Microbial fuel cell sensors for water and wastewater monitoring

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Abstract

Microbial fuel cells (MFCs) use bacteria to convert chemical energy in organic substrate to electrical energy. MFC biosensoric capabilities are based on its ability to detect a change in current or voltage caused by introduction of an analyte. The operating principle is the linear relationship between analyte concentration and current or voltage output in the biosensor. The objective of this review is to provide an overview of the application of MFC biosensors for water and wastewater monitoring through biological/chemical oxygen demand (BOD/COD) or toxicity. While most reviews focus on operating principles and designs/configurations of MFC biosensors, this review also covers microbial consortium, current generation mechanisms and performance indicators. Factors affecting operation and challenges of the technology are also discussed. MFC biosensors have been widely investigated because of their simple architecture, cost effective construction, and potential self-sustaining power source. An efficient MFC biosensor must be capable of rapid and precise in-situ monitoring of a target compound, have long operational stability with low maintenance requirements and quick recovery after sudden disturbance. MFC biosensor performance is limited by several factors including electron transfer at the anode, substrate concentration, internal resistance of the system and environmental variables (i.e. temperature and pH). These limitations could be minimized by improving the reactor materials, design and operation. However, practical applications have proven that improving one factor does not necessarily result in an overall improvement

in performance. The key to an efficient biosensor is establishing a combination of conditions that allow optimal performance of the biosensor without compromising sensitivity or stability.

Keywords: Bioelectrochemical systems, Biosensors, Environmental monitoring, Water quality, Pollution.

1 Introduction

Within bio-electrochemical systems (BES), microbial fuel cells (MFCs) generate electricity through electrochemical reactions that convert the chemical energy in organic substrate to electrical energy. The use of bacteria to generate electricity was first discovered in 1911 (Potter 1911). MFCs have progressed, over the last century, from application for electricity production from organic waste, to use for environmental monitoring in the last fifteen years (Karube et al. 1977; Kim, Chang, et al. 2003; Kim and Kwon 1999; Matsunaga, Karube, and Suzuki 1980). MFC biosensors can be made using simple architecture and low-cost materials. They could be self-sustaining and may not require an external power source. In water and wastewater monitoring, MFCs can offer the capability of simultaneous treatment of contaminated medium and generation of a measured concentration response within a short response time, in contrast with traditional methods (Schneider et al. 2016; Sun et al. 2015; Jiang et al. 2018). Most reviews focus on operating principles and designs/configurations of MFC biosensors. This review provides an overview of the application of MFC biosensors for water and wastewater monitoring using biological/chemical oxygen demand (BOD/COD) or toxicity. It will cover the operating principles of MFC biosensors for water quality monitoring, with emphasis on microbial consortium in both anode and cathode, electron transfer and current generation mechanism. Factors affecting performance, challenges of the technology as well as future perspectives will also be discussed.

In MFCs, an organic substrate is oxidized by electroactive bacteria. This results in release of electrons that are transferred from the anode, via an external circuit, to a terminal electron acceptor (TEA), such as oxygen, at the cathode, thereby generating electric current. Protons from the anodic chamber diffuse across a proton exchange membrane (PEM) into the cathodic chamber and react with the electrons and oxygen. A typical microbial fuel cell (Figure 1) consists of a vessel comprising two electrodes (anode and cathode) separated by an ion-exchange membrane and with a resistor connected across the electrodes. The electric current generated is measured by a voltammeter as the potential difference between the two electrodes. The data is recorded on a computer using a data logging software.

2 Operating principles

The biosensing element of MFC biosensors will be an electroactive microorganism at the anode or cathode electrode surface. In other words, redox reactions of the metabolic pathways of the microorganisms are responsible for the electric current or voltage generated. Any analyte that modifies these reactions will likely affect the current output. The nature of the analyte can either enhance or diminish current output, depending on the type of interaction that takes place between the analyte and bacteria or its metabolic pathway (Chang et al. 2005). Since microorganisms act as biocatalyst, then any analyte that alters their ability to transfer electrons will cause a change in the generated current or voltage. The biosensoric capabilities of the MFC is based on the ability to detect this change and involves correlating the analyte concentration to the current or voltage output.

