Review



# Biosensors Based on Microbial Fuel Cells: A Brief Review

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# Biosensors Based on Microbial Fuel Cells: A Brief Review

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**Abstract:** Microbial fuel cell (MFCs) devices utilise the metabolic processes of bacteria to generate electricity from various organic substrates. Due to the enormous amount of energy that organic waste produces, scientists are highly interested in advanced MFCs. MFCs serve as a multidisciplinary research platform at the engineering and natural sciences intersection. MFCs have various uses besides being used as energy sources, such as sensing capabilities. Despite showing considerable promise, only a few marine sediment MFCs have been deployed in real-world applications to supply current for low-power devices. It is now required to maintain track of the work being done by research groups worldwide and regularly compile significant discoveries due to the rising quantity of research publications. Review papers are a traditional beginning point for a literature review for new scholars. This review is a fast reckoner that directs readers to pertinent reviews and research publications detailing significant advances in microbial fuel cell research during the previous two decades. An overview of key advances in MFC research over the past two decades is provided as a quick reference in this review article. In addition, the report identifies research gaps that, if filled, could bring this technology closer to real-world use.

**Keywords:** MFCs; wastewater treatment; Bio-sensors; alternate energy

## Introduction

A bioelectrochemical system (BES) combines biological and electrochemical processes to convert chemical energy to electrical energy or the other way around [1, 2, 3]. Microbes like bacteria, archaea, or fungi are employed in a BES to catalyse processes that result in the production of electrons, which can either be captured as energy or used to drive chemical reactions **[4, 5]**. BESs have a wide range of applications, including biosensors, energy generation, and wastewater treatment. Microbial electrolysis cells (MECs) and microbial fuel cells (MFCs) are the two primary categories of BES **[6-8]**. MFC is a bioelectrochemical device that can generate energy by using electrons obtained from anaerobic substrate oxidation **[9-11]**. MFC technology has received a lot of interest in recent years **[12-17]**.

Microorganisms oxidise organic materials in an MFC to produce electrons, which are then collected at an electrode. Microorganisms at an electrode in a MEC are given electrons, which they utilize to break down carbon dioxide or other substrates into products like hydrogen. To produce energy, MFCs specifically use microbes to catalyse electrochemical reactions. MFCs typically have anodic and cathodic chambers that are separated by a proton exchange membrane (PEM), as depicted in **Figure 1**, though single chamber MFCs with or without an air-cathode are also possible **[18-21]**. 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 transmitted through an external circuit to the cathode. Thus, MFCs are able to produce energy directly from a range of organic and inorganic chemicals by using microbes as electrocatalysts **[22-24]**.

