for wastewater treatment, but their compact size, modular design, and energy-neutral operation provide them additional flexibility and the possibility to treat wastewater without a sewer or power grid.

However, there are several obstacles, such as high capital expenditures, that must be overcome for these systems to be used commercially. When compared to traditional reactors used in wastewater treatment, BESs cost more since they include electrodes, current collectors, wiring, and membranes. If a BES is used to entirely or partly replace an activated sludge system, it is anticipated that the value of the product created and the cost savings from less aeration needs will be sufficient to pay for the system's initial capital expenditure. With the use of various settings and artificial nutrients, including acetate, high energy productivity has been achieved. However, the system's effectiveness when utilising household wastewater is often substantially lower. Real wastewater has a low conductivity, which results in significant losses. Additionally, the limited alkalinity causes pH decreases that are constrained and reduce microbial activity. High microbial growth rates and particle concentrations may cause serious issues including separator fouling and pipe obstruction. Additionally, the management of current production is supported by the complexity of organic compounds in home wastewater, which involves many interactions and contests between various microorganisms. The chemical oxygen demand (COD) concentrations of the treated residential wastewater effluent are remained high, with 60-220 and 23-164 mg l1 in continuous flow and fed-batch modes, respectively. This is true even when MFCs are used for wastewater treatment. The biochemical oxygen demand (BOD), phosphorus,

and nitrogen permitted values for BES waste must thus be reached by post-treatment. Therefore, reducing energy use and maximising energy recovery from wastewater are the ultimate goals of BESs.

As a biosensor, the MFC

In the ScienceDirect database, a search for "microbial fuel cell" and "sensor" turned up 67 papers covering this topic from 2007 to 2017. This topic has recently had a tremendous growth in interest, as shown by the fact that almost 75% of these publications were published in the previous five years. Regarding the fictitious potential productivity of an MFC employing acetate as the substrate and oxygen as the electron acceptor. A MFC is prone to a variety of losses, including those brought on by activation, ohmic, and mass transfer. The output voltage will never reach the anticipated hypothetical rate as a result of these losses. The produced output power and voltage for MFCs have been much improved, but the findings have not yet been very encouraging for scaling up and commercial applications. MFC biosensors may be thought of as a different approach that makes use of the MFCs' insufficient power to easily and sustainably monitor target analytes in aquatic environments in situ or online. The developed anodic biofilm's electroactive metabolic activity, in which electrons are transferred from this biofilm to the anode surface via one of two methods—direct electron transfer using nanowires or direct contact, or mediated electron transfer using mediators or extracellular matrix—is directly related to the electrical current produced by an MFC. Any changes in their intricate metabolic processes might affect how much power is produced. This current variance may be reduced by maintaining operating constants such as feed conductivity, pH, and temperature.

The produced anodic biofilm, which serves as a bioreceptor, is essential to the scientific theory of employing MFCs as electrochemical biosensors (the recognition element). Response to a clear fluctuation causes the anode's electron flow rate to vary, acting as a transducer to turn the current change into a quantifiable signal. Other amperometric biosensor types need an applied external voltage to oxidise the analyte or substrate in order to produce a suitably functioning biosensor. Coupled fuel oxidation on the anode surface and oxidant reduction on the cathode surface ensures the driving potential of MFCs. The difficult part of the process is the anodic oxidation processes because they need nonsaturated fuel, which changes the amount of biodegradable organic matter in the feed, directly affecting how many electrons are transported to the anode and influencing the output current. Under these conditions, MFCs may be used as biosensors to measure the amount of organic carbon in water. The presence of anomalous materials in the feed will cause a quick change in the current output if the MFC is used in saturated fuel conditions with all other parameters, including pH, temperature, salinity, and constant anode electrode potential. Natural mixed microbe cultures have been used extensively, despite research on the use of pure cultures. The use of mixed microbial cultures ensures improved stability and produces MFC biosensors that are more effective. Because the bioreceptor cannot be effectively immobilised, the electroactive biofilm forms spontaneously during enrichment on the biocompatible surface of the anode electrode, enabling the MFC setup to physiologically monitor hazardous components in water.

