Recent Trends and Advances in Microbial Electrochemical Sensing Technologies: An Overview

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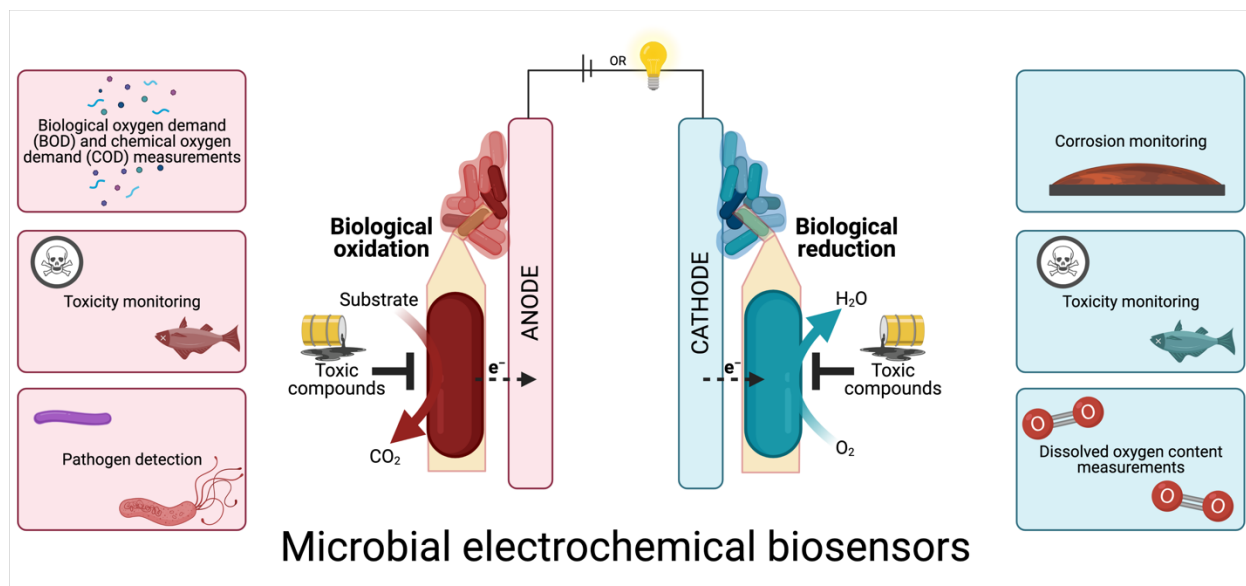
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Graphical Abstract

Microbial electrochemical biosensors

Abstract

Microbial electrochemical systems have been utilized as technologies for generating electricity through organic matter and wastewater treatments. Recently, growing research attention has been devoted to the development of microbial electrochemical sensors as biosensing platforms. Microbial electrochemical sensors are a type of microbial electrochemical technology capable of sensing through the anodic or the cathodic electroactive microorganisms and/or biofilms. Herein, we review and summarize the recent advances in the design of microbial electrochemical sensing approaches with a specific overview and discussion of microbial electrochemical anodic and cathodic sensing. Particular emphasis is given on the recent trends for different MES applications in biosensing, including toxicity monitoring, pathogen detection, corrosion monitoring, as well as measurements of biological oxygen demand, chemical oxygen demand, and dissolved oxygen. We conclude with perspectives and outlooks to understand the shortcomings in the design and applications of microbial electrochemical sensing platforms.

Keywords

Microbial electrochemical sensors, dissolved oxygen, BOD measurements, toxicity, pathogen detection

Introduction

Microbial electrochemical technologies (METs) are bioelectrochemical systems (BESs) in which microorganisms act as biocatalysts promoting or enhancing specific oxidation or reduction reactions [1]. In METs, an oxidation reaction occurs at the anode and a reduction reaction occurs at the cathode, both of which can be catalyzed by microorganisms. These microorganisms are named electroactive bacteria, and through their metabolism, they are capable of releasing electrons to the electrode (anode) or accepting electrons from the electrode (cathode) [2]. The electron transfer (ET) involves different mechanisms that can be grouped in four different categories: (i) direct through outer membrane cytochromes, (ii) direct through nanowires, (iii) indirect through mediators, and (iv) indirect through catabolites [3]. Direct electron transfer is often the preferred mechanism for METs as it is the least energy-consuming [3]. With the first discovery of direct electron transfer occurring at the end of the 1990s, the interest in METs grew exponentially. Several experiments have been conducted to identify and characterize electroactive bacteria composing electroactive biofilms [4]. It was also shown that METs are capable of using diverse simple and more complex wastewaters [5]. A synergistic approach can be envisioned in which fermentative bacteria break down complex molecules and electroactive bacteria are able to further degrade smaller and simpler molecules while directly (or indirectly) transferring electrons to the anode [4]. Similarly, electroactive bacteria and biofilms are also actively involved in reduction reactions at the cathode [4].