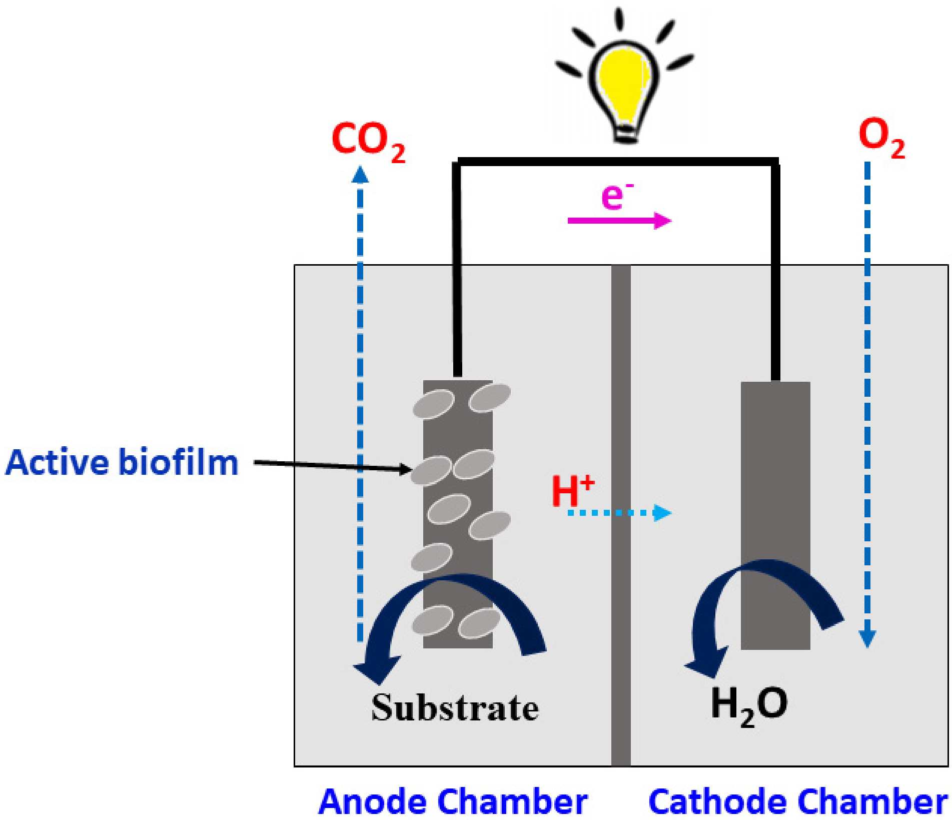


**Figure 1.** Schematic diagram of a MFC.

As illustrated in Figure 2, MFCs have recently found applications in wastewater treatment **[25, 26]** microbial solar cells **[27]**, bioelectricity generation **[28, 29]**, industrial chemicals recovery **[30]**, pollutant removal **[31]**, microbial desalination cells **[32, 33]**, sensors **[34-36]**, hydrogen production **[37, 38]**, bioremediation **[39, 40]**, and energy recovery **[41, 42]**.

