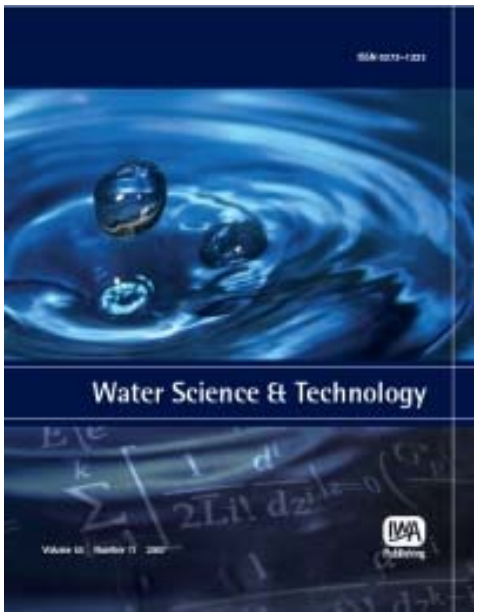

Microbial fuel cell-based biosensors for environmental monitoring: a review

Jian-Zhong Sun, Gakai Peter Kingori, Rong-Wei Si, Dan-Dan Zhai, Zhi-Hong Liao, De-Zhen Sun, Tao Zheng and Yang-Chun Yong

ABSTRACT

The microbial fuel cell (MFC) is an innovative technology that was initially designed to harness energy from organic waste using microorganisms. It is striking how many promising applications beyond energy production have been explored in recent decades. In particular, MFC-based biosensors are considered to be the next generation biosensing technology for environmental monitoring. This review describes recent advances in this emerging technology of MFC-based biosensors, with a special emphasis on monitoring of biochemical oxygen demand and toxicity in the environment. The progress confirms that MFC-based biosensors could be used as self-powered portable biosensing devices with great potential in long-term and remote environmental monitoring.

Key words | biochemical oxygen demand, biosensor, environmental monitoring, microbial fuel cell, toxicity

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INTRODUCTION

The microbial fuel cell (MFC) is an innovative device in which the chemical energy stored in organic waste is efficiently converted to electricity with the help of microorganisms (Lovley 2006; Zhao *et al.* 2009; Yong *et al.* 2012). In an MFC, microbes oxidize electron donors (organic substrates) with diverse metabolic enzymes, which results in the release of electrons from the substrates. The released electrons are then passed to the solid anode via intracellular and extracellular electron transfer pathways. The electrons collected by the anode are then transferred through the external circuit to the cathode, which is separated from the anode by a proton exchange membrane. The electrons flow through the external circuit and thus create an electric current, while the organic substrates/waste in the anodic chamber are also oxidized/degraded (Lovley 2006). Thus, MFCs are considered to be a promising technology with simultaneous bioenergy generation and environmental pollution treatment (Yong *et al.* 2011; Tao *et al.* 2013; Xu 2013; Yong *et al.* 2013; Yong *et al.* 2014). More impressively, various novel and fascinating applications derived from MFC technology, including H_2 production by microbial electrolysis cells (Cheng & Logan 2007), biocommodities synthesis by microbial electrosynthesis cells (Lovley & Nevin 2013), water desalination by microbial desalination cells (Kim & Logan 2011), waste heat recovery by microbial reverse-electrodialysis cells (Cusick *et al.* 2012) and environment monitoring by MFC-based sensing devices (Chang *et al.* 2004; Chang *et al.* 2005; Si *et al.* 2015) have been developed. Among these, MFC-based sensing devices are of great interest to

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researchers as they have the potential to achieve self-powered, remote and *in situ* monitoring of environmental pollution. The IUPAC definition of electrical biosensors is: 'An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element' (Thevenot *et al.* 2001). Based on this definition, those MFC sensing devices that use bacteria in the anodic chamber as the biological recognition element to directly generate an electrical output in response to exogenously added analyte could be considered as biosensors.