Problems in using biosensors to evaluate water quality

Due to their low cost, disposable nature, and simple, compact form, biosensors have a significant potential to monitor and manage the quality of water and wastewater treatment in different treatment facilities. A biological recognition component is incorporated with a physical transducer in the biosensor mechanism to convert the biological response to an electrical signal depending on the analyte content. Additionally, it enables real-time monitoring, which lowers the expense of gathering and transporting water samples for additional analysis. Enzymatic biosensors are a common technology that differentiate the target analyte with high sensitivity. They are based on an electrochemical way of detection. However, they face a number of difficulties, such as high costs associated with enzyme purification, laborious immobilisation processes, and subpar durability and stability, which is related to a loss of enzyme function.

When large amounts are required, the use of bacterial cells offers an alternative to the enzymatic method. Since microbial cells contain a broad array of enzymes, microbial biosensors are very adaptable and selective to a wide range of target analytes. Conductometry, potentiometry, and amperometry are three electrochemical approaches that are often used for microbial sensors. Currently, microbial biosensors are mostly used to monitor water quality; however, a small number of commercial prototypes have also been used to detect water toxicity. The exceptional capacity of certain microbial strains to endure extremes in temperature, salinity, high alkalinity, and acidity opens up a bright future for water monitoring in industrial wastes. Microbial biosensors, however, face a number of difficulties.

Due to the need of substrate and product penetration into the cells, they include restricted selectivity, low recognition limits, significant possibility for contamination with other strains, and limited mass transmission. These issues make it difficult to use biosensors on-site. Biosensors are inadequate for the in situ monitoring of unexpected shocks in wastewater because they use a particular set of microorganisms and can only detect a limited number of target chemicals. Additionally, it has not been shown that enzymes or microorganisms immobilise on the surfaces of biosensors in severe water conditions, and they have a short lifespan, such as a few hours or days, which makes them unsuitable for long-term quality monitoring during wastewater treatment procedures. In addition, the present biosensors need additional dissolved oxygen (DO), conductivity, and pH probes to accurately assess changes in different parameters. Long-term remote real-time monitoring in difficult environments is presently restricted as a result of all these barriers. Additionally, the biosensor's slow reaction time makes it difficult to take prompt action to counteract the consequences of shock. For instance, anaerobic granule biosensors were used as an early warning system for the presence of copper or phenol in wastewater influent, but the biosensor's practical on-line detection applications were hampered by the response time delay.

Toxicity in water

The effectiveness of wastewater treatment facilities may be directly impacted by the variations in wastewater characteristics caused by municipal and industrial operations. The presence of certain

organic and/or inorganic contaminants, such as nitrate, organic carbon matters, and detergents, may cause variance in the usual operations of wastewater treatment facilities. Toxins, such as chromium, cyanide, or nickel, can cause serious, permanent damage to the biomass. The majority of wastewater quality measures undergo chemical analysis off-site, which adds time to the process of getting back to regular operation. In order to ensure environmental water safety and community health, developing real-time shock sensors are already proving to be crucial for monitoring the quality of wastewater influent as an online early timely alert. The latest generation of biosensors makes use of higher creatures like fish, algae, or bioluminescent microorganisms. Different signals, such as variations in movement or light intensity, are produced by these sensors in response. The fundamental disadvantage of these biosensors is that a transducer is required to transform the signal produced by the organisms into an electrical signal that can be connected to other variables. Because of the signals' measuring challenges or nonlinear trend, relying on a transducer might result in inaccuracies. As a result, new sensors should be designed such that data may be readily and reliably translated from directly collected signals. Electrical signals provide a direct method of measurement that is advantageous since it is transducer-free and simple to interpret. As real-time sensors for toxicant detection in water, many MFC-based biosensors have been created. The MFC may act as a sensor to track chemical toxicants in water since the presence of chemical toxicants can reduce the electrical current produced by MFCs. MFC sensors do, however, take a while to respond, and it might be difficult to ensure sensitivity when ambient circumstances change. The reaction time delay may be reduced by improving the construction and components of MFC sensors.

Transient fatty acids

Several bioprocesses, including anaerobic digestion, are measured for their volatile fatty acid concentrations to determine their effectiveness. Propionate, acetate, and butyrate are often the most readily accessible VFAs produced, making them significant factors in gauging the efficiency of bioprocesses. The development of a simple and affordable sensor, such as an MFC, that can identify tiny chain VFAs would enable its use in this crucial bioprocess. On the other hand, dependable microbial biocatalysts at the anode chamber are required for the use of MFCs as transducers. Conductive polymer and/or carbon composite electrodes were used to increase the sensor signal's repeatability, stability over time, and quick responsiveness to variations in the acetic, propionic, and butyric acid concentrations. By modifying the surface of the anode carbon-based electrode with functionalized polymeric coatings, such as poly(pyrrole) and polyacrylamide, the negatively charged biofilm immobilisation was improved. Additionally, by using these polymers to modify the anode electrode, it is possible to improve the sensor's reaction time, stability, and output of voltage and current. The use of the beneficial poly pyrrole functionalization was credited with the greatest improvements in sensitivity, stability, and repeatability when compared to pristine electrodes. The signals may be produced by organic acid concentrations in aqueous solutions ranging from 0 to 60 mg l-1.

The tremendous increase in world population makes it very difficult to get clean fresh water and treat wastewater. In order to provide an early warning of the presence of toxins and gather more

pertinent environmental data in real time, it is essential that onsite water quality sensors be effective, practical, quick, inexpensive, and easy to construct. The current small-scale devices created to function with high sensitivity have the most promise to enable MFC technology to satisfy these needs. Comparatively to other types of microbial biosensors, such as microbial bioluminescence-based biosensors, which depend on intricate and expensive transducers and electrical components to function, MFC-based biosensors have a simple and affordable construction.

MFC biosensors are a stand-alone technology that can test a variety of analytical objectives in real-time, but when compared to other conventional analytical devices, they still need to have enhanced stability, repeatability, sensitivity, and selectivity. Therefore, MFC-based biosensors will gain recognition and soon be authorised as a standard analytical method with the improvement of the analytical features. Researchers should concentrate on developing low-cost and straightforward MFC-based sensor device designs by employing affordable and robust electrode and membrane materials. By using inexpensive and efficient components, such as electrodes made of graphene, the overall cost of the MFC biosensor may be decreased, resulting in the production of scaled-up commercial units that are competitively priced and productive. The identification of new bacterial strains that can sustain biofilm formation and MFC activity in the presence of a wider spectrum of hazardous chemicals than those found in typical wastewaters also needs additional effort. MFCs have a great deal of potential to be reliable sensors that operate in difficult settings and realistic situations while yet delivering a satisfactory output response.

CHAPTER 12

NOVEL APPLICATIONS OF MICROBIAL FUEL CELLS IN SENSORS AND BIOSENSORS

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Innovative technologies that integrate biological redox catalytic activity with abiotic electrochemical processes and physics are being developed as a result of bio-electrochemical systems (BESs). BESs are often categorised according on the purposes for which they are used, such as the production of electricity, chemicals (such as formate or methane), and water desalination. Regarding the subject at hand, BESs that produce energy are classified as bio-electrochemical fuel cells, which are further separated into microbial fuel cells and enzymatic fuel cells (EFCs). To produce energy, MFCs specifically utilise microorganisms to catalyse electrochemical processes. MFCs typically have a proton exchange membrane between the anodic and cathodic chambers, while single chamber MFCs with or without an air-cathode are also possible. In the anodic chamber, microorganisms oxidise the organic or inorganic substance to release protons and electrons. The produced electrons are collected at the anode and sent via an external circuit to the cathode. Thus, MFCs are able to produce energy directly from a wide range of organic and inorganic chemicals by employing microbes as electrocatalysts. The cathodic processes in MFCs are very few in comparison to the almost infinite number of possible anodic reactions, and they often involve the reduction of oxygen to generate water or hydroxyl ions. However, it must be noted that there is still a lot of opportunity for advancement in terms of practical application and the lowering of production costs in the reactor configurations and electrolyte designs of MFCs.

A novel dual-purpose for MFCs as an energy generation method for wastewater treatment has recently been presented, resulting from the adaptability of feedstock utilised as anodic fuel. In reality, with a competitive life-cycle evaluation, MFCs might provide a self-sustainable or even a net positive energy production while eliminating pollutants. As an example, an artificial wetland recently adopted an MFC system. A built wetland (CW) is a technique for treating water that uses natural processes to enhance the quality of the water. This research has shown that while an MFC/CW system has a lower environmental effect than regular CW systems, it costs 1.5 times as much to operate.

The topic of this study and other current research efforts is the use of MFCs in the supply of electricity for distant electronic devices such sensors and biosensors. A sensor is, generally speaking, an electrical device that has the ability to recognise and measure changes in attributes and events happening in its immediate surroundings. The measured input data is transformed into an output electrical voltage or current and ultimately sent to other electronic devices, most often

a computer processor. Based on the enormous amount of work done by academics over the last several decades, assessing water quality, including pH, temperature, and heavy metal concentrations, is one of the most important uses of wireless sensors today. A biological or biomimetic component serves as a sensitive detector for an interest analyte, as shown in the particular situation of biosensors. A physico-chemical component or transducer converts the signal produced by the interaction with the analyte into a physically measurable signal that can be processed by an electronic signal processor.

The biochemical specificity of a biosensor's biologically active component or bioreceptor determines how well it performs its intended purpose. Biosensors may be categorised as enzymatic biosensors, immunosensors, genosensors or DNA biosensors, or tissue-based biosensors depending on the kind of receptor. According on the transduction method, biosensors may also be categorised into a number of groups, including electrochemical, optical, piezoelectric, and thermal/calorimetric biosensors. The food business, the medical area, the maritime industry, and other industries all have significant uses for biosensors.

MFC-based DNA biosensors, which are novel devices capable of specific-sequencing without the need for external power, have recently been presented as systems for detecting DNA. A dual-chambered MFC system was used as a power source to build an analytical instrument for the observation and analysis of the DNA immobilisation and hybridization events with the assistance of Danesh Gostar Hamgam Ba Sanat Company (Babol, Iran). The hybridization of the DNA probe was specifically controlled by the electrical output of the MFC, and any discrimination of complementary DNA sequences—such as non-complementary sequences or one-base mismatched sequences—was disclosed. The experiment's findings, which used human serum as a sample, demonstrated great sensitivity, notable stability, and tolerable repeatability.

Even more fascinating, because to their inherent sensitivity to biological and physico-chemical factors, MFCs themselves may be used as self-powered biosensors. Among the most intriguing applications are those that monitor microbial activity, biocorrosion caused by microbial biofilms, biochemical oxygen demand (BOD), toxicants, and pH and temperature changes. In comparison to traditional (bio)sensors, MFC-based (bio)sensors provide better stability and sensitivity. This review is separated into two distinct parts based on the aforementioned circumstances. The purpose of the first part is to discuss the advantages and disadvantages of using MFC integration as a power source for sensors and biosensors as well as the key elements affecting the performance of their power output, such as pH, temperature, and organic loading rate (OLR). The application of MFCs as self-powered biosensors for toxicants and BOD is described in the second part.

Sensor and Biosensor MFC Power Supply

The majority of industrial sensor and biosensor equipment are typically powered by electrochemical batteries (such as lithium batteries), which have a finite and relatively short lifespan and must be frequently recharged or replaced. It is now a hotly sought task to find effective and self-renewable energy sources that can provide adequate power for distant devices,

when battery replacement is neither practical nor easy. Accordingly, self-renewable MFCs emerge as a long-term alternate power source for biosensors and remote monitoring sensors, as schematically shown in Figure 3. Remote sensors that use MFC power supply instead of traditional batteries have much reduced operating costs and environmental dangers.

A appropriate feedstock, either organic or inorganic, for which substantial study has been carried out, is a crucial component for the MFC energy efficiency. The low power outputs fundamental to MFCs and the unstable electric potentials produced below the working potential of traditional electronic components in sensors, despite all the benefits, provide the most difficult obstacles to further increasing the use of MFCs in sensors. In order to give logical solutions to overcome the aforementioned limitations of MFCs, operating principles of MFCs as energy sources, as well as power needs and detailed performance of power management systems for distant sensors, have been researched. As a consequence, connecting many MFCs in series or parallel is one method for increasing the power output of MFCs. A recent remote biosensor based on MFCs connected in parallel that was created by Jiang *et al.* as part of a warning system to stop the usage of a dangerous water stream is a good example of this. According to the guiding idea that only the bioanode would be employed as the detecting and transducing element, the sensor was developed with a cathode-shared integrating four MFCs.

When subjected to a pH shift from 6 to 4, the sensor displayed an instant voltage drop. The cathode-shared MFC sensor array's small form ensured the detection's plausibility, and what's more intriguing is that the quantity of integrated MFC sensors could be adjusted in accordance with the monitoring need. Additionally, it has been shown that an MFC is capable of operating sensors and integrated telemetry systems for the wireless transmission of distant signals when equipped with the proper energy management system. The need for a high-power source is one of the issues with these devices' utilisation, however. The open circuit voltage (OCV) of numerous anaerobic fluidized bed microbial fuel cells or series designs when compared to the total of OCV of the individual cells. The use of ultra-capacitors to store the low energy generated by the MFC and deliver it only when the level of output energy is sufficient to operate the relevant remote device appears to be a more practical strategy for improving the operability of MFCs than the parallel or series design-based approach. Additionally, the electric potentials of MFCs have been effectively increased and stabilised by using DC/DC converters.

A novel form of floating microbial fuel cell (f-MFC) using this technology has recently been developed for the powering and data transmission of a distant environmental sensor. More specifically, ten active MFCs produced a cell potential between 100 and 800 mV. After around 20 to 30 days of startup, power output reached a high of 3-3.5 mW. A power management system based on a DC/DC converter was included into the devices. The DC/DC converter-equipped tested f-MFC effectively enabled the provision of basic electronic devices (a buzzer and an LED light) as well as the transfer of distant data. Interestingly, numerous aesthetically planned "floating gardens" MFCs were installed in the purpose of demonstrative activities at the 2015 Milan World Exposition EXPO2015 employing thick rice plants to assure the long-term

performance. Some of the "floating garden" MFCs remained operational for more than a year, which served as evidence of the potential application of such cells.

Finally, because they may have an impact on the power and electric potential outputs of MFCs, a number of environmental factors, including temperature, pH, organic loading rate (OLR), as well as the presence of toxicants and inhibitors, should be strictly taken into account when designing MFCs. To project the parts and the design of an MFC-driven sensor to scale-up for practical applications, among other things, it is necessary to comprehend the effect of those critical factors.

Temperature Effect

The system's kinetic and mass transfer processes are connected to many MFC characteristics including activation energy, mass transfer coefficient, and solution conductivity. Changes in temperature have a major impact on those processes, which has an impact on how well the MFC generates energy. The impact of temperature, on the other hand, varies depending on the kind of microbe and the feedstock used. According to the unique properties of the MFC, various optimal temperatures will be needed. Numerous studies have shown that raising the reaction temperature in the anode chamber significantly increases power production. This increase in power density has been linked to an improvement in microbial metabolism and membrane permeability as well as a decrease in ohmic resistance brought on by a greater liquid solution conductivity as a result of raising the temperature.

It is noteworthy to note that temperature fluctuations may also influence biofilm formation, since the growth of biofilms near the anode has a significant effect on anodic biocatalytic activity and, therefore, on energy production. Accordingly, certain studies demonstrate that the biofilm growth in an MFC and, subsequently, the starting time are both influenced by the beginning temperature. Therefore, faster biofilm stabilisation and MFC operation at higher temperatures result in better performance.

Despite the aforementioned, certain microbes prefer low-temperature conditions to work at their best, which may be advantageous for MFC-driven sensors that are installed in cold environments. However, microbial processes slow down at very low temperatures, and MFCs ultimately lose their ability to function.

Effect of pH

The pH is a crucial element in MFCs, as it is in other biological systems, having an impact on both cathodic reactions and anodic microbial activity. The oxidation of organic material in the anode compartment of an MFC generates protons, which move through the PEM into the cathode compartment where they typically interact with oxygen to form water molecules. The reaction media in the microbial anode chamber will become more acidic as a result of proton accumulation brought on by continuous operation and/or restricted proton diffusion and migration across the membrane. On the other hand, the pH in the cathode compartment is likewise impacted by the creation and migration of protons from the anode. In fact, the oxygen

reduction reaction (ORR), under imbalanced replenishment of protons, continuously consumes protons, favouring alkalinization. States that energy production eventually declines as a result of the cathode chamber's pH rise.

In fact, oxygen reduction benefits from low operating pH, which raises an MFC's current output. However, an anode pH that is too low may have a detrimental impact on the performance and durability of the biofilm by reducing bacterial activity and, as a result, the production of electrons and protons. Despite having an impact on biofilm formation, the specific response to pH variations will have an impact on other important factors, including ion concentration, membrane potential, and proton motive force. This is because the response is highly dependent on the type of microbe and the conditions required for its growth. Because each compartment often has a distinct optimal pH, the performance for energy production in traditional double-chamber MFCs relies on the pH gradient between the anode and cathode compartments. On the other hand, combined effects of the electrolyte pH on both anodic and cathodic processes dictate the performance of single-chamber MFCs, which have just one electrolyte. The majority of MFCs are said to operate best at pH values between 6 and 9, with neutral pH often being ideal. However, higher pH levels, between 8 and 10, are where air-cathode MFCs function best.

For pH fluctuations of 1 unit in MFCs, changes in current densities exceeding a factor of 4 have been seen. To maintain steady-state current output, active pH management using chemical buffers such phosphate, bicarbonate borax, or synthetic zwitterionic compounds has been suggested. The use of chemical buffers, although maintaining a constant pH, hinders the performance of the MFC owing to interactions with the electroactive microorganisms, electrodes, and/or proton exchange membrane. Chemical buffers are thus not practical for actual applications due to the inescapable severe influence on the power output and an inherent rise in operating expense resulting from their usage. A potential and cost-effective solution to the pH-buffering difficulty in MFCs is the harnessing of local sources of carbon dioxide for the in situ formation of a carbonate/bicarbonate buffer by reacting with hydroxide ions in the MFC cathode.

As an alternative, hybrid systems between single and dual chamber MFCs have been developed to provide self-sustaining pH control MFCs. In further detail, the self-sustaining elimination of accumulated protons in the effluent of the dual chamber MFC was carried out by an aerobic microbial oxidation of acetate by the biofilm connected to the air cathode of a single chamber MFC. Comparing the hybrid to the MFC made up of an isolated single or dual chamber, it has been shown that the hybrid displayed the best electricity production performance and the most effective conversion of acetate into electricity at high power levels. Additionally, the hybrid stack displayed a steady operating period of around 15 hours, which was about twice as long as that achieved using isolated MFCs. This was made possible by the helpful function of self-sustaining pH management by SC-MFCs.

Effect of Organic Loading Rate

As the rate of biological oxygen demand (BOD) per volume unit of solution, organic loading rate (OLR) may be formally described. BOD is a proximate indicator of how much oxygen aerobic

organisms need to oxidise the organic materials (often regarded as contaminants) present in a solution under isothermal circumstances. In order to measure the incoming mass rate of organic matter in a reactor for the treatment of wastewater or sludge, OLR is often utilised. Despite the fact that inorganic substrates may also be employed, it is important to highlight that the possible use for MFCs is as a self-powered reactor technology in a waste water/sludge treatment. As a result, OLR applied to a reactor is often referred to as volumetric loading rate, and it is measured in kg of BOD per reactor volume per day. The potential for substrate conversion in an MFC may also be determined by defining the organic loading rate per unit weight of microbial mass.

In order for MFCs to work well, running at the optimal sludge loading rate (SLR) or OLR is essential. This is because both power density and current efficiency rely on the substrate conversion rate, which is directly connected to OLR. As a result, raising the OLR up to a limit typically boosts energy production, after which greater loading rates reduce the MFC's output power. Because there is more feedstock available for oxidation in the anode due to the larger OLR, the electroactive biofilm's metabolic activity is favoured, which accounts for the increased power production. On the other hand, a higher OLR has been linked to reduced anode to cathode electron transfer efficiency, which impedes an MFC's ability to function properly. Compared to a conventional whole-cell biosensor, MFC is a self-powered biosensor.

The MFCs are appropriate to function as biosensors because of their great sensitivity to a variety of environmental and biological stimuli. Therefore, self-powered devices, i.e., operated without any external electricity source, may be developed to run autonomously for the distant detection and quantification of required parameters by suitably integrating energy production and sensing functions from MFCs. Indeed, a number of evaluations have already been published in relation to those distinct and brand-new uses proposed for MFCs as electrical biosensors.

Electrical biosensor is a self-contained integrated device that provides particular analytical information utilising a biological recognition element in contact with an electrochemical transduction element (anode/cathode electrodes in MFCs). Enzymes, fluorescent proteins, and other fluorescence/pigment molecules make up the majority of the biological components for analyte identification in traditional biosensors. A typical biosensor's uses for remote environmental monitoring are significantly constrained by the need for an external power source to run all electronic parts, such as the transducer and others, which convert, amplify, and transmit signals. The bacteria in an MFC, on the other hand, act as the biological sensing element and produce an electrical current that can be measured and output via the anode/cathode electrodes of the MFC. MFC-based biosensors do not need an external transducer since the analytical output signal is already measured as an electric current. As a result, MFC-based biosensors are thought to be more cost- and energy-efficient than traditional biosensors and are recyclable and sustainable. The most apparent uses for MFC-based biosensors, based on what has been mentioned so far, seem to be monitoring microbial activity and associated parameters, including such biochemical oxygen demand (BOD) or detection of inhibitors and toxicants. Their applications for quick monitoring of environmental variables including pH, temperature,

composition, or concentration of organic matter, as well as other parameters relating to the quality of water effluents, are particularly promising.

Biosensors based on MFC for BOD Detection

A well-known crucial measure for evaluating the quality of water is the biochemical oxygen demand (BOD). Concretely, the most common technique for determining the content of biodegradable organics in wastewater is the five-day BOD test method (BOD5). A water sample is incubated for five days at 20 °C as part of the test by measuring the quantity of dissolved oxygen (DO) before and after. This procedure is labor-intensive and time-consuming, and it needs much skill to provide repeatable results. Therefore, compared to a five-day BOD test, BOD evaluation using MFC-based biosensing seems to be a quicker, easier alternative technique with improved repeatability and accuracy. As a result, during the last several years, MFCs have been steadily incorporated as a BOD measurement method. The BOD measurement in an MFC is based on the proportionality between the quantity of oxidable organic matter present in the feedstock/fuel used to power the MFC system and the current efficiency of the cell (also known as Coulomb- or Faraday-efficiency).

The most significant benefits of an MFC-based BOD biosensor over traditional ones are accuracy and repeatability, quick reaction (rapid monitoring), long-term operational stability, and little maintenance service. An MFC-based device for remote BOD detection, for instance, was created by Kim *et al.* and displays more than 5 years' worth of steady current generation. Temperature, pH, conductivity, inorganic solid concentration, and other factors have been shown to have a substantial impact on the sensor's sensitivity. However, a number of other important factors, such as the level of dissolved oxygen in the cathode chamber, external resistance, effluent flow rate, and any other factor that will affect the MFC's ability to generate electricity, also have a significant impact on the stability and sensitivity of MFC-based biosensors for BOD monitoring.

Low metabolic rates and restricted bacterial and organic material variety are the fundamental drawbacks of MFC-based BOD biosensors. The most practical method for improving the performance and applicability of MFC-based sensors for BOD detection is currently thought to be research aimed at identifying versatile and highly active electrogenic microorganisms capable of efficiently oxidising various types of organic substances.

Monitoring microbial activity and contaminants in groundwater is a specific focus of BOD control. The majority of monitoring techniques are often expensive, time-consuming, and unsuitable for the subterranean environment. MFC BOD sensors provide quick, easy, non-invasive, and affordable alternatives. A brand-new submersible microbial fuel cell with this objective in mind. The groundwater BOD and microbiological activity were monitored in situ using the cell as a self-powered sensor. The cell identified microbial activity and BOD in less than 3 hours using actual polluted groundwater, with variations ranging from 15% to 22% and 6% to 16%, respectively.

More recently, employing MnO2 catalysts, Kharkwal *et al.* produced an MFC-based BOD sensor with long-term stability. In particular, manganese dioxide was employed as a brand-new, cost-effective cathode to lower MFC manufacturing costs in preparation for potential scale-up applications. Both genuine household wastewater and sodium acetate solution were used to evaluate the cell. With minor fluctuations in the range of 3% to 12%, the BOD levels predicted by the biosensor and the BOD5 readings agreed. It was shown that the problematic MFC BOD biosensor was stable for more than 1.5 years.

MFC-Based Biosensors for Detecting Water Toxicity

A key factor in determining the required steps for supplying safe water with the right level of quality for consumption by people, animals, and crops is the detection of toxicity in water. Due to the fact that any toxicant in the aqueous feedstock would alter the metabolic activity of microorganisms and, as a result, the rate of substrate intake, which is, as was previously said above, directly connected to the current output of an MFC, MFCs seem to be suitable sensitive systems. By keeping an eye on changes in the electric current caused by MFCs, it is possible to identify any change in the presence or concentration of toxicants in flowing water with ease, saving time and money (on personnel, reactants, and analytical equipment) compared to traditional approaches.

The straightforward and planar silicon-based miniaturised MFC, created by Dávila and colleagues in 2011 as a fast response toxicity biosensor, is one of the most important developments in the recent ten years. Regardless of the biosensing application, a silicon-based MFC was found to have a maximum power density of roughly 6.5 W/cm2. Finally, an MFC-based toxicity sensor's performance and sensitivity to various toxicants heavily rely on the kind of electroactive microorganisms utilised in the cell. To create the right devices in accordance with the necessary monitoring demands, it is crucial to choose microorganisms with specifically improved sensitivity to certain toxicants.

This work, which has caught the attention of academics from all around the globe, demonstrates the promise of MFCs in the area of sensors and biosensors. MFC is renowned for having a poor power output when compared to other electrical energy sources. However, MFCs may be used as a reliable, long-lasting power supply in low-power electronic devices like remote sensors. Due to the remarkable sensitivity shown to BOD, DO, and toxicity in water nowadays, MFCs may also be thought of as self-powered biosensors for doing so. Fast reaction times, affordable operation, and the ability to run on their own power are just a few of the impressive benefits of the devices described above, which hold great potential for expanding applications.

QUESTIONS FOR PRACTICE

1. What is MFC? Describe the principle mechanism of MFC.
2. What are the components of MFC? Describe the functions of various componants.
3. What kind of anode is used in microbial fuel cells?
4. What kind of bacteria is used in microbial leaching?
5. What are the applications of microbial fuel cells?
6. What kind of microbial fuel cells are there?
7. What is the microbial fuel cell's voltage?
8. What components make up the microbial fuel cell?
9. What microorganisms are utilized in microbial leaching, and what is their name?
10. What substance is used in fuel cells?

REFERENCE BOOKS FOR FURTHER READING

1. Microbial Fuel Cells by Bruce E. Logan · 2008 Publisher:Wiley

2. Microbial Fuel Cell: A Bioelectrochemical System that Converts Waste to Watts 1st ed. 2018.

3. Microbial Electrochemical and Fuel Cells: Fundamentals and Applications (Woodhead Publishing Series in Energy) 1st ed.

4. Integrated Microbial Fuel Cells for Wastewater Treatment; Rouzbeh Abbassi (editor), Asheesh Kumar Yadav (editor), Faisal Khan (editor), Vikram Garaniya (editor)

5. Microbial fuel cells: electricity generation from organic wastes by microbes. Authors: Guo Kun Guo Kun, D. J. Hassett, T. Y. Gu

6. PEM Fuel Cell Durability Handbook, Two-Volume Set Edited ByHaijiang Wang, Hui Li, Xiao-Zi Yuan 1st ed. 2018

7. Microbial fuel cells: novel biotechnology for energy generation; Korneel Rabaey; Willy Verstraete

8. Micro Fuel Cells: Principles and Applications; 1st Edition, 2009