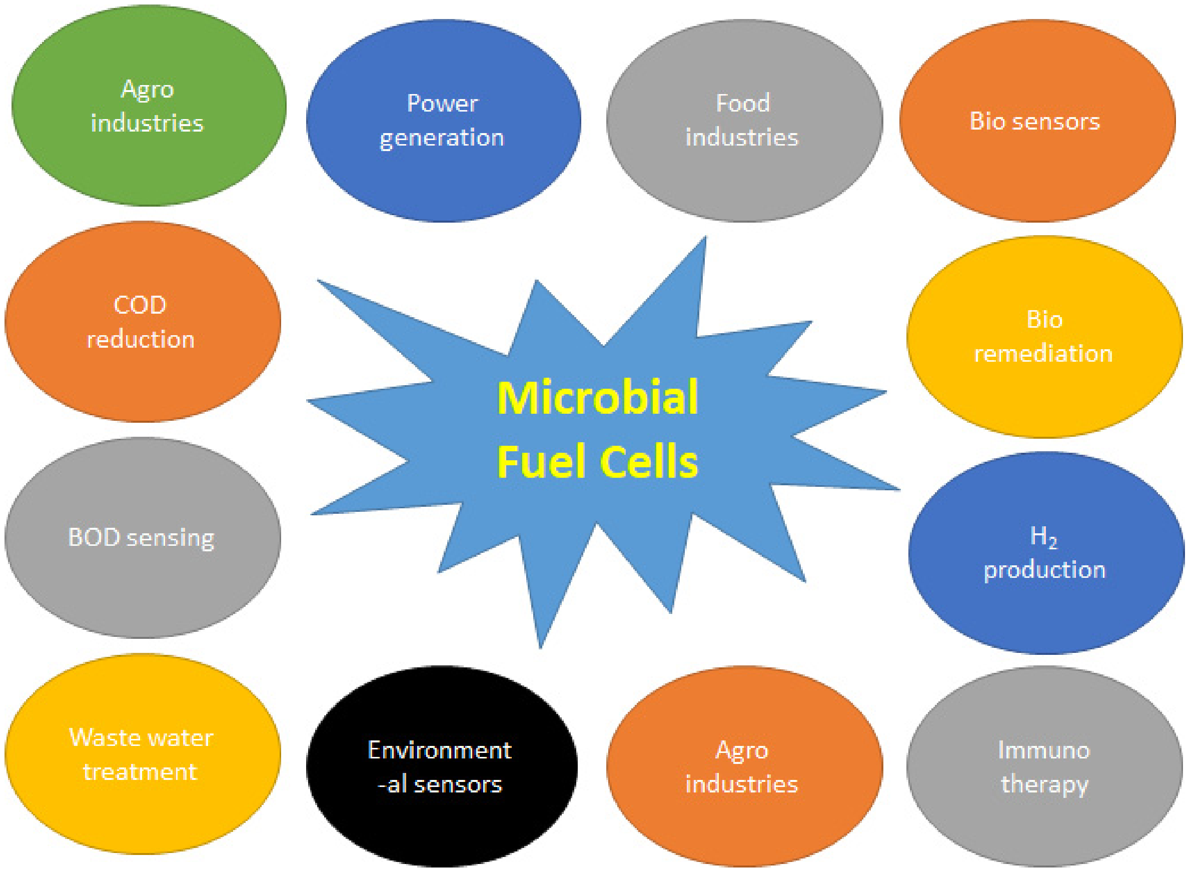


**Figure 2.** Applications of MFCs.

MFCs can be utilised for a number of applications, including sensors. The microorganisms are utilised in an MFC-based sensor to identify and quantify the presence of specific chemicals in a sample. The microorganisms in the MFC, for instance, will absorb glucose and produce electricity as a by-product if the MFC is intended to detect glucose **[43]**. The concentration of glucose in the sample can then be calculated from the amount of power generated. MFC based sensors have a number of advantages to conventional ones, such as the potential for usage in remote or inaccessible sites and the capacity to work under low-power settings. They do however, also have substantial disadvantages, including as the microorganisms' dependence on a constant supply of nutrients and their sensitivity to changes in temperature and pH. MFC-based sensors are generally promising for a variety of applications, such as environmental monitoring, medical diagnostics, and food safety testing **[44]**. The performance and dependability of these sensors are now being improved, and new applications for this technology are being explored.

In fact, with a competitive life-cycle evaluation, MFCs might have an energy output that is self-sustaining or even net-positive while removing pollutants **[45-50]**. As an illustration, 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 **[51]**. This study has shown that although an MFC/CW system has a lower environmental impact than regular CW systems, it costs 1.5 times as much to operate **[52]**.

The analytical tool known as a "biosensor" transforms a biological response into a signal that can be measured and processed. The bioreceptor, which precisely interacts with the target analyte, and the transducer, which transforms this connection into an electronic signal, are the two primary parts of a standard biosensor. The transducer response can then be analysed, displayed, and further amplified by a detector circuit. Therefore, a biosensor is an independent, integrated system that combines a biological recognition component with an external transducer **(Figure 3)**. Biosensors can be categorized into electrochemical, calorimetric, optical, and piezoelectric types depending on the type of transducer they use **[53]**.