In the light of the rapid development of microbiology, biosensors have attracted more and more attention and have become an alternative tool for sensitive, fast, and selective detection/quantification of various analytes (D'Souza 2001; Yong & Zhong 2009). It became one of the most important methods for environmental monitoring (Dong *et al.* 2011). In particular, the whole-cell biosensor holds great potential for developing long-term and cost-effective environmental monitoring due to its capability of self-regeneration and self-replication. It has the unique property of providing bioavailability information, which is not possible by any other analytical methods (Yong & Zhong 2009; Dong *et al.* 2011; Yong & Zhong 2013; Si *et al.*, 2015). However, traditional whole-cell biosensors usually use fluorescence proteins, enzyme activity and fluorescence/pigment molecules as indicators. To get a readable/quantitative signal, these molecules/indicators should be measured using electrically-powered equipment such as fluoro/ultraviolet-visible spectrometers, microscopes, etc. (Yong & Zhong 2009; Dong *et al.* 2011). Then, the measured electrical output by the equipment will represent the response of the biosensor (Figure 1). So, an external power source and costly equipment are indispensible for traditional biosensors, which seriously limits their application in remote and long-term environmental monitoring, where a suitable power source and the relevant analysis equipment are usually unavailable.

In contrast, for an MFC-based biosensor, the bacteria can sense the analyte and then give a corresponding response on its output electric current, in which the sensing step and electrical signal transition step are integrated and can be completed in one step without a signal transducer and external power source (Figure 1) (Chang *et al.* 2004; Kim *et al.* 2006; Peixoto *et al.* 2011). The most interesting aspect of the MFC-based biosensor is that it does not need a transducer to convert the output to an electrical signal because the measured signal is already an electrical current (Peixoto *et al.* 2011). These unique characteristics facilitate the

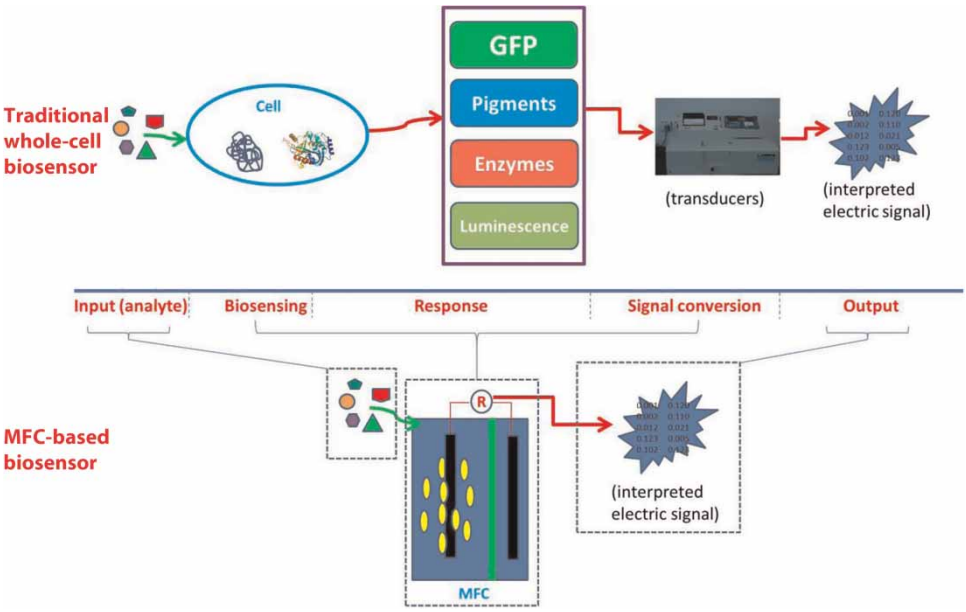

Figure 1 | Comparison of the biosensing process between a traditional whole-cell biosensor (top panel) and an MFC-based biosensor (bottom panel). Traditional whole-cell biosensors usually use green fluorescence proteins (GFP), pigment molecules, enzyme activity and luminescence as indicators. They are measured using electrically powered equipment hence the need for a transducer. The MFC-based biosensor uses organic matter as fuel to generate current and it does not need a transducer because the measured signal is already an electrical current.

fabrication of disposable and portable biosensor devices, which perfectly meet the requirement for long-term and remote sensing. This review will mainly focus on this new generation of self-powered biosensors based on MFC techniques.