Until now, METs have been studied mainly for the possibility of generating electricity through organic matter and wastewater treatment. Less but growing attention has been devoted to the microbial electrosynthesis of value-added products (VAPs), including hydrogen and organic compounds. However, recently, particular focus has been given to the use of METs as biosensing platforms. In this sense, a biosensor is defined as an analytical tool capable of utilizing biological matter and transducing its interactions with an analyte of interest into a valuable electrical output [6]. In parallel, electroactive biofilms can lose their intrinsic electroactivity in the presence of toxic compounds for inhibition sensing applications. In this review, a recent overlook of the METs for sensing platforms is presented with specific discussion related to microbial electrochemical anodic and cathodic sensing.

Discussion

Categories of MET sensors

The biocatalysts utilized in METs are composed of electroactive bacteria arranged in a more complex biofilm structure present at one or both electrodes. Once the biofilm is well established and oxidants or reductants are constantly provided at the same concentration, the current output is theoretically stable over time as steady-state metabolic activity and current response are reached. Both anodic and cathodic biofilms are sensitive to the operational conditions whose variations, in turn, lead to an increase or decrease of the current output. The variation of oxidant or reductant concentration provided at the cathode and the anode, respectively, could lead to a positive or negative response. Importantly, the biofilm can be absent at the cathode, but still, the variation in oxidant concentration might affect the response of the overall microbial electrochemical sensor (MES). In parallel, the addition of toxic compounds might negatively affect the biofilm or electroactive bacteria activity. This interaction might be reversible and instantaneous with the recovery of the biofilm functionalities or completely irreversible with deleterious effects on the biofilm activity. The principal and most important MES categories related to the anode and cathode response are reported in Figure 1.
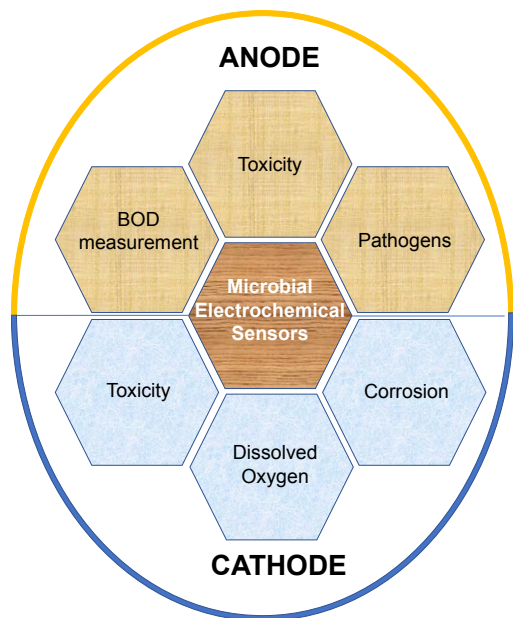
Figure 1. Microbial electrochemical sensors (MESs) and most promising biosensing targets.