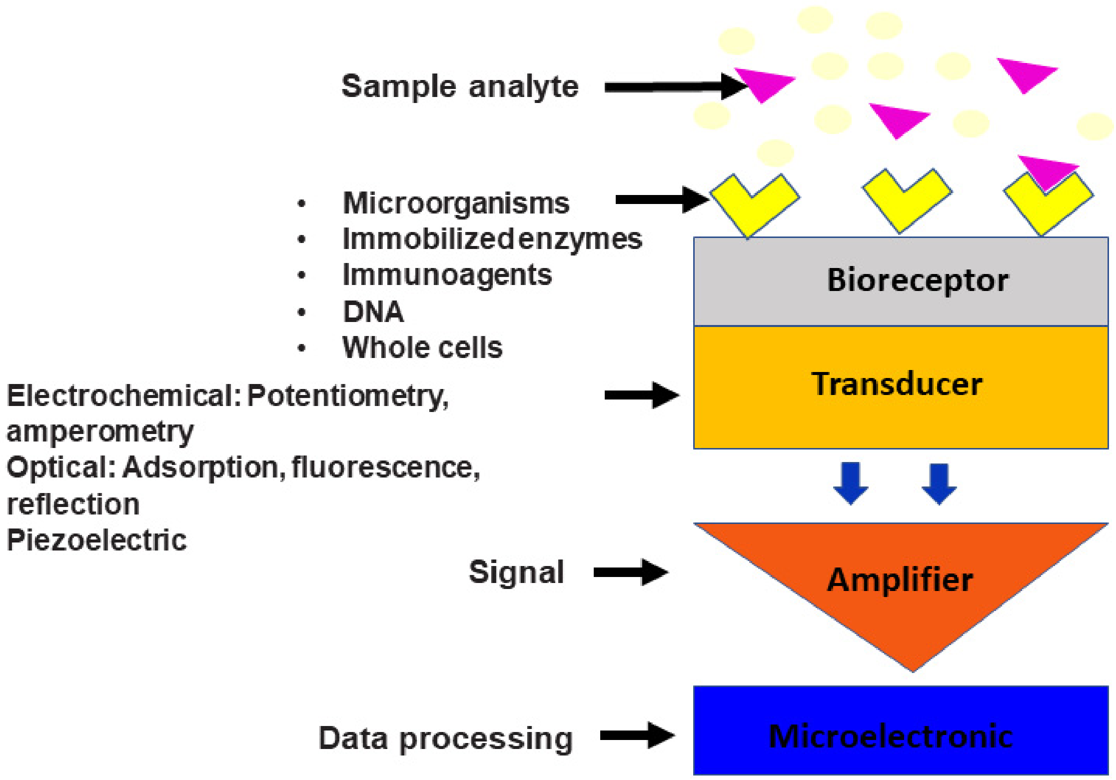


**Figure 3.** Schematic of a biosensor.

Even more fascinating, because to their inherent sensitivity to biological and physico-chemical factors, MFCs themselves can be used as self-powered biosensors. Among the most intriguing applications **[54,55]** are those that monitor microbial activity **[56]**, bio corrosion caused by microbial biofilms **[57]**, biochemical oxygen demand (BOD) **[58]**, toxicants **[59]**, pH and temperature changes **[60]**. In comparison to conventional (bio) sensors, MFC-based (bio) sensors offer higher stability and sensitivity **[61]**.

This review is separated into two distinct sections based on the aforementioned circumstances. This article was written with the primary goal of assisting novices in sorting through the voluminous scientific literature on MFCs that is readily available by pointing them towards targeted reviews and pertinent breakthrough research articles highlighting key advancements in the area.

**Microbial biosensors**

MFCs can be utilized in many different ways, including as sensors. The microorganisms are utilised in an MFC-based sensor to identify and quantify the presence of specific chemicals in a sample. The microorganisms in the MFC, for instance, will absorb glucose and produce electricity as a by-product if the MFC is intended to detect glucose. The concentration of glucose in the sample can then be calculated from the amount of power generated.

MFC-based sensors provide several advantages over traditional ones, including the ability to work in remote or inaccessible locations and in low-power circumstances. However, they also have significant drawbacks, such as the necessity for a steady supply of nutrients for the microbes and their susceptibility to temperature and pH variations. However, enzymes can result in very expensive biosensors. Enzymes frequently need co-factors and/or more enzymes in order to produce a legible signal **[62]**. An appealing alternative is to use complete cells, which already have all the necessary co-factors and enzymes. As a result, microbial sensors have attracted a lot of attention lately **[63]**. Microbial sensors are distinguished by significantly improved operational stability and the potential for long-term usage but have lesser selectivity than pure enzyme-based sensors **[64]**. By creating bacteria with new metabolic pathways tailored to particular needs, synthetic biology can be used to get around the microbial sensors' low selectivity **[65]**. Using recombinant DNA technology, it is possible to express several enzymes on the surface of microorganisms in order to boost selectivity and sensitivity. Without the target substrate entering the cell, the expressed enzymes engage in direct interactions with it **[66]**. Instead of monitoring the amounts of specific chemical components, microbial sensors are also employed as early warning toxicity systems that quickly assess the toxicity of a water sample. Early warning systems are essential for ensuring prompt action in the event of inadvertent contamination, which enhances system management reactions to incidents **[67]**.

MFC-based sensors are generally promising for a variety of applications, such as environmental monitoring, medical diagnostics, and food safety testing. The performance and dependability of these sensors are now being improved, and new uses for this technology are being explored.

**MFCs as an electrochemical sensor**

The capacity of microbial fuel cells (MFCs) to produce electrical signals in response to environmental changes makes them suitable for use as electrochemical sensors. The anode and cathode components of MFCs are separated by a membrane. Microorganisms that oxidise organic molecules to produce electrons and protons often colonise the anode. The electrons move to the cathode, where they interact with an oxygen-containing terminal electron acceptor to produce a current. When MFCs are utilised as electrochemical sensors, the microorganisms on the anode can adapt their metabolism and produce various amounts of electrons in response to environmental changes. As a result, the current produced by the MFC may change. These fluctuations can be measured and utilised as a signal to identify environmental changes. For instance, by monitoring variations in the current generated by the MFC in response to the presence of the contaminant, it has been possible to utilise MFCs to identify contaminants in water, such as heavy metals or organic pollutants. MFCs have been investigated as a technique to power sensors or other electronic devices in distant or low-resource locations, in addition to their use as sensors. MFCs' capacity to produce electricity from organic compounds in the environment may one day offer an affordable and dependable source of power for devices like environmental monitoring systems.

The simplicity and affordability of the MFC technology are its key advantages. In reality, no external transducers are required because the system's distinctive current change can instantly detect the presence of a pollutant in the feeding stream. Mixed cultures of naturally occurring microbes, derived from anaerobic sludge, are typically used, despite reports of the use of pure cultures **[68]**. The use of mixed cultures ensures improved stability and has been demonstrated to produce MFC biosensors that perform better **[69,64]**. The electroactive biofilm naturally forms on the biocompatible surface of the anode during the enrichment process, eliminating the requirement for time-consuming immobilization processes of the sensing element **[70]**. The potential for on-site, continuous operation to allow real-time monitoring is an intriguing feature of MFC-based sensors **[64]**. Additionally, the possibility of self-powering operations is made possible by the electricity produced by the MFC **[71]**.

Overall, the use of MFCs as electrochemical sensors has the potential to offer a sensitive, focused, and long-lasting way to identify environmental changes. To improve MFC design and their performance as sensors, more study is necessary.

**MFC sensor design**

Microorganisms' metabolic processes can be used in microbial fuel cells (MFCs) to turn organic material into electricity. MFCs have many uses as sensors, including the detection of water contaminants and the observation of soil health.

The choice of microorganisms affects the MFC sensor's performance. The sensor performance of MFCs depends on the electrode. High-conductivity material is used as the anode. Graphite, conductive polymers, and carbon fibres are generally used as anodes. The target analyte that needs to be detected must be taken into account while choosing the substrate for the MFC sensor. If the sensor is meant to detect pollutants in water, for example, the substrate should be chosen to encourage the growth of microorganisms that can metabolize the toxins. A data gathering system that can gauge the MFC sensor's electrical output ought to be included. An ammeter or a voltmeter

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can be used for this. The MFC sensor needs to be calibrated before use to make sure it is correctly detecting the target analyte. By subjecting the sensor to known concentrations of the analyte and observing the electrical output, this can be accomplished.

Da'vila et al. (2011) reported the creation of the first microsized MFC biosensor using soft lithography **[72]**. Recently, a layer-by-layer fast prototyping 3-D printing approach was used to manufacture a small, microscopic air-cathode MFC sensor **[73]**. When compared to larger-scale devices, miniature MFCs have higher current densities, and the "miniaturization and multiplication" approach is probably the most practical way to increase the power output for real-world applications **[74]**. However, the MFC technology is still in its early stages of miniaturization, and other obstacles must be solved before they can be used in real applications, including as high internal resistances **[75, 76]**.

**MFCs as BOD sensors**

The amount of oxygen needed by microbes to break down organic matter in water is measured by a process called biochemical oxygen demand (BOD). Water with high BOD levels may include toxins that are bad for both human and aquatic health **[77, 78].** Since microbial fuel cells (MFCs) can monitor their electrical output, which is related to how much organic matter they are consuming, they can be utilised as BOD sensors **[79, 80].**

MFCs have also received a lot of attention as BOD sensors. The amount of organic matter supplied into the system affects the number of electrons produced, which affects the current output if the MFC is run at nonsaturated fuel concentration. Therefore, it is possible to connect the current produced by an MFC to the feeding solution's BOD value. The BOD value can also be related to the charge in a batch-wise feed mode of operation **[81]**. According to Karube et al. 1977, the first MFC-type BOD sensor was described **[82]**. The sensor demonstrated that the coulomb produced by the MFC was directly proportional to the concentration of glucose in water by using the bacterium *Clostridium butyricum* immobilised on the anode surface. According to Kim et al. (1999), the first mediatorless MFC-type BOD sensor used the metal-reducing bacteria *Shewanella putrefacients* **[83]**. However, the same team demonstrated in 2003 that the utilisation of mixed cultures at the anode, derived from activated sludge, would result in improved assessments of the content of biodegradable organics in wastewater. Additionally, using mixed cultures rather than pure organisms resulted in a sensor with far improved stability and stable performance, which were shown to last up to 5 years after operation **[84]**. The sensor can benefit from operating in a temperature-controlled environment in terms of stability and repeatability, and this is typically recommended. MFC-type BOD sensors in particular have been used at temperatures between 30-33 °C **[85, 64]**, however good performance has also been shown at lower temperatures (20 to 25 °C) **[81]**. Nitrate and oxygen, two-electron acceptors with high redox potential that may be present in certain industrial wastewaters in large concentrations, may interfere with the MFC sensor's ability to monitor BOD. In this regard, Chang et al. (2005) found that the MFC sensor's accuracy in nitrate- and oxygen-rich wastewater was enhanced by the application of respiratory inhibitors such as azide and cyanite **[86]**. A small-scale air-cathode MFC (2 mL total anodic volume) made using quick prototyping layer-by-layer 3-D printing was able to achieve a reaction time of as little as 2 minutes **[87]**. The main MFC-based BOD sensors that employ mixed bacteria cultures at the anode are included in **Table 1** along with their literature citations. The dynamic range, reaction time, and sensitivity of the MFC sensors are contrasted. As stated, a single-chamber arrangement improves sensor reaction time. With a small design (anodic chamber volume: 2 ml), it is similarly influenced by the anodic volume, reaching a value of 2.8 minutes. When the biocatalysts at the anode were refreshed for each sample analysis, the response time was similarly very quick (up to 3 minutes) **[88]**. A typical single chamber MFC-based biosensor is presented in Figure 4 **[89]**.