2.1 Microbial consortium

In MFCs, biofilms are formed on an anodic surface after inoculation, and a sustained substrate supply. Most electrodes employed, when using microbial biocatalysts, are made of carbon materials. Pure strains inoculated in the anodic side of MFC biosensors, include *Shewanella putrefaciens* IR-1, *Shewanella oneidensis, Geobacter sulfurreducens* (DSM 12127), *Serratia marsecens*, *Bacillus subtilis*, *Bacillus licheniformis*, *Trichosporon cutaneum*, *Klebsiella oxytoca*, *Hansennula anomala, Pseudomonas putida*, *Torulopsis candida*, *Proteus vulgaris*, *Clostridium butyricum*, *Aeromonas formicans*, *Psuedomonas syringae*, *Escherichia coli*, *Moraxella*, *Saccharomyces cerevisiae* (Liu, Björnsson, and Mattiasson 2000; Riedel et al. 1988; Tan, Li, and Neoh 1993; Chee, Nomura, and Karube 1999; Kim, Chang, et al. 2003; Su et al. 2011; Kim and Kwon 1999; Dávila et al. 2011; Kim et al. 1999). Most of the strains previously listed require the use of mediators in order to facilitate electron exchange between microbes and electrodes. One of the first reported mediator-less MFC sensors was a lactate sensor using a pure culture at the anode (Kim et al. 1999). Later, it was found that when using pure cultures of bacteria for a specific substrate, bacteria could be responsive to different types of substrates, which can give false positives. As a result, MFC sensors should be better suited to give information on broader parameters (such as BOD). This should also be adequate when using biofilms of mixed microbial culture. Mixed cultures yield higher power densities and more stable than pure culture biofilms because of increased capacity to utilize a broader range of substrates as fuel (Du, Li, and Gu 2007; Logan et al. 2006; Lei, Chen, and Mulchandani 2006). Additionally, mixed culture inocula may be sourced naturally from the environment including marine sediments, freshwater sediments, garden soil, activated sludge or anaerobic sludge. Biocathodes may also be employed in certain configurations, where the cathode serves as electron donor for a microbial biofilm on its surface such as

Thiobacillus ferrooxidans (Du, Li, and Gu 2007). Other phyla found in biocathode communities include *Proteobacteria*, *Bacteroidetes*, *Chlorobi* and *Actinobacteria* (Chen et al. 2008). Mixed microbial cultures, sourced from the environment, can also been used in MFC cathodic electrodes as the response element (Velasquez-Orta et al. 2017).

2.2 Analyte

A wide range of bacterial substrates have been employed in MFCs biosensors as the analyte. These include different forms of carbohydrates (acetate, arabitol, mannitol, glucose, galatitol, sucrose, xylose, cellulose), chemicals (nitrilotriacetic acid, phenol, fumarate, lactate, pyruvate, butyrate), waste (farm manure, real or synthetic wastewaters) and algae biomass (Pant et al. 2010; Velasquez-Orta, Curtis, and Logan 2009). In MFC biosensors reviewed for this communication, acetate, glucose and glutamate were the most common substrates utilized by electroactive bacteria. This is not surprising as these compounds are not only simple to oxidize, but also have a high energy store. Furthermore, glucose and glutamic acid are the carbon sources used to calibrate the standardised BOD_5 test (APHA 2005). The substrate can be supplied in batch mode, where the reactor is fed at periodic intervals, or in continuous mode, where the substrate is fed into the system at a specified flowrate. Although batch mode operations have been most commonly studied, a decline in current can occur due to starvation caused by insufficient fuel supply. When the reactor is maintained in continuous mode a constant current can be maintained, thereby providing more stability to the system. The operation mode of an MFC impacts on its performance as it can influence the electron transfer mechanism. For example, electron shuttles can be lost in continuous mode which would be thermodynamically unfavourable for the bacteria producing shuttles, therefore bacteria capable of direct electron transfer to the anode surface would likely be the dominant species (Lovley 2006). This observation suggests some level of adaptation in MFC microbial consortiums to favour the growth and proliferation of bacteria that can thrive best in the environmental conditions, thereby highlighting the dynamic nature of the consortium to changes in the environment.

2.3 Electron transfer mechanism

Although there is still considerable debate on the specifics of electron transfer mechanisms in microbial fuel cells, most literature identify two types of electron transfer (ET) mechanisms, namely mediated and non-mediated. Non-mediated ET involves direct contact between the bacteria and the electrode surface. Electrons are transferred directly from the bacteria to the electrode using nanowires (conductive pili) or membrane associated cytochromes e.g. *Geobacter sulfurreducens*, *Shewanella oneidensis*, and *Thermincola potens* (Logan 2008),

(Philips et al. 2016; Logan 2008). In mediated ET, electron shuttles are used to transport electrons between the bacteria and the electrode surface. This contact is made by soluble electron shuttles such as natural mediators secreted by the electroactive bacteria or chemical reagents (artificial redox mediators) (Abrevaya et al. 2015; Logan et al. 2006). Flavins or redox endogenous mediators such as 2-amino-3-dicarboxy-1,4-naphthoquinone and pyocyanin can facilitate electron shuttling between the bacteria and the anode (Santoro et al. 2017; Velasquez-Orta et al. 2010). Artificial redox mediators such as flavins, thionine, neutral red, methylene blue, and anthraquinone-2,6-disulfonate (AQDS) facilitate electron transfer especially for bacteria that are unable to transfer electrons on their own; they are often used in small quantities (Abrevaya et al. 2015; Logan et al. 2006).