Anodic microbial electrochemical sensors

Electroactive bacteria and biofilms populating the anode can pair their oxidative metabolisms with electron transfer to the anode. This interaction forms the basis of METs capable of sensing a metabolic substrate of interest by measuring the electroactive bacteria metabolic activity via a current response [7]. Common applications of anodic MESs are biological or chemical oxygen demand (BOD/COD) measurements, toxicity measurements, and pathogen detection.
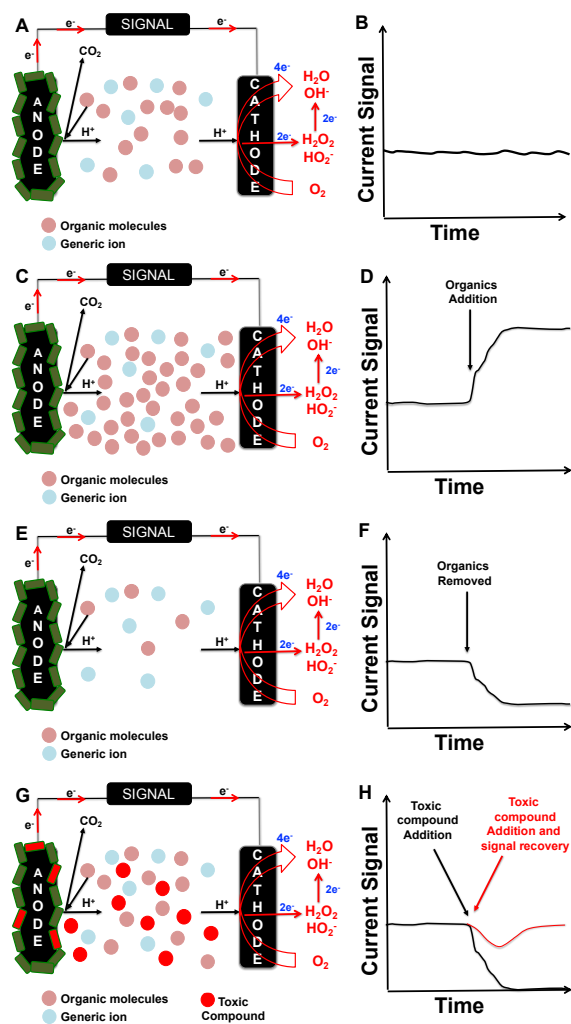
Figure 2. Anodic electroactive biofilm operating as a biosensor. A constant and similar concentration of organics is provided (A) and a steady-state current output is obtained (B). Organic molecules, as biological oxygen demand (BOD), are added in the MET system (C) and the current output increases proportionally with the increase in organics (D). Similarly, as the BOD concentration decreased (E), the signal output would decrease (F). The addition of a toxic

compound in the MET system (G) might lead to a total decrease of the signal or a partial decrease in the signal followed by a recovery of the signal (H).

BOD/COD measurement

The anodic electroactive bacteria are sensitive to variations of the substrate provided for which they are capable of respiring and pairing with electron transfer. Once the MET system is in steady-state conditions (Figure 2.A and B), an increase in organic substrate concentration should provide an increase in the current produced (Figure 2.C and D) [8]. Naturally, this is true until a threshold is reached (saturation) and a further increase in organics concentration does not lead to a further increase in current output recorded. In parallel, if the concentration of organics is decreased, this is reflected in a decrease in the signal output (Figure 2.E and F). Therefore, biosensors based on METs can act as an online sensor for biological oxygen demand (BOD) or chemical oxygen demand (COD), reducing the response times dramatically compared to traditional toxic colorimetric detection kits which have response times of 5 days and a few hours for BOD and COD, respectively. In fact, MET biosensors, after the proper calibration, can immediately display the current output and be correlated with the organic matter concentration in an aqueous medium.

Toxicity at the anode

The influence of toxic substances (e.g., heavy metals, antibiotics, pesticides) affecting the metabolisms of electroactive bacteria has been utilized for the development of toxicity biosensors. Recent works focused on maximizing the sensitivity of these devices by investigating various operational modes (e.g., continuous or transient operation) [9], as well as their miniaturization [10,11]. Interestingly, electroactive bacteria capable of withstanding relatively high concentrations of various heavy metals such as Cr, Cd, and Ni for the development of shock sensors have been reported, enabling cost-effective and portable microbial sensors. Importantly, it was shown that the electrical signal could be restored when the concentration of metals remained below a certain threshold, as shown in Figure 2.G and 2.H [12]. Efforts have been directed into developing portable devices that have the capability to operate in self-powered mode, thus not requiring connection to