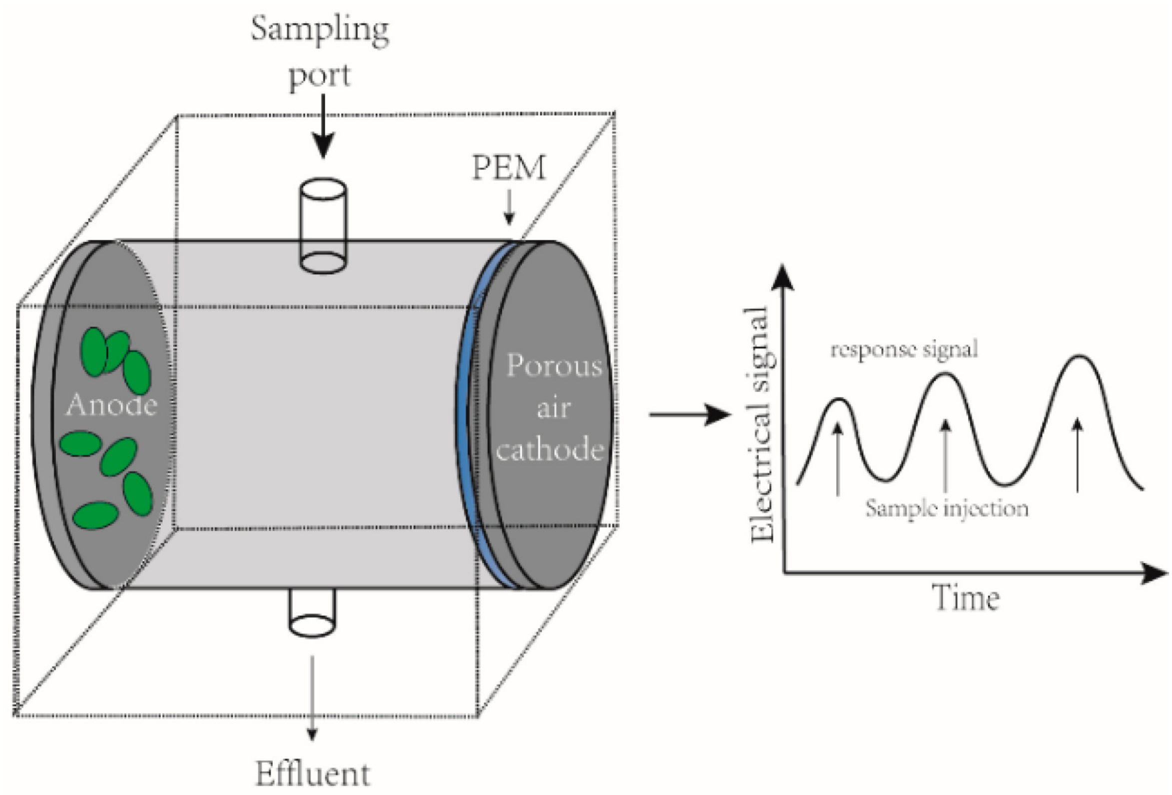


**Figure 4.** Single-chamber MFC-based BOD biosensor [89].

**Table 1.** MFCs as BOD/COD sensors.

| Entry | Configuration | Source of carbon | Volume of anodic chamber (cm3) | Sensitivity (µA mM21 cm22) | Linearity range[a] | Time for response | Reference |
|---|---|---|---|---|---|---|---|
| **1** | Two chambers | Glucose and glutamic acid | 25 | ND | 80–150 ppm[a] | 30 min | **[64]** |
| **2** | Single chamber | Glucose | 12.6 | 0.08 | 50–350 ppm[a] 100–500 ppm[b] | 40 min | **[81]** |
| **3** | Two chambers | Wastewater from a starch processing plant | 25 | ND | 0–206 ppm[b] | 30 min–1 h | **[84]** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4** | Two chambers | Glucose and glutamic acid Glucose | 20 | 0.15 | 20–100 ppm[a] | 1 h | **[86]** |
| **5** | Single chamber | Single chamber | 2 | 0.05 | 3–164 ppm[a] | 2.8 min | **[87]** |
| **6** | Two chambers MFC coupled with an anaerobic reactor that provides a stable anaerobic consortium | Glucose | 100 | ND | 1–25 g $l^{-1c}$ | 3 min | **[88]** |
| **7** | Single chamber | Glucose and glutamic acid | 73 | ND | 5–120 ppm[a] | 2.2 h | **[90]** |
| **8** | Submerged anode coupled with a cathode chamber | Acetate, glucose, wastewater | NA | 0.1 | 10–250 ppm | 40 min | **[91]** |

[a]Curve of Current *vs* BOD/COD. [b]Curve of Coulombs *vs* BOD/COD. [c]Curve of Cell voltage *vs* concentration of glucose.

**Detection of toxicants in water by MFCs**

Every day, thousands of contaminants from industrial, agricultural, and home activities pollute water systems around the world. Traditional pollutants such as heavy metals are included, as are emerging contaminants such as pesticides, medications, and personal-care goods. On the other hand, toxicity bioassays that measure the lethality and vital activities of complex organisms, like fishes, daphnia, or algae, after being exposed to the target toxicant, are used to determine the extent of the potential risks that these pollutants have on the aquatic biota and human health **[92, 93]**. These bioassays have a number of drawbacks, including poor sensitivity and repeatability, lengthy detection periods, and offline measurements. As a result, there has been an increase in interest in creating real-time sensors in recent years. The problem is also to identify developing contaminants, such as the aforementioned medications and their metabolites.