In MFC biosensors, redox mediators provide the link between the bacteria and the electrodes by harvesting electrons from the bacterial cells, during which they are reduced, to the anode surface where the electrons are released, and the mediators are reoxidized. They are then made available for the transport of more electrons. This mechanism facilitates electron transport in the organism that are unable to produce they own natural mediators or do not have nanowires. Bacteria such as *Shewanella putrefaciens* did not require added mediators for electron transfer while thionine and hydrogen was used to facilitate electron transfer when *Proteus vulgaris*, *Clostridium butyricum* and *Aeromonas formicans* were utilized in MFC biosensors (Matsunaga, Karube, and Suzuki 1980; Thurston, Bennetto, and Delaney 1985; Kim et al. 2002; Karube et al. 1977). The use of external mediators may not promote the stability of the biosensors (Chang et al. 2004). In several cases where anaerobic or activated sludge was used as the enrichment medium for the biosensors, mediators are not used (Chang et al. 2004; Kim et al. 2007; Kim, Youn, et al. 2003). This may be due to the ability of the diverse microbial community present in the inocula to utilize various electron shuttle mechanisms. Due to the external mediator's potential toxicity and cost, there is current little interest in adding redox compounds to MFC sensors.

2.4 Current or voltage generation

Current is the most commonly calibrated signal generated by MFC biosensors as it can be monitored and recorded in real-time and it is an indirect measure of the concentration of a target analyte. A plot of the current generated versus the concentration of the analyte, is known as a calibration curve. It is a means of quantifying the concentration of the analyte and defines the nature of the relationship between the analyte concentration and current output. The relationship between biological oxygen demand (BOD; a standard water industry method for quantifying unspecified or unknown concentrations of biodegradable organic substrates) and MFC current output has been shown to be linear up to an identified saturation concentration (upper limit). Beyond this limit, higher

concentrations of the organic substrate cannot be measured as the bacteria are unable to oxidize further the substrate until concentration levels subside (Jiang et al. 2018). This sensing parameter is particularly relevant for water quality monitoring (in correlation with BOD or COD). It was recently demonstrated that the dynamic range could be significantly extended (2-3 fold) using a three-stage MFC reactor configuration assembled hydraulically in series (Spurr et al. 2018). A wide linear range is crucial for application to monitor wastewaters from urban or industrial sources or for volatile fatty acid (VFA) monitoring during anaerobic digestion to prevent VFA build up in the reactor, which could lead to a system failure. Membrane fouling or unfavourable pH shifts can also cause systematic failure of the MFCs.

Changes in the open circuit potential, or voltage, can also been used as the response element in MFC biosensors (Wang et al. 2018; Liu, Lei, and Li 2014). In an ideal system, when there is no current flow through the system (open circuit potential), the potential difference between the anode and the cathode is referred to as the overall electromotive force or cell potential (E_{emf}) and is related to Gibbs free energy (ΔG). The relationship between the cell potential, temperature and concentration of the reactants can be expressed by the Nernst equation. In reality, with MFCs including those used as biosensors, the actual potential (E_{MFC}) is less than E_{emf} because of energy losses through the energy used to start the reaction (activation losses, η_{act}), overcome internal resistance (ohmic losses, η_{ohm}) and energy losses due to mass transport within the system (concentration losses, η_{concn})(Logan et al. 2006; Esfandyari et al. 2017). The anode potential must therefore be kept at a low potential, enough to drive the reactions but minimize activation losses. Activation losses can also be minimized by increasing the anode surface area and operating temperature (Logan et al. 2006). Use of membrane or electrode materials with low resistance, shortening the distance between electrodes and increasing electrolyte conductivity can reduce ohmic overpotential and increase current output in the biosensor (Logan et al. 2006; Bard, Inzelt, and Scholz 2008). Concentration losses, mainly due to mass transfer limitations, can also be a result of limited removal of oxidized species from the anode or supply of reduced species. Concentration losses can be minimized by using buffer solutions to maintain the pH of the electrolyte within an acceptable range (Logan 2008) or by improving mixing near electrodes.

3 Performance indicators

An efficient MFC biosensor must be capable of rapid and precise *in-situ* monitoring of compounds, have long operational stability, low maintenance requirements and short recovery time (Kim et al. 2007). The efficiency of an MFC biosensor is measured by parameters such as response time, detection limit, sensitivity, recovery time,

and stability. These indicators can be expressed in terms of the current generated, coulombic efficiency or yield and power or current density with reference to the anode or cathode surface area. Electroanalytical techniques such as cyclic voltammetry, chronoamperometry, chronopotentiometry, electrical impedance spectroscopy as well as polarization curves and peak current and power density can be used to study and characterize MFC to optimize their performance (Logan 2008) and may be applicable to improve MFC bio-sensing performance.