a power source [13,14]. It should be noted that toxicity microbial electrochemical sensors did not display high selectivity. However, the influence that various toxic compounds have on the response of these sensors paves the way for an on-line and early monitoring of toxic events. Very recently, intact photosynthetic bacteria and isolated chloroplasts have been utilized to obtain sun-light powered herbicide biosensors that could detect the concentrations of widely utilized herbicides within the limits set by the U.S. Environmental Protection Agency [15,16].
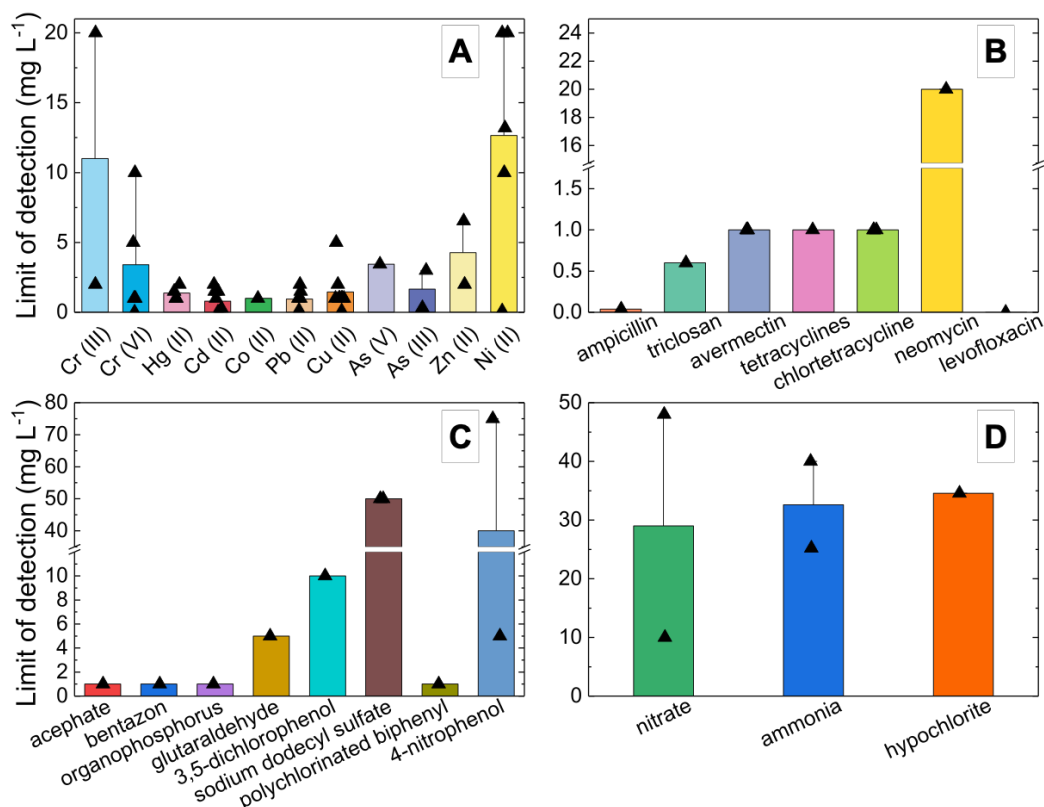
Figure 3. Detection limit reported in different studies summarized in Ref. [7] using MESs in order to detect: (A) heavy metals, (B) antibiotics, (C) other organic toxicants, and (D) other inorganic toxicants. Figure adapted and rearranged with permission from Ref. [7]. Copyright (2021) Elsevier.

Microbial electrochemical sensors for pathogen detection

MESs have also shown promise as devices for rapid detection and monitoring of pathogens and developing bacterial infectious diseases [17-20]. Namely, these sensing platforms offer a means for the fast, sensitive, and qualitative detection of relevant pathogenic microorganisms, cellularly-derived metabolites, signaling molecules, and/or hormones. The detection of cellular signaling

molecules glucose and L-lactate have been demonstrated using MESs based on *Gluconobacter oxidans* [21] and *Hansenula polymorpha* [22], respectively. In the context of clinical applications for pathogen diagnostics, MESs have been developed for the detection of electrochemically active pathogenic microorganisms in clinically relevant samples [17-20]. In these MES designs, there is no biological catalyst on the electrode surface. However, the pathogenic bacteria present in clinical samples act as catalysts through the secretion of redox-active mediators as secondary metabolites, giving a detectable signal only when the pathogen of interest is present. For instance, MESs with unique array-based geometries have been designed for sensitive and real-time detection of the opportunistic human pathogen *Pseudomonas aeruginosa* via the generation of redox-active phenazine metabolites produced during infection stages [20,23-26]. Thus, MESs show promise as analytical devices for pathogen detection in environmental monitoring and medical diagnostics, offering various attractive advantages such as fast response, low cost, and versatility. Possible designs of wearable [27] and self-powered [28] MESs offer exciting future directions in this research field.

## 2.3. Cathodic microbial electrochemical sensors

Electroactive bacteria and biofilms can also populate the cathode and are capable of pairing a biological reduction reaction with electron transfer to the cathode. Commonly this reduction reaction involves the abiotic or biotic catalyzed oxygen reduction reaction (ORR) [7]. The applications of MES utilizing the cathode include corrosion sensing, dissolved oxