Efficient and Ecofriendly Water/Wastewater Quality Monitoring with Microbial Fuel Cell Biosensors

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ABSTRACT

In terms of current global issues, the world is primarily threatened by two crucial challenges: environmental pollution and water scarcity. Concerning the potential threats of water scarcity and contamination, the sustainability of water and treatment of sewage are amongst important priorities for any society attempting to avoid these threats. As a result, the properties of water and wastewater must be continuously monitored, and the quality should be maintained at standard levels. Furthermore, due to water scarcity and sanitary concerns, the used form of water known as wastewater must be treated and reused. Hence, environmental protection is one of the essential aspects of modern governance and of the priorities of Oman Vision 2040. Quality-determining parameters such as COD, BOD, and toxicity are the most common parameters to estimate the amount of pollution in water/wastewater which their detection methods are really challenging, i.e., time-consuming, laborious and produce hazardous chemicals. Besides, water quality measurement is a daily task in all industrial and agricultural plants with huge costs. Therefore, this research work assesses the potential of using biosensors, based on the green technology of microbial fuel cell, as environmentally friendly, portable and inexpensive tools for monitoring the water/wastewater quality in an efficient and sustainable pathway.

Introduction

The globe is rushing to develop new, more efficient, and environmentally friendly industrial machinery. This research effort aims to develop an MFC-based biosensor for online quality monitoring in industrial wastewater. Wastewater represents one of the major threats to life on earth, thus it needs to be treated and its quality continually monitored. When wastewater escapes, it contaminates land and groundwater, which is the main threat it poses. Additionally, it contributes to the spread of pathogens and microbes that can harm human health. Furthermore, the death of aquatic organisms caused by microbes consuming the dissolved oxygen in the water creates an unbalanced environment. In addition, climate change has brought up a variety of problems, including a water deficit. Thus, the only option is to protect the current sources, such as groundwater and wastewater, through ongoing quality monitoring (Sun *et al.*, 2015).

Sensors are the best device we can select to monitor wastewater quality. Depending on its particular application and the parameters that must be evaluated, the optimum monitoring tool for wastewater quality must be chosen. pH, dissolved oxygen (DO), turbidity, conductivity, temperature, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) are one of the basic parameters that frequently appear in wastewater. Sensors are devices that recognize physical stimuli in the environment, react to them, and translate them into electrical impulses. These signals can subsequently be processed and evaluated, to offer information about the environment or to manage a system. One sort of sensor that can recognize and react to biological substances or impulses is the biological sensor. These sensors are frequently employed in applications related to medicine, healthcare, environmental monitoring, and food safety.

The biosensor is one sort of biological sensor, and it generally comprises an enzyme or antibody together with a transducer that transforms the biological signal into an electrical signal (Zhang *et al.*, 2022).

Microbial fuel cells (MFCs) are used as the transduction mechanism in MFC-based biosensors, which are biosensors. Devices called MFCs produce energy from the metabolic processes of microorganisms like bacteria or algae. The electrical signal produced by the MFC is used by MFC-based biosensors to identify and measure the presence of particular analytes in a sample. A biological recognition substance, such as an enzyme, antibody, or DNA probe, that specifically binds to the target analyte is often connected with the MFC in MFC-based biosensors. The electrical signal produced by the MFC changes when the target analyte binds to the recognition element, which may be monitored and utilized to calculate the analyte's concentration. In comparison to conventional biosensors, MFC-based biosensors have several advantages, such as high sensitivity, quick response times, and the capacity to function in challenging environments like soil or wastewater. They have been utilized in a variety of functions, such as environmental monitoring, biomedical diagnostics, and testing for food safety. They do, however, have certain drawbacks, namely the requirement for a constant source of fuel and the chance of interference from other microbes in the sample (Cui, Lai and Tang, 2019).

History of MFC-Based Biosensor

In 1791, Luigi Galvani, an Italian physician, and scientist who explored the nature and effects of electricity in animal tissue was the first to identify a bioelectric phenomenon when he saw the twitching of an isolated frog leg after passing a brief electrical discharge through it. The term "bioelectricity" was developed as a result of the discovery. In 1838, Welsh physical scientist and lawyer William Robert Grove devised the "Grove cell" wet-cell battery (Flimban *et al.*, 2019). This is a two-fluid electric cell that generates roughly 12 amps of current at 1.8 volts by using amalgamated zinc in dilute sulfuric acid and a platinum cathode in strong nitric acid, separated by a porous ceramic pot. By relating physical properties and chemical reactions, Friedrich Wilhelm Ostwald, a pioneer in the field of physical chemistry, experimentally determined the interconnections between the various components of the fuel cell: electrodes, electrolytes, oxidizing and reducing agents, anions, and cations in 1893 (Flimban *et al.*, 2019).

Michael Cresse Potter, a professor of Botany at the University of Durham in the United Kingdom, proved in 1910 that organisms may transmit current and create voltage while studying how bacteria break down organic molecules (Flimban *et al.*, 2019). In 1911, Potter discovered electrical energy in cell cultures of Saccharomyces cerevisiae and Escherichia coli using platinum electrodes. This discovery prompted him to construct a crude microbial fuel cell. Cohen of Cambridge, UK, revived Potter's notion in 1931 when he described how a batch of biological fuel cells generated more than 35 V (Flimban *et al.*, 2019). The fuel cell concept gained prominence when the Space Administration and the National Aeronautics and Space Administration indicated an interest in turning organic waste into power on extended space journeys in the 1960s. Bacteria and algae were the first species used in biological fuel cells. During this time, the Rohrback group developed the first biological fuel cell, which used Clostridium butyricum as a biomaterial to generate hydrogen through glucose fermentation. The use of biological fuel cells as a source of energy became commercially available quite quickly, but was generally useless, eventually disappearing from the market (Flimban *et al.*, 2019).

Williams established in 1966 that rice husk is a viable source of lignocellulose since fermentation produces various important enzymes and biofuels, resulting in 40 mA at 6 V using biological fuel cells. M. J. Allen and H. Peter Bennetto of Kings College, UK, pioneered the fuel cell revolution by developing improved biological fuel cells that used varied bacteria to increase both the reaction rate and the efficiency of electron-transfer systems (Flimban *et al.*, 2019). They combined an understanding of the electron transport mechanism with significant technical improvements. In 1999, the Byung Hong Kim group at the Korean Institute of Science and Technology discovered certain electrochemically active bacterial species that did not require mediator molecules to transfer electrons to electrodes. Using electrochemical experiments, they discovered that Shewanella sp. is capable of electrochemical reactions. Chaudhuri and Lovely showed that in the presence of Fe3+, the R. ferrireducens bacterium may recover up to 83% of the electrons lost during glucose oxidation (without the use of a mediator). Figure 1 displays a schematic depiction of the timeframe of the previous history (Flimban *et al.*, 2019).

Concept and Mechanism

Microbial fuel cell (MFC)-based biosensors are a type of electrochemical biosensors that use microorganisms as biocatalysts to convert chemical energy into electrical energy. These devices are capable of detecting various target analytes, such as organic compounds, heavy metals, and toxins in environmental, clinical, and food safety monitoring. The general methodology and mechanism of MFC-based biosensors involve the following stages. First is the selection of microorganisms capable of oxidizing the target analyte and generating electrons as a byproduct of their metabolism. The selection of microorganisms can be based on their electrochemical activity, substrate specificity, and resistance to environmental stresses. Second is the design and construction of MFC, which consists of two compartments: an anode and a cathode separated by a proton exchange membrane (PEM) or a salt bridge. The anode is typically made of a conductive material, such as graphite, carbon fiber, or a conductive polymer, and is inoculated with the selected microorganisms (Cui et al., 2019). The cathode is usually made of a noble metal, such as platinum, and is exposed to the air or another electron acceptor. The third step is the detection of the target analyte, which is based on the changes in the electrical output of the MFC. When the microorganisms oxidize the target analyte at the anode, they transfer the electrons to the electrode surface, generating a current that can be measured by an external circuit. The changes in the current output of the MFC are directly proportional to the concentration of the analyte being detected (Cui et al., 2019).

Overall, the principal mechanism of MFC-based biosensors involves the transfer of electrons from the microorganisms to the electrode surface via an electron transfer pathway. The electrons are generated by the oxidation of the target analyte by the microorganisms, which results in the production of protons and other metabolic byproducts. In addition, the electrons generated by the microorganisms are transferred to the anode through a series of redox reactions mediated by electron transport proteins, such as cytochromes, quinones, and flavoproteins. The anode acts as an electron acceptor, receiving the electrons and generating a current that can be measured by an external circuit. Moreover, the cathode, on the other hand, acts as an electron donor, receiving electrons from the external circuit and reducing the electron acceptor, such as oxygen or another oxidizing agent. The reduction of the electron acceptor generates a current that completes the circuit and allows the MFC to function as a biosensor (Cui et al., 2019).

Parameters

Chemical Oxygen Demand (COD)

Chemical and biochemical oxygen demand are the primary metrics used to assess mine effluent organic matter content (COD and BOD, respectively). Although precise and rapid measurement is vital, current procedures are time-consuming, produce chemical compounds that are dangerous to the environment, and need the use of specialist experts. As a result, they are unsuited for real-time monitoring, making it difficult to respond quickly to contamination occurrences (Corbella *et al.*, 2019).

The major pollutant impact of wastewater is organic matter (Li *et al.*, 2018). Analyzing chemical oxygen demand (COD) or biological oxygen demand (BOD), which represent the level of chemical and biological oxidation, respectively, might help predict organic matter contamination in water bodies (Li *et al.*, 2018). COD is the mass concentration of oxygen equal to the amount of dichromate consumed by dissolved and suspended matter when a sample (water or sludge) is treated with that oxidant under prescribed conditions (Geerdink, van den Hurk and Epema, 2017). COD is a more accurate depiction of organic matter than BOD since BOD is difficult to standardize and takes a long time to calculate (Li *et al.*, 2018). Moreover, BOD provides no information regarding the oxidation state of an organic molecule (Li *et al.*, 2018).

Traditional COD assessment methods (dichromate colorimetric methodology) employ several very toxic/hazardous chemicals, including silver sulfate, potassium dichromate, and sulfuric acid. All of them have the potential to affect the environment in which we live. Also, the composition of the samples may easily interfere with the results. Since silver sulfate is used as a catalyst during digestion, halides in water samples will precipitate with Ag2SO4, impacting the effectiveness of organic oxidation and, thus, the accuracy of COD determination. Moreover, the presence of reduced inorganic species in water samples accounts for stoichiometric oxidation, necessitating further modifications before the final results, exposing another shortcoming of the traditional approach (Xu et al., 2017).

Microbial Fuel Cells are bioelectrochemical devices that generate energy by oxidizing organic molecules utilizing exoelectrogenic bacteria as catalysts. In addition to being used for green energy production, MFCs have the potential to be used as a tool for continuous or semi-continuous organic matter concentration measurement. MFCs provide an electrical signal that can be associated with organic matter content. The flexibility to install MFCs in situ, monitor them online, avoid expensive laboratory operations that need the addition of chemicals, and the lack of a transducer are the key advantages of employing MFCs as biosensor devices (Corbella et al., 2019).

Biochemical Oxygen Demand (BOD)

An attractive new approach to detect biological oxygen demand (BOD) is microbial fuel cell (MFC)-based biosensors. The amount of oxygen that is consumed by bacteria and other organisms during the aerobic process of decomposition of organic matter is known as biochemical oxygen demand (BOD). Additionally, it has experienced a thorough and quick development over the past 20 years, making it one of the most extensively used metrics for evaluating water quality (Cui et al., 2019). A crucial metric for describing biodegradable organic matter in water and wastewater is the biochemical oxygen demand (BOD). In addition, From BOD assessment to toxicity detection, DO identification, microbial activity monitoring in water/wastewater plants, or as a power supply for other sensors, it has a wide range of applications. Additionally, it demonstrates distinct advantages in numerous scenarios, including low cost, in situ evaluation, simple operation, and straightforward building. Due to their unique benefits, which primarily include improved durability and sustainability, the lack of test kits and reduced maintenance, and the fact that some MFC-based biosensors are commercially accessible, they have also been attracting growing research interest. MFC-based biosensors may someday become recognized as standard practices as a result of advancements in materials and microbiology, particularly electrogenic bacteria (Cui et al., 2019).

Microbial fuel cells (MFCs) have been used for environmental monitoring as whole-cell-based biosensors. MFCs, on the other hand, are gadgets that use microorganisms as a form of catalyst to turn chemical energy into electricity. They can be classified into two categories based on configuration: single-chamber MFCs and dual-chamber MFCs. An ion exchange membrane (IEM) separates the anodic and cathodic chambers in dual-chamber MFCs, whereas anodic chambers and air cathodes make up the majority of single-chamber MFCs. When organic substances in water or wastewater are oxidized by electroactive microorganisms, electrons, and protons are produced (Cui et al., 2019). The positive electrode collects electrons from the oxidation process, which are subsequently transferred to the negative electrode via a resistance-equipped external circuit. other protons and cations, such as Na+ and K+, move to the cathode via the IEM in the meantime to maintain charge balance. Finally, the electrons and protons will join with the oxygen to make water when it serves as an electron acceptor. And the electrons are delivered as an electrical signal to the data logger so that it can be displayed as data to show how much or how concentrated an organic compound is in the water sample (Cui et al., 2019).

Heavy Metals

One of the main problems with heavy metal persistence, toxicity, and bioaccumulation has been considered to be environmental contamination. The ecosystem and human health are directly threatened by the accumulation of heavy metal ions in wastewater, including Cu^{2+}, Cd^{2+}, Cr^{6+}, Pb^{2+}, As, and others (Bashir et al., 2019) (Do *et al.*, 2022). Because heavy metals are easily accumulated in the human body and cannot be broken down by nature. An excessive buildup of heavy metals can affect the human central nervous system, lower energy levels, and damage vital bodily organs like the liver, kidneys, lungs, and other vital organs (Wang *et al.*, 2023). Additionally, heavy metals pose a serious risk to human health since they can disrupt chromosomal stability, result in neuropathy, and harm the liver. As an illustration, copper is a heavy metal that is essential to numerous processes, such as electron transport, oxidation, and oxygenation of substrates. However, a high copper level can cause adverse effects that are hazardous to both humans and the ecosystem, including nausea, vomiting, diarrhea, tremors, rigidity, and even death (Zhang *et al.*, 2022).

Chemical analysis techniques such as ion chromatography, atomic absorption spectrometry, inductively coupled plasma mass spectroscopy, colorimetric techniques, gas chromatography-mass spectroscopy, flame atomic absorption spectroscopy, inductively coupled plasma optical emission spectroscopy, and inductively coupled plasma-mass spectroscopy are being used to detect chromium in water samples. However, they require expensive equipment, extra chemical compounds, specialist training, and lengthy measurement durations. Microbial fuel cells (MFCs) have emerged as biosensors for managing wastewater quality. Microbial fuel cells (MFCs) are technologies that directly transform the chemical energy in organic materials into electrical energy through the metabolism of bacteria. They have received a lot of interest due to their attributes of energy efficiency, affordability, simplicity of use, and sustainability (Wu *et al.*, 2017)

MFCs use electroactive bacteria (EAB) as biocatalysts and have anaerobic and aerobic cathode compartments divided by a proton exchange membrane (PEM). To create an electrical current, electrons are first delivered to the anode electrode and then move via an external resistance to the cathode. However, toxic pollutants can inhibit EAB activity and high concentrations of pollutants can cause irreversible damage, restricting their usefulness. In heavy metal monitoring the best condition is the optimal pH and temperature for the development of a community of electrogenic microorganisms were found to be 7.0-8.0 and 25 °C, respectively. A too-high temperature may favor the growth of methanogens, which is detrimental to the ability of the MFC biosensors to generate electricity, the best external resistance 1000 Ω was selected and the best value of organic substrate concentration -300 mg L^{-1} was selected. The linear response was seen throughout a wider range of Cu^{2+} and arsenic detection limits. These findings suggest that their suggested approach can perform well as a biosensor indicator for heavy metals (Do *et al.*, 2022).

Table 1. Details of previous studies done on MFC based biosensor in the literature.

MFC Configuration	Reactor	Definition	Application	Anode	cathode	Linear range	Response time (min)	Reference
Biochemical oxygen demand (BOD)	Double	a chemical method for estimating the amount of dissolved oxygen required by aerobic living organisms in a body of water to break down organic material contained in a water sample at a particular temperature during a specific period.	They are used to measure the amount of oxygen utilized by these organisms over a given time.	Carbon brush	Carbon brush	20-500 mL	NA	(Zhou *et al.*, 2018)
Biochemical oxygen	Single		Used to assess the quality of	Carbon felt	AC-air cathode	200 mg/L	NA	(Wang *et al.*, 2018)

demand (BOD)			water and wastewater.					
Chemical oxygen demand (COD)		Its the amount of dissolved oxygen required to oxidize organic chemical compounds such as petroleum in water.	They are used to determine the short-term impact of wastewater effluents on receiving water oxygen levels.	Carbon cloth (330μm , wosl1009, tech co, Ltd)	Pt film (40μm, wrights of Lymm)	NA	10 min	(Xiao *et al.*, 2020)
Chemical oxygen demand (COD)		The COD is the number of oxygen equivalents consumed in the oxidation of organic compounds by solid oxidizing agents, including dichromates and permanganates, and it indicates the number of organic contaminants in the sample.	used to estimate the levels of organic contaminants in wastewater	Cu electrodes nano-PbO2-modified electrodes	Cuo Ti PbO2 disc	(5 to 3000 mg/L) and a low detection limit (1.84 mg/L)	NA	(Yao, Wang and Zhou, 2014)
Volatile fatty acids (VFAs)	Double	Linear short-chain aliphatic mono-carboxylate molecules are the building blocks of many organic chemicals, such as acetic acid, propionic acid, and butyric acid; VFAs have two (acetic acid) to six (caproic acid) carbon atoms.	The concentration of volatile fatty acids (VFAs) is one of the essential metrics for monitoring bioprocesses such as anaerobic digestion and microbial fuel cells.	Ag	AgCl	<40 mg/L	1-2 min	(Kaur *et al.*, 2013)
Dissolved oxygen (Do)		Free and non-compound oxygen in water or other liquids is referred to as dissolved oxygen (DO), and it is engaged in various biochemical and physiological processes.	Data on dissolved oxygen provide vital information about biological and metabolic reactions in aquatic settings.	Four pieces of graphite felt	Two pieces of plain carbon cloth	NA	3.3 min	(Markfort and Hondzo, 2009)

Conclusion

The development of efficient and environmentally friendly industrial technologies is a global priority. Monitoring the quality of wastewater is crucial in preventing its contamination and protecting the environment, human health, and aquatic organisms. Biosensors, particularly MFC-based biosensors, are a highly sensitive and efficient tool for continuous monitoring of various wastewater parameters such as pH, BOD, COD, heavy metals, and dissolved oxygen. They offer a quick response time and are suitable for use in difficult environments. To further improve the effectiveness of MFC-based biosensors in wastewater monitoring, future research should focus on developing robust and efficient fuel cells that can generate stable power output for long-term use. Additionally, the use of advanced technologies such as artificial intelligence and machine learning can enhance the accuracy and speed of data analysis, leading to better decision-making and timely response to potential contamination events. Overall, the adoption of biosensors in wastewater treatment plants holds great promise for protecting the environment and ensuring sustainable industrial practices.

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