The time duration for current to achieve 95% of the steady state current after a change in current output due to the presence of a target compound is one method used to calculate the response time of the biosensor (Di Lorenzo, Curtis, Head, Velasquez-Orta, et al. 2009). A short response time is vital for rapid monitoring. The minimum quantity of a compound that can be measured by the biosensor is its detection limit. The lower the quantity that can be detected, the higher the performance of the biosensor– this is especially important for monitoring drinking water resources where contaminants can be present in trace amounts. The sensitivity of an MFC biosensor is the change in current per unit change in the concentration of the compound, and is determined by the anodic biofilm. It is measured in relation to the anode surface area as shown below:

$$\text{Sensitivity} = \frac{\Delta I}{\Delta c}\frac{1}{A} \qquad 1$$

where Δ I (μA) is unit change in current, Δ c (mM) is unit change in concentration, and A (m^2) is the anode surface area. Biosensors with larger unit changes in current appear to be more sensitive (Stein, Hamelers, and Buisman 2012). Sensitivity can be increased by improving electron recovery either by enhancing electroactive bacteria in the anode or by inhibiting the activity of other bacteria that could compete with the bacteria for substrate (Jiang et al. 2018). It has been reported that low external resistance can improve the response time and sensitivity (Pasternak, Greenman, and Ieropoulos 2017).

A good biosensor should be able to recover rapidly after periods of starvation, non-usage, sudden disturbance or toxicity presence. Recovery time is determined by the nature of the compounds or periods without fuel, anodic biofilm and operational conditions in the sensor. Bacteria in the biofilm that are able to recover quickly from any stress or damage caused by such periods demonstrate resilience and this may lengthen the operational stability of the biosensor in which they are utilized. A biosensor with long operational stability and low maintenance provides increased reliability for water or wastewater monitoring. Recovery time has been shown to increase with prolonged periods of starvation but this can be improved by electrode modification (Chang

et al. 2004; Kaur et al. 2014). Whereas, low resistance and increased concentrations of analyte can lengthen the time it takes for a biosensor to recover (Pasternak, Greenman, and Ieropoulos 2017).

4 Environmental monitoring

Organic compounds are the primary pollutants in wastewaters and are difficult to characterize, hence analytical techniques such as biological oxygen demand (BOD) and chemical oxygen demand (COD) are used to monitor the amount of organic matter in wastewater. BOD is a measure of the oxygen consumed from biological degradation of organic pollution in water and is widely regulated for assessment of water quality, while COD is a measure of the amount of oxygen required for complete chemical oxidation of organic matter to carbon dioxide. As previously mentioned, the current produced in the MFC biosensor is proportional to the concentration of the organic substrate. By measuring and calibrating this current, the amount of organic content in water or wastewater can be estimated.

Existing literature shows that MFCs have been widely validated against chemical oxygen demand (COD) or biological oxygen demand (BOD) sensors for use in monitoring of water quality (Ayyaru and Dharmalingam 2014; Chang et al. 2004; Di Lorenzo, Curtis, Head, and Scott 2009; Di Lorenzo, Curtis, Head, Velasquez-Orta, et al. 2009; Di Lorenzo et al. 2014; Kang et al. 2003; Karube et al. 1977; Kim, Chang, et al. 2003; Kim, Youn, et al. 2003; Kumlanghan et al. 2007; Liu, Lei, and Li 2014; Zhang and Angelidaki 2011; Pasternak, Greenman, and Ieropoulos 2017). A recent review of MFC biosensors for environmental monitoring described the use of self-powered dual-chambered MFC designs for monitoring biological oxygen demand (BOD) and toxicity with response times of five minutes to ten hours, and high stability of up to five years in one instance (Sun et al. 2015). The electricity generation was directly related the concentration of organic matter or toxins in the wastewater: whereas the presence of organic matter favoured increased current generation, introduction of toxins caused a decrease in current generation. MFCs have been used as volatile fatty acid (VFA) sensors to monitor biological processes (e.g. liquid waste) in anaerobic digestion during wastewater treatment, as VFA accumulation inhibits microbial activity, decreases the efficiency of COD removal and can cause system failure (Kaur et al. 2013; Kretzschmar et al. 2017). The most common VFAs analysed are acetate, propionate and butyrate. MFC biosensors used to monitor toxicity in water rely on the metabolic activity of the biofilm to sense the presence of toxicants. The introduction of a toxic substance into the MFC reactor causes an inhibition in the metabolic activity of the electroactive bacteria, resulting in decline in signal or current output. The performance indicators for toxicity biosensors are current density and power output (Sun et al. 2015; Stein et al. 2012).

BOD and toxicity sensors are the most widely studied MFC biosensors for water quality monitoring (Table 1), utilizing mostly synthetic media containing either acetate, glucose and/or glutamic acid as the fuel source. Continuous operation, which resembles the closest approach to real-world application for real-time monitoring, has been the predominant feeding mode. Although response time, and method for calculating such, varied between the biosensors, the majority recorded response times within 1 hour, confirming the suitability of MFC biosensors for rapid water and wastewater monitoring, compared to the standard offline tests including BOD which requires five days sample incubation and COD which requires two hours heating and 30 minutes cooling of samples. Response time could be improved by reducing anodic volume or employing high substrate concentrations at low feeding rates (Moon et al. 2004).. A decrease in current density following the introduction of known concentrations of a toxicant (Nickel, Ni) was also observed in an microbial electrolysis cell (MEC) biosensor using different ion exchange membranes, which suggested that microbial activity was inhibited by the presence of chemical toxicants/pollutants (Stein, Hamelers, and Buisman 2012). Addition of toxic substances, such as organophosphorus compounds, mercury and cadmium has decreased current generation by inhibiting electron transfer mechanism of electroactive micro-organisms (Kim et al. 2007). Biosensor response signals also decreased after introduction of the chromium or iron (Liu, Lei, and Li 2014) but increased when using nitrate or acetate The magnitude of the response was linearly correlated to the concentration of the tested analytes, with distinguishing voltage signal changes between toxic and non-toxic analytes.

The double chamber is a common configuration used for MFC biosensors, and some studies utilise a single chamber MFC that enables low maintenance requirements. A single-chamber MFC was used to monitor COD removal and VFA concentrations in four types of industrial wastewater (Velasquez-Orta et al. 2011). Submersible MFCs were also tested to monitor changes within an activated sludge tank or in groundwater, giving an indication of microbial activity or organic matter loads (Zhang and Angelidaki 2011; Xu et al. 2014). More recently, various modifications of sediment MFC biosensors (sediment/bulk liquid, sediment/sediment, bulk liquid/air, and bulk liquid/bulk liquid) have been investigated for in-situ monitoring of crude faecal contamination in groundwater (Velasquez-Orta et al. 2017). Here a cathodic electrode, used as the sensing element, was exposed to the analyte (water) producing a decrease in current output after faecal contamination.

5 Challenges

Limitations of the performance of MFC biosensors include substrate concentration, high internal resistance of the system, diffusion of oxygen into the anode chamber, the presence of alternate terminal electron

acceptors at the anode, proton permeability across the PEM, oxygen supply and consumption in the cathode chamber as well as the interference of environmental factors such as temperature, pH and ionic conductivity of the electrolyte used (Kim et al. 2007; Larrosa-Guerrero et al. 2010; Li et al. 2017; Schneider et al. 2016; Gil et al. 2003). Other challenges of MFC biosensors include specificity, sensitivity, standardization, micro-organisms used for the anodic biofilm and scalability for mass production.

5.1 Reactor configuration.

The internal resistance of an MFC biosensor depends on the design and configuration of the system, and in turn determines its performance. MFC performance can be enhanced by reducing the distance between electrodes, and by using miniature reactor sizes (Ringeisen et al. 2006). A VFA MFC sensor using polypyrrole-modified carbon electrode as the working electrode favoured bacterial attachment to the electrode surface and improved electron transfer rate, and the recovery time of the sensor was enhanced (<2-10 mins) when compared with the unmodified electrodes (10-30 minutes) (Kretzschmar et al. 2017). The use of natural polymers, such as agarose and polyacrylamide, in the presence of mediators, also increased the start-up time and stability of the sensors (Kaur et al. 2014).

For electroactive bacteria to proliferate, the anode must be maintained under anaerobic conditions. Oxygen diffusion into the anode chamber leads to loss of the organic substrate through aerobic respiration and results in low MFC performance. Biosensor designs that minimize oxygen diffusion into the anodic chamber and lower the density of anodic biofilms have been shown to improve sensitivity. Lowering the flow or shear rate also increases the sensitivity of the biosensor (Chang et al. 2004; Shen et al. 2013). The use of a sulfonated poly ether membrane, which prevented oxygen diffusion, was reported as being more effective than Nafion^{TM}, allowing detection of up to 650ppm glucose concentration (Ayyaru and Dharmalingam 2014), however its applicability in MFC biosensors for BOD measurements still needs to be determined. Zhang and Angelidaki (2011) demonstrated the use of a submersible microbial fuel cell biosensor for monitoring BOD in groundwater where the anaerobic conditions were maintained in the anode by immersing it in a subsurface environment. The compact reactor design also minimized ohmic losses within the system. Although using a membraneless configuration promoted proton diffusion into the cathode, however this did not improve performance as the process was inhibited by high concentrations of cations (Kim, Chang, and Gadd 2007). It has also been reported that membraneless systems are advantageous because internal resistance is reduced and no pH gradient is formed between the anode and the cathode. Nevertheless, such configurations are susceptible to biofouling of the cathode which reduces system

performance (Logan 2010). Electrode and membrane fouling has reportedly led to diminished MFC performance after more than 6 months of operation (Kim, Youn, et al. 2003).

From the above, it can be seen that the internal resistance is a major limitation in the operation and performance of an MFC biosensor. The lower the internal resistance of the system, the higher its performance. Internal resistance of the reactor can be reduced by optimizing the configuration either by reducing the distance between electrodes or by using membranes or separators that permit the diffusion of protons to the cathode chamber while preventing the influx of oxygen into the anode chamber. It is also important to select electrodes with surface characteristics that enhance electron transfer to provide adequate support for the bacterial biofilm and the cathode and membrane materials must be such that enhances oxygen reduction and proton diffusion while preventing its diffusion into the anode chamber, respectively. Although single chamber configurations are simpler and cheaper, oxygen diffusion into the anode and membrane fouling are on-going challenges with this design. Not using a reactor, as proposed in sediment MFCs, result in a system difficult to control. In such systems, the interpretation of the current output would need measurement of other variables.

5.2 Operational conditions.

Operating conditions such as pH, temperature, conductivity as well as the redox potentials of the electrodes and the feeding mode impact the performance of MFC biosensors. A change in temperature can influence both the reaction kinetics and the thermodynamics of an MFC biosensor. Temperature significantly affects the metabolism of the bacteria which is one of the most important factors affect performance, in addition to electrode potentials, activation energy, mass transfer process and conductivity of the electrolyte. Amongst all these changes are seen in the current generation and BOD or COD removal of the biosensor. For example, increase in temperature increased COD and VFA removal, as well as conductivity in a single chamber MFC biosensor used to monitor COD (Larrosa-Guerrero et al. 2010; Oliveira et al. 2013). Current output increases with increasing temperature and conductivity (Zhang and Angelidaki 2011). At pH values above 8, current output reduced, which suggested that microbial metabolism is influenced by the pH (Yang et al. 2013). The current output was therefore diminished under this condition. The most suitable pH range for MFCs has been reported as 6 – 8. pH changes can be controlled by using phosphate, bicarbonate, borax or synthetic zwitterionic buffers (Oliveira et al. 2013; Gil et al. 2003). These examples indicate that acidic or very alkaline conditions in the anodic chamber have negative impacts on biofilm stability and electron transfer. The effect of environmental parameters on the performance of modified MFCs used to monitor faecal contamination in groundwater showed a decline in

dissolved oxygen concentration and increase in current output after a contamination event. Current output declined with increase in temperature (Velasquez-Orta et al. 2017). Current output was, however, low when these MFCs were tested in real groundwater well. The difference in response was attributed to different soil, water and microbial characteristics of the actual groundwater well when compared with the laboratory tests. This example illustrates how unpredictable environmental conditions may affect biosensor performance in-situ. Further research is required to determine MFC performance with real wastewaters and design MFC biosensors that can measure response signal without the interference of environmental factors.

In a continuous mode single-chambered MFC used to monitor copper toxicity, Shen et al. (2013) showed that decreasing the flow rate of the reactor and maintaining an anoxic environment in the anodic compartment improved the biofilm density, and enhanced its sensitivity. Nevertheless, Moon et al. (2004) reported that lowering the feeding rate improve response time but did not improve sensitivity; to improve sensitivity the feeding was increased from 0.053 ml min^{-1} to 0.65 ml min^{-1} . This shows that improving one factor or indicator does not necessarily result in an overall improvement in performance. The key to an efficient biosensor is establishing a combination of conditions that allow the biosensor to perform at its best without compromising its sensitivity or stability. This is an example of some of the complexities required to optimize the performance of an MFC biosensor.

5.3 Microbial consortium.

When using mixed cultures, a diversity of micro-organisms are enriched in microbial fuel cells, most of which, if not all are electroactive bacteria (Du, Li, and Gu 2007; Kim, Chang, and Moon 2006; Logan 2008; Santoro et al. 2017). As earlier discussed, electron transfer mechanism and proton diffusion across the anodic chamber can hinder efficient current generation. MFC performance is dependent on electron transfer mechanism of the microbial biofilm in the anodic chamber, however, this mechanism has not been thoroughly understood. (Li et al. 2017; Schneider et al. 2016; Sun et al. 2015; Velasquez-Orta et al. 2017). For instance, although mixed cultures produced higher current output, when isolates from this consortium were used as pure culture, they generated lower current than expected (Logan and Regan 2006). Most microorganisms in MFC reactors are unable to thrive in severe weather (e.g. low temperatures) or ionic conditions (e.g. starvation). There is need to develop biosensors using bacteria that can flourish in extreme environments (Lei, Chen, and Mulchandani 2006).

5.4 Standardization.

Even though MFC biosensors can detect the presence of toxic substances, the quantification of these substances is still a challenge as the sensor only measures the signal response to the change caused by the toxic substance. In recent times, calibration curves have been produced to establish a relationship between the current output and the concentration of the toxic substance (Jiang et al. 2018). While this may be acceptable for laboratory investigations or use as early-warning detection systems, but in-situ or online monitoring would require devices that can provide information on the precise amount of the target analyte present in the water being analysed. Various configurations, conditions and methods of measurements have been employed to characterize MFC biosensors, hence it can sometimes be difficult to make a comprehensive comparison of the performance of these sensors. Although most literature use current, current density or power density to describe MFC performance, this may not be suitable for MFC biosensors where the key focus is detection or monitoring of analyses rather than power generation. A common platform or standard would provide a means of establishing the specific minimum requirements of an efficient biosensor. In addition, use of different synthetic calibrants and variation between validation methods including BOD_5, COD, DOC and substrate concentration make comparison of sensing ranges difficult.

5.5 Specificity.

This is also another area that is not clear. So far, the investigation of MFC biosensors for water and wastewater monitoring has predominantly been conducted in the laboratory, with few field trials. Laboratory experiments are conducted under highly controlled conditions and measure very specific (mostly, single) parameters to provide clarity on the reactions occurring and the influencing factors. In reality, the interaction of biotic and abiotic environment factors with the biosensor performance involves more complex reactions. MFC modelling can be used to design biosensors that are adaptable to these interactions. With the use of fixed anode potential for measurement of response signal, the use of different anode potentials for sensing different compounds has been proposed. This concept requires further investigation.

6 Conclusions

MFC biosensors can be used for rapid monitoring of BOD and COD during anaerobic digestion in wastewater treatment plants. They can also be employed as toxicity sensors for monitoring chemical compounds such as nickel, cadmium, chromium and organophosphate compounds in water, with the capacity to measure

concentrations as low as 1 mg L^{-1}. Limitations of the performance of MFC biosensors include electron transfer from the biofilm to the anode, substrate concentration, internal resistance of the system, proton permeability across the PEM, oxygen supply and consumption in the cathode chamber as well as the interference of environmental factors such as temperature, pH and ionic conductivity of the electrolyte. Practical applications have demonstrated that of modification of reactor design, configuration and materials is usually required to manage these limitations. There is indeed a plethora of practical evidence of the rapid, sensitive and in-situ capabilities of MFC biosensors. However, a wide range of configurations and methods have been employed for these investigations, making it difficult to establish basic standards against which an efficient biosensor can be measured. Nevertheless, its usefulness for real time water and wastewater monitoring is undeniable. Further investigations need to be conducted to develop biosensors using bacteria that can flourish in extreme environments and utilize a wider range of substrates as fuel source as well as compact miniaturized MFC biosensors for field applications. Reactor designs that minimize internal resistance while improving response time, specificity and sensitivity are the focus of on-going research efforts. A common platform or standard would provide a means of establishing the specific minimum requirements of an efficient biosensor.

7 Future perspectives

Most biosensors, especially for BOD and COD, are only able to detect overall response such total organic carbon without distinguishing between the various forms of carbon present. Although, recent studies have explored development of sensors for target contaminants, the ability of a biosensor to detect a single analyte in a complex matrix is still a progressive area of research for MFC biosensors. A foremost concern with MFC biosensors is scalability, and membrane electrode assembly consisting of arrays of MFCs are more preferable for boosting performance. Simpler and cost-effective material remain the sensible choice for scaling up reactors. Genetic engineering of target genes of electroactive bacteria may be further explored as a means of enhancing the electrogenic activities of the microbial consortium. Although this is not the main focus of these biosensors, power management systems are also being developed for storing the energy generated by MFC in order to put it to other relevant uses such as powering remote sensors or small lighting devices.

The promising potentials of MFC biosensors for water and wastewater monitoring as a low cost, low energy solution to water management problems will continue drive further refinement of this technology. MFC biosensors could also be integrated with wastewater management treatment plants to monitor the effectiveness of the treatment process.

Table 1. *Microbial fuel cell-based biosensors for water and wastewater monitoring*

	MFC configuration	Electrode material		Substrate	Analyte	Mode of operation	Current output (mA)	Response time (min)	Detection limit/range (mg L^{-1})	Stability	Reference
		Anode	Cathode								
1	Double chamber with AEM	Lead	Carbon with platinum	Glucose and glutamic acid	BOD	Batch	ND	30 - 40	14 - 190	40 days	(Karube et al. 1977)
2	Double chamber using CEM	Graphite felt	Graphite felt	Wastewater from starch processing plant	COD	Batch	1,7	ND	50	3 yrs	(Gil et al. 2003)
3	Double chamber using CEM	Graphite felt	Graphite felt	Wastewater from starch processing plant	BOD	Batch	1.1*	30 - 60	2.5 - 206	5 yrs	(Kim, Chang, et al. 2003)
4	Double chamber using CEM	Graphite felt	Graphite felt	Glucose and glutamic acid	BOD	Continuous	ND	30	91-142	> 1 yr	(Kim, Youn, et al. 2003)
5	Double chamber using CEM	Graphite felt	Platinum-coated graphite felt	Glucose and glutamate in surface water and artificial wastewater	BOD	Continuous	0.01-0.02	ND	2 - 6	> 8 weeks	(Kang et al. 2003)
6	Double chamber using CEM	Graphite felt	Graphite felt	Glucose and glutamic acid	BOD	Continuous	3.7 -5.2	60	20-200	> 1 yr	(Chang et al. 2004)
7	Double chamber using CEM	Graphite felt	Graphite felt	Glucose and glutamic acid	BOD	Continuous	1.9*	5-36	50-100	> 2 yrs	(Moon et al. 2004)
8	Double chamber using CEM	Graphite felt	Graphite felt	Glucose and glutamic acid	Hg, Cd, Pb,	Continuous	0.026-0.040	20 -120	1-10 (OrgP) 0.01-1 (Cd) 0.1-1 (Pb)	1 year	(Kim et al. 2007)

9	Double chamber (using PEM) coupled an anaerobic reactor	Graphite rod	Graphite roll	Glucose	PCBs, OP BOD	Batch	0.0013	3-5	25	ND	(Kumlanghan et al. 2007)
10	Double chamber using CEM, AEM, cation selective or bipolar membrane,	Graphite	Graphite	Synthetic wastewater, Acetate	Nickel	Continuous	ND	120	13.2 – 187.6	ND	(Stein, Hamelers, and Buisman 2012; ter Heijne et al. 2008)
11	Single chamber	Graphite rod	Carbon fibre paper with carbon nanoparticles	Acetate	BOD	Batch	0.02-1	300 - 1200	32-1280	ND	(Modin and Wilén 2012)
12	Single chamber	Graphite granules	Carbon supported with platinum catalyst	Glucose, Artificial water	COD	Continuous	0.01-0.09	40	50-500	> 7 months	(Di Lorenzo, Curtis, Head, Velasquez-Orta, et al. 2009)
13	Double chamber (submerged) using CEM	Toray carbon paper	Toray carbon paper/ Platinum (Pt)	Acetate, glucose. wastewater	Microbes , BOD, COD	Batch	0.1	40	10-250	> 5 months	(Zhang and Angelidaki 2011)

14	Single chamber (submerged) using CEM	Toray carbon paper	Wet proof carbon paper/ Platinum (Pt)	Domestic wastewater	BOD	Batch	0.2	30-60	17-183	ND	(Peixoto et al. 2011)
15	Single chamber	Carbon felt	Carbon cloth	Glucose and glutamic acid	BOD	Continuous	0.00047[#]	132	5-120	ND	(Yang et al. 2013)
16	Single chamber with air cathode using PEM	Carbon cloth	Carbon cloth	Potassium acetate	COD, Cd	Continuous	ND	2.8	3-164 (COD) 0.0001 – 0.1 (Cd)	ND	(Di Lorenzo et al. 2014)
17	Single chamber with air cathode	Carbon cloth	Platinum coated carbon cloth	ND	Cr, Fe, NO_3, NaOAc	Batch	0.00023[#]	35 - 40	1-8 (Cr) 1-18 (Fe, NO_3)	ND	(Liu, Lei, and Li 2014)
18	Single chamber with PEM	Carbon cloth	Carbon cloth coated with Pt	Artificial wastewater, Glucose	BOD	Batch	0.88	80	50-1000	ND	(Ayyaru and Dharmalingam 2014)
19	Double chamber with CEM	Granular carbon	K3[Fe(CN)6] / graphite felt	Acetate	COD	Continuous	21.6	120	200	ND	(Wu et al. 2015)
20	Double chamber	ND	ND	Glucose, Methionine, Acetate, Glycerol	BOD	Continuous	0.0196	10-15	235	ND	(Hsieh et al. 2016)
21	Double chamber with CEM	Polished graphite rods	Polished graphite rods	Glucose and glutamic acid	BOD	Batch	0.031	60-1260	50-250	60 days	(Anam et al. 2017)
22	4 single chambers connected in parallel	Carbon fibre veil	Carbon fibre veil	Human urine	COD	Batch	18.1	3	15 - 150	5 months	(Pasternak, Greenman, and Ieropoulos 2017)

23	3-stage single chamber with CEM	Carbon cloth	Carbon with platinum catalyst	Glucose and glutamic acid	BOD	Continuous	0.58	150	25-750	2 years	(Spurr et al. 2018)		

CEM. cation exchange membrane; PEM. proton exchange membrane ; AEM. anion exchange membrane ; ND. No data available;* as reported in (Peixoto et al. 2011);[#] calculated from available data

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Figures & Captions

Figure 1. Schematic diagram of a conventional Microbial Fuel Cell.