MFC-BASED BIOSENSORS FOR ORGANIC CARBON MONITORING IN WATER

Traditional methods of organic carbon monitoring

Organic matter including nitrogenous compounds, proteins, amino acids, carbohydrates and lipids are the main pollutants in domestic wastewater, which may cause serious environmental problems such as eutrophication (Kim *et al.* 2006). Thus, organic matter content is considered to be one of the most important parameters that need continuous monitoring during wastewater treatment and environmental assessment (Bourgeois *et al.* 2001). However, detailed characterization of this organic matter in the wastewater is difficult and so bulk parameters such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are usually used to describe the total amount of pollutant organic matter in water (Bourgeois *et al.* 2001). BOD is a measure of the organic pollution of water that can be degraded biologically. It usually takes 5–7 days of incubation at $20 \pm 1\ ^{\circ}C$ in the dark, hence BOD_5 and BOD_7, and is expressed in milligrams O_2 per litre, while COD indirectly measures the organic compounds in water and is expressed in milligrams per litre (mg/L) (Chang *et al.* 2004; Moon *et al.* 2004; Di Lorenzo *et al.* 2009; Chiappini *et al.* 2010; Jouanneau *et al.* 2014; Liu *et al.* 2014). COD is the measure of the organic matter in water that can be fully oxidized to carbon dioxide by strong oxidizing agents (e.g. potassium dichromate) under acidic conditions. However, these traditional methods should use external powered equipment as a transducer to generate an electrical signal. In addition, the operation of these methods usually requires professional personnel and are time-consuming and expensive (Peixoto *et al.* 2011). Therefore, development of cost-effective and portable methods for BOD or COD determination is of great interest to people involved in the water industry.

MFC-based biosensors for BOD monitoring

As the organic matter is used as the fuel for an MFC to generate current, the concentration change of the organic matter in water directly affects the output electricity. In particular, the steady current output usually correlates with the concentration of the organic matter, while the charge output correlates with the total amount of organic matter (Kim *et al.* 2003; Peixoto *et al.* 2011) (Figure 2(a) and 2(b)). Thus, it is feasible to correlate the current change with the concentration fluctuation of the organic matter.

Different research groups have developed several MFC-based biosensors to measure the BOD concentration of real or artificial wastewater (Table 1). Most such biosensor devices are designed based on a dual-chamber MFC, in which the anaerobic anodic chamber is designed as the detection part. With the inoculation of different activated

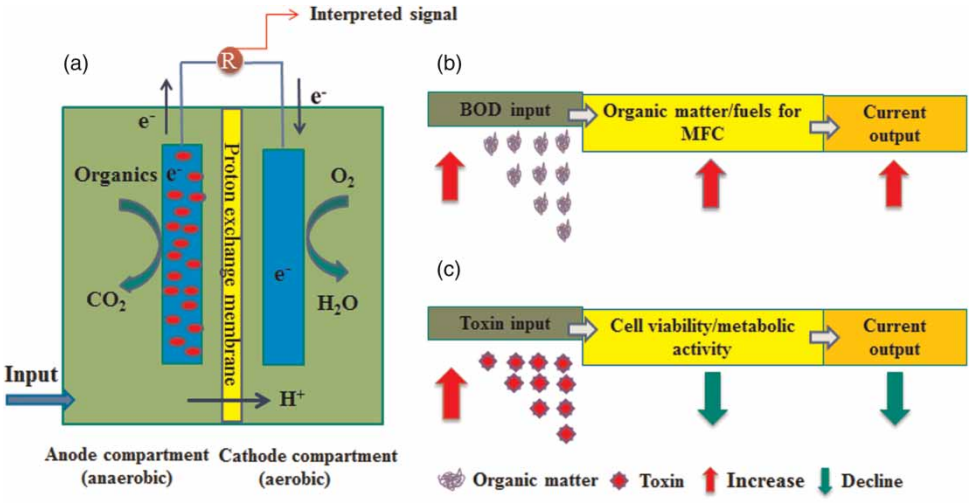

Figure 2 | (a) Schematic diagram of an MFC. (b) Mechanism for MFC-based BOD monitoring. Increased BOD input provides more organic matter/fuel for the MFC, which in turn results in an increase in current output. (c) Mechanism for MFC-based toxicity monitoring. Increased toxin input will repress/inhibit the cell viability/metabolic activity, which directly reduces the current output.

Table 1 | Microbial fuel cell-based biosensors for detection of organic matter

Reactor	Analytes	Biofilm enrichment	Stability	Measure range	Response time	Reference
D[a]	Artificial wastewater	AS (4 weeks)	>2 months	20–200 mg BOD/L	60 min	Peixoto *et al.* (2011)
D	Wastewater	AS	>5 years	2.6–206 mg BOD/L	30 min to 10 h	Kaur *et al.* (2013)
D	Artificial wastewater	AS	NA[b]	20–200 mg BOD/L	5 min	Kim *et al.* (2010)
D	Artificial wastewater	Oligotrophic bacteria	NA	2–10 mg BOD/L	60 min	Liu *et al.* (2011)
D	VFA[c]	AnS (~4–25 days)	NA	5–40 mg VFA/L	1 min to 20 h	Modin & Wilen (2012)
S	Wastewater	AS (3 weeks)	NA	17–183 mg BOD/L	30 min to 10 h	Kim *et al.* (2006)
S	Artificial wastewater	AnS (30 days)	>25 days	50–750 mg BOD/L	79 min to 10 h	Feng *et al.* (2013)
S	Artificial wastewater	AS + AnS (80 days)	NA	32–1280 mg BOD/L	5–20 h	Kim *et al.* (2009)

[a]Single-chamber MFC (S), dual-chamber MFC (D), activated sludge (AS), anaerobic sludge (AnS).
[b]NA, not available.
[c]VFA, volatile fatty acid.

sludges, the enrichment time for anodic biofilms varies from 3 to 8 weeks (Peixoto *et al.* 2011). After the mature anode biofilm is formed, the wastewater to be analysed (analyte input) is injected into the anodic chamber and the MFC output fluctuation is monitored with simple devices and serves as the biosensor output. Primary metabolisms including current generation in the MFC are dependent on the limiting substrate concentration in Monod's equation. Typically, the BOD concentration is correlated with the maximum current output or total charge output. For the maximum current output, the linear relationship is only observed at low BOD concentration, while a much wider BOD concentration range can be covered when correlated with the total charge output. Kim *et al.* demonstrated an MFC with over 5 years of stable current generation, where coulomb generated from the MFC was directly proportional to the strength of wastewaters, which provided the opportunity to use it as a BOD sensor. However, improvement of coulomb efficiency of MFCs, enabling a direct calculation of BOD from coulomb remains a critical issue as oxygen diffuses through the cation-specific membrane, usually resulting in low coulomb yield and low electrical output (Kim *et al.* 2003; Peixoto *et al.* 2011). Volatile fatty acids (VFAs) have been denoted as parameters for monitoring wastewater treatment processes such as anaerobic digestion (Kim *et al.* 2010). Kaur *et al.* (2013) obtained a good correlation between the concentration of individual VFA species and the total charge delivered from the MFC. Thus, the MFC-based biosensor was constructed to analyse the concentrations of individual VFAs (acetate, propionate and butyrate) in wastewater (Kaur *et al.* 2013).

With the help of the self-power property of MFC-based biosensors, the BOD concentration can be monitored with rapid response times, varying from 5 min to 10 h and with high stability (Table 1) (Kim *et al.* 2003). The operational stability of this MFC-based sensor is superior to the conventional BOD sensors, which is essential for a reliable sensor system. More importantly, the MFC-based biosensors were fairly robust, and maintained their performance for years (up to 5 years) while being exposed to a wide range of BOD concentrations (Kim *et al.* 2003; Feng & Harper 2013). This characteristic suggested that the MFC-based biosensor could serve continuously as a BOD biosensor during permanent service in the field (Feng & Harper 2013).

Challenges and solutions for MFC-based BOD biosensor

As the MFC chamber is closed and the anodic compartment should be maintained in an anaerobic condition, it is hard to realize *in situ* or online monitoring using a conventional dual-chamber MFC-based biosensor. Thus, several new reactor design strategies have been developed to overcome the problem of achieving *in situ* or online monitoring. Peixoto *et al.* (2011) developed a dual-chamber submersible MFC-based BOD sensor which was directly submerged in a wastewater channel or anaerobic reactor for *in situ* monitoring. In this case, the submerged open anode chamber directly contacted with the domestic wastewater to be analysed, while the closed cathode was continuously sparged with air (Pt-covered wet-proof carbon paper). The current density output showed a linear relationship with BOD_5 concentration, ranging from 17 ± 0.5 to 78 ± 7.6 mg O_2/L and with a fast response (from 30 min to 10 h). As the MFC output signals strongly depend on the bacterial community of the anode biofilm, MFC operation parameters, water chemistry and pollutant bioavailability may vary between

systems. Hence, application of MFC-based biosensors in BOD monitoring is a complicated task, and advanced data processing techniques are indispensable in interpreting the relationships between BOD and the MFC output. Chang *et al.* (2004) developed an MFC-based BOD sensor system for real-time wastewater monitoring. The wastewater was purged with nitrogen gas to maintain the anaerobic condition and then continuously injected into the anodic chamber using a peristaltic pump. At a hydraulic retention time of 1.05 h, BOD concentration up to 100 mg/L showed good linear correlation with the MFC output. When the BOD concentration of the injected wastewater changed, the current output changed along with the BOD and reached a new steady state within about 60 min, which demonstrated potential for use in real-time monitoring, although the response time still needs to be improved.

New MFC membranes have been developed to overcome the problems caused by the oxygen inhibition. Oxygen diffusion into the anodic chamber through the proton exchange membrane is the main problem encountered for MFC-based BOD biosensors (Moon *et al.* 2005; Liu *et al.* 2011; Feng *et al.* 2013). In the dual-chamber MFC-based biosensor, the cathodic chamber is usually equipped with a Pt catalyst while oxygen is continuously purged in. A Nafion proton exchange membrane is usually used to separate the cathodic chamber from the anodic chamber. However, leakage of oxygen into the anode chamber may inhibit the growth of obligate anaerobes and results in loss of organic matter due to aerobic respiration. Thus, it may subsequently lead to low sensitivity and inaccuracies in BOD measurement. Ayyaru & Dharmalingam (2014) developed a single-chamber MFC equipped with a sulfonated poly ether ether ketone membrane (instead of a Nafion membrane) for BOD monitoring. This new membrane showed lower oxygen permeability than the Nafion membrane and thus resulted in better MFC performance. When used as a BOD biosensor, it showed a 62.5% wider BOD sensing range (up to 650 ppm) than that equipped with the Nafion membrane. Membrane-electrode assembly (MEA) is another promising strategy to improve MFC performance. MEA was also applied to an MFC-based BOD biosensor and increased its sensitivity (Kim *et al.* 2009; Ayyaru & Dharmalingam 2014).

The problems associated with the presence of electron acceptors and pH shifts in MFC-based BOD biosensors have been established. The electron acceptors (such as nitrate, which usually exists in wastewater) largely reduced MFC output signal and thus resulted in low sensitivity for BOD monitoring (Kim *et al.* 2009). Chang *et al.* (2005) developed a simple and practical strategy to overcome this problem. By addition of respiration inhibitors such as azide and cyanide, the MFC output and the sensing performance did not change in the wastewater without nitrate. In contrast, the addition of such respiration inhibitors eliminated the detrimental effect from nitrate, and the BOD sensing performance was resumed (Chang *et al.* 2005). This finding provided a new approach to accurate measurement of BOD in wastewater with nitrate or other electron acceptors. However, possible water contamination from toxic azide and cyanide added into that BOD biosensor should be taken into consideration, and new non-toxic and eco-friendly strategies should be further developed. High internal resistance and pH shifts are other limitations encountered with MFC-based BOD biosensors. Modin & Wilen (2012) demonstrated a design that overcame these two limitations. The design involved application of an external voltage that boosted the current generation in the system, which allowed the microorganisms at the anode to generate current at their full potential. By omitting the ion exchange membrane between the cathode and anode, it was observed that decreasing analyte pH did not affect the sensor. BOD concentration is correlated with the transferred charge, hence through batch-wise feeding, microbial kinetics does not limit the range of the sensor, which would be the case if current was correlated with BOD concentration (Modin & Wilen 2012). At present, MFC-based biosensors have been commercialized to meet the requirements in application, e.g. HABS-2000 and HABS-2001 developed by the Korea Institute of Science & Engineering. However, the stability and sensitivity of the biosensor still need to be improved and the response time should be reduced to compete with modern analytical methods.

MFC-BASED BIOSENSORS FOR TOXICITY DETECTION

Traditional methods of toxicity monitoring

Toxicity is one of the most important parameters for water quality inspection, as the toxic compounds are harmful to the health of human beings and other living creatures. Thus, toxicity detection is indispensable in wastewater treatment, drinking water processing and water environmental monitoring (Kim *et al.* 2007; Davila *et al.* 2011; Stein *et al.* 2012a). By using traditional physical-chemical detection methods, the presence of known toxicants could be

determined via various techniques. However, these methods cannot provide key information about unknown toxicants such as biotoxicity and bioavailability (D'Souza 2001). Therefore, detection of toxicity using biosensors is of great interest due to their unique potential to identify the bioavailability of the toxicants as well as the general toxicity toward living organisms. If bacteria are exposed to chemical toxicants, their metabolic activity or cell viability will be inhibited. This inhibition can be monitored by various approaches such as fluorescent stains for cell viability or enzyme/respiration assays for metabolic activity. Therefore, various whole-cell toxicity biosensors were developed by integrating different physical-chemical equipment. Helped by this equipment, the biotoxicity detected by the bacteria could be transduced to an electrical signal, which can be easily identified or further processed (Kim *et al.* 2007; Davila *et al.* 2011; Stein *et al.* 2012a). However, the need for external power supported transducers limits their practical applications.

MFC-based biosensors for toxicant monitoring

As the microbial metabolism is the sole driving force for the conversion of chemical energy into electricity, the MFC output mainly depends on the bacterial viability and activity. Thus, if the microbial activity is inhibited, the MFC output will decrease. The toxicants that bacteria are exposed to determine the inhibition of bacterial activity and thus the decrease of MFC output (Stein *et al.* 2010, 2012a) (Figure 2(a) and 2(c)). Therefore, MFC is feasible to serve as a toxicity biosensor (Table 2). For conventional two-chamber, MFC was used as the toxicity biosensor, where the anodic chamber was the detection part. Usually, the anode chamber could be operated in continuous mode by continuous feeding with medium or wastewater to be analysed, and thus achieve online monitoring (Stein *et al.* 2012a). The current density output, anode potential, or power output could serve as the MFC biosensor output signal (Davila *et al.* 2011). Kim *et al.* (2007) demonstrated that addition of various toxic substrates (such as organophosphorus, lead, mercury and polychlorinated biphenyls) caused significant decrease in MFC current output. For example, addition of organophosphorus, lead, mercury, or polychlorinated biphenyls at low concentrations of 1 mg/L resulted in 61%, 46%, 28% or 38% decrease in current output, respectively, when compared to the control. When mixed heavy metals (1 mg/L Cd and 1 mg/L Pb) were applied, ~76% inhibition ratio was obtained (Kim *et al.* 2007). Moreover, the MFC-based biosensors were successfully applied for fast monitoring of acidic toxicity (Shen *et al.* 2012), formaldehyde acute toxicity (Davila *et al.* 2011), surfactant toxicity (sodium dodecyl sulfate (Stein *et al.* 2012b) and toxicity caused by heavy metals (such as nickel and copper) (Stein *et al.* 2012a; Shen *et al.* 2013). By using pure cultures of *Shewanella oneidenesis* MR-1 as the anodic bacteria, an MFC-like bioelectrochemical system was constructed for formaldehyde quantification (Wang *et al.* 2013). The current output of this biosensor showed a linear response to formaldehyde up to a concentration of 0.08%, which indicated the feasibility of using this biosensor for formaldehyde toxicity quantification. Moreover, attention should be paid once the samples contained toxic azide and/or cyanide compounds. Unlike other toxic compounds which exhibit decline in current in MFC due to microbial activity inhibition when used in higher concentration, the addition of azide and cyanide in oligotrophic MFCs and MFCs fed with air-saturated artificial wastewater containing nitrate exhibited an increase in current output. The respiratory inhibitors eliminated the inhibitory effects of the electron acceptors on the current generation from MFCs, due to the fact that azide inhibits nitrate reduction and terminal oxidase competitively (Chang *et al.* 2005).

Table 2 | Microbial fuel cell-based biosensors for toxicity detection

Reactor	Toxicant	Biofilm enrichment	HRT	LCD	Anode potential	Reference
D[a]	SDS	EBC	NA	50 mg/L	>− 0.4V (Ag/AgCl)	Stein *et al.* (2012b)
D	Ni^{+}	EBC	45 min	22.7 mg/L	−0.4V (Ag/AgCl)	Stein *et al.* (2012a)
S	Cu^{2+}	Wastewater (2 months)	1–20 min	5 mg/L	Without control	Shen *et al.* (2013)
D	Cu^{2+}	EBC	~47 min	~93 mg/L	−0.15 ~ −0.4V (Ag/AgCl)	Stein *et al.* (2010)
D (microsize)	Formaldehyde	GS	NA	0.1%	Without control	Davila *et al.* (2011)
EC	Formaldehyde	SO	NA	0.01%	0.3V (SCE)	Wang *et al.* (2013)

[a]Single-chamber MFC (S), dual-chamber MFC (D), *Shewanella oneidensis* MR-1 (SO), *Geobacter sulfurreducens* (GS), sodium dodecyl sulfate (SDS), enriched bacterial community from long-term operated MFC (EBC), hydraulic retention time (HRT), lowest concentration detected (LCD), saturated calomel electrode (SCE), three-electrode electrochemical cell (EC).

Improvement of MFC-based toxicity biosensors

The operational parameters significantly influenced the sensitivity of the MFC-based toxicity biosensors. As the decrease in current output is the indicator for the presence of toxicants, a stable and high baseline current output (MFC without toxicant) is essential to obtain high accuracy and sensitivity (Kim *et al.* 2007). The conventional MFC operational parameters such as anode potential and external resistance significantly affected the biosensor sensitivity. The electrode potential determines the energy level of the electrons that passed to the anode and thus affects the electron transfer efficiency and the current output. Usually, a higher anode potential resulted in faster electron transfer and higher current output. For example, in a conventional two-chamber MFC, the average current density for an MFC with anode potential of -0.3 V (1.3 A/m^2) is about 10 times that delivered from an MFC with a lower anode potential of -0.4V (0.12 A/m^2) (Stein *et al.* 2012a). Thus, optimization of the anode potential is important for MFC-based biosensors. By comparison of different controlled anode potential, higher anode potential usually resulted in higher current output and higher sensitivity toward different toxicants (such as nickel, SDS) (Stein *et al.* 2012a, b). Moreover, external resistance directly determined the MFC current output and thus affected its sensitivity toward toxicants. Usually, lower external resistance resulted in higher current output and higher sensitivity when exposed to toxicants (Stein *et al.* 2012a). In contrast to anode potential and external resistance, among four different membranes (cation exchange, anion exchange, monovalent cation exchange, and bipolar membrane) used in MFC, the influence of membrane type on the biosensor sensitivity for nickel detection is negligible (Stein *et al.* 2012a). Besides, shear stress induced from water flow or nitrogen sparging is another determining factor for MFC-based toxicity biosensors. Hydrodynamic shear rates were proved to affect the biofilm structure and production of extracellular polymeric substances (EPS), which influenced the interaction between toxicants and biofilm, and thus influenced the biosensor sensitivity to heavy metals (Shen *et al.* 2013). Low shear rate resulted in formation of an anode biofilm with lower biomass density, higher porosity and lower EPS content, which in turn resulted in improved biosensor sensitivity to copper toxicity (Shen *et al.* 2013).

Trace and high throughput measurement is of great interest in the field of environmental monitoring. For normal MFCs, the volume of the anodic chamber is about ten to hundreds milliliter and it is still not small enough to achieve trace and high throughput detection. In view of this, a silicon-based micro-fabricated MFC array was designed and used for toxicity monitoring (Davila *et al.* 2011). The MFC arrays are fabricated on a 6×6 mm silicon plate, while each MFC had an anode compartment with working volume of 144 μl. The micro-fabricated MFC was able to give an efficient performance and was validated to be a toxicity sensor toward 0.1% formaldehyde. The compact design provides the possibility to incorporate this device into other equipment, or served as portable sensor device, or sensor array for high throughput toxicity biosensing (Davila *et al.* 2011). More impressively, a commercial MFC-based biosensor for detecting toxicity is now available, e.g., biological toxicity test system HATOX-2000 invented by a company in Korea has been applied for monitoring comprehensive water toxicity.

CONCLUDING REMARKS AND PERSPECTIVES

Recent progress on MFC-based biosensors used for BOD and toxicity sensing have been thoroughly reviewed. The design of the MFC-type biosensor integrated the advantages of the whole-cell biosensor and the self-powered MFC device. This unique design provides featured compact sensor configuration, in which the microorganisms directly generate readable electric signal output without any external powered transducer. In terms of applicability, many research groups have developed different MFC configurations, operation control strategy, mathematic models, as well as different microbial enrichment/strains to improve the performance of MFC-based biosensors. Laboratory tests with real or artificial wastewater and field tests were also conducted. Attempts at online, *in situ* or real-time environmental monitoring performed by different research groups are also included in this review.

Following various research and advancements in MFC-based biosensors, there is a promising opportunity to translate the remarkable progress of this technology into practical application. However, there are still limitations both in fundamental and application aspects that should be addressed. The selectivity of the MFC-based biosensor, one of the most important metrics for biosensing, has not yet received the consideration it deserves. Other research focuses such as development of novel MFC devices and operation control strategies to meet the requirement of high throughput and trace monitoring are also of great importance and should be given more attention. The fundamental studies to unveil the underlying mechanism for various operational control

strategies (such as potential control which is unfavorable for portable device design) are also vital to simplify or develop novel operation procedures. In all, MFC-based biosensors have a promising future in developing portable and self-powered sensing devices for *in situ*, online, high-throughput, highly selective; and sensitive environmental monitoring.

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