The detection of intoxicants in water can be accomplished using MFCs by monitoring variations in the device's electrical output in response to the presence of the target intoxicant. The fundamental

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idea behind this method is that the target intoxicant is consumed by the microorganisms in the MFCs as a substrate, which produces an electrical current that can be monitored. The first study on the use of MFCs to identify toxicants in water was published by Kim et al. in 2007 **[94]**. The presence of chemicals including mercury, diazinon, polychlorinated biphenyl, and lead in the feed, as well as the injection time and concentration of the toxic substances, as noticed by the scientists, caused a quick decline in current output. The classification is challenging because numerous different chemicals, including organics and heavy metals, have been investigated. **Table 2** lists the most recent MFC sensors reported along with the category of chemicals found. For the most part, the MFC sensors listed in **Table 2** use mixed microorganisms at the anode. According to Stein et al. (2010), using a regulated anode potential while operating the device could help tune the specificity of MFC sensors **[95]**. In this method, a variety of substances might be simultaneously identified in water by operating many MFC sensors, each at a different anode potential. Recombinant bacteria can also be used to address selectivity, resulting in single analyte MFC sensors. The application of a genetically altered Shewanella oneidensis in an MFC sensor for the detection of arabinose was described by Golitsch et al. (2013) **[96]**. By employing a genetically altered *Escherichia coli* for the overexpression of the enzyme xylose dehydrogenase onto the membrane surface, Xia et al. (2013) reported the detection of xylose **[97]**. Figure 5 depicts a typical dual-chamber microbial fuel cell used as a toxicity biosensor. It also shows how hazardous components can impact the activity of electrogenic microbes in biofilms, resulting in an abrupt change (either fall or rise) in voltage **[98]**.

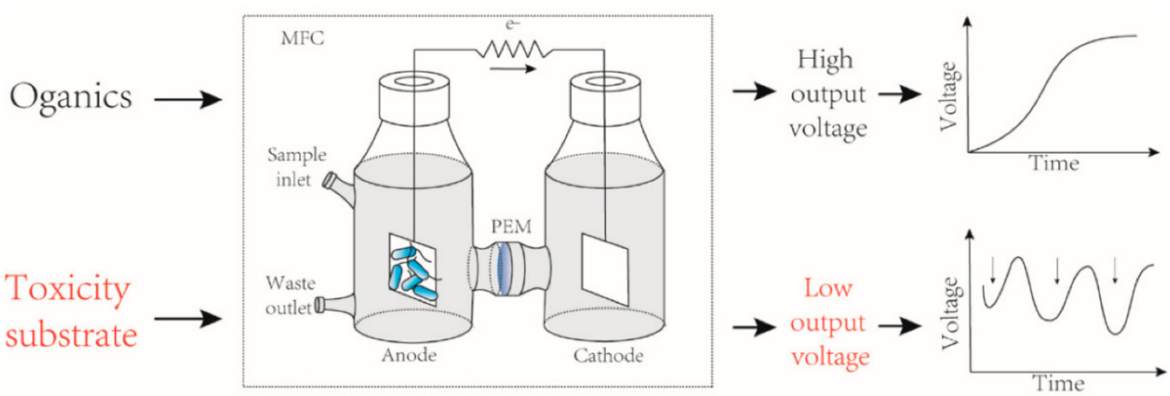


**Figure 5.** Dual-chamber microbial fuel cell used as a toxicity biosensor **[98].**

It is also challenging to quantitatively identify contaminants with MFC sensors. Although many authors claim that the MFC sensors respond positively to the presence of pollutants, as can be seen from **Table 2**, a calibration curve is typically missing, which makes it challenging to do quantitative measurements. Irreversible anode biofilm damage is a concern of employing MFCs as toxicant sensors. This damage can be brought on by either the toxicant's composition or its dose **[99, 87]**. Wang et al. (2013) demonstrated linear responses to formaldehyde only for lengthy contact times over 18 h and proposed long contact times as a means to obtain proportionate responses **[100]**.

**Table 2.** Sensing toxicants by MFCs.

| **Entry** | **Device** | **Dynamic range** | **Bioreceptor** | **Time of Measurement** | **Pollutant** | **Control** | **References** |
|---|---|---|---|---|---|---|---|
| **1** | Air-cathode single | 1–25 μg l$^{-1}$ | Mixed bacteria | 6 min | Cd | External resistance (1 kΩ) | **[87]** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | chamber | | | | | | |
| **2** | Two-chamber MFC | ND | Mixed bacteria | 20 min–2 h | Diazin, Pb, Hg, PCBs | External resistor (500 Ω) | **[94]** |
| **3** | Two-chamber MFC | 0–200 mg $l^{-1}$ | Mixed bacteria | 2 h | Ni, Cu | Poised anode potential (−0.4 V) | **[95]** |
| **4** | Two-chamber MFC | ND | *Geobacter sulfurreducens* | 3 min | Formaldehyde | Fixed current of 1 μA | **[99]** |
| **5** | Three electrode set-up | 0.01–0.10% (in volume) | *S. oneidensis* MR-1 | 2–18 h | Formaldehyde | Poised anode potential (0 mV) | **[100]** |
| **6** | Two-chamber MFC | ND | Mixed bacteria | 2 h | Sodium dodecyl sulfate (SDS) | Poised anode potential (−470 to 400 mV), external resistance (0–1000 Ω), and fixed current (0.05 mA) | **[101]** |
| **7** | Air-cathode single chamber | ND | Mixed bacteria | 1.2 h | $Cr^{6+}$ | External resistance (480 Ω) | **[102]** |

## Conclusions

MFCs are an innovative technology that takes advantage of some microbes' capacity to produce electricity from organic materials. Particularly for distant or off-grid places, MFCs have the potential to provide sustainable and renewable energy sources. Because a biological event at the anode is directly converted to an electrical signal by MFCs, they offer an appealing way to assess the quality of a biological sample. They have been shown to be a reliable substitute for the conventional BOD test for determining the strength of wastewater. The capacity to operate in situ, online, and with excellent operational stability is a huge advantage for MFCs in particular. MFCs are always being improved for increased performance, effectiveness, and durability by researchers. The initial proof of concept results are highly encouraging, but further research is needed to properly understand the range of possibilities this technology opens up. Miniaturization and synthetic biology may present intriguing opportunities in this regard. There are still certain issues that need to be resolved before MFCs can be commercially viable, despite the apparent advantages. In comparison to other energy sources, these drawbacks include the high cost of materials and poor power output. MFCs are a promising field of study with significant potential for the production of sustainable energy, and current research will continue to enhance their viability and efficiency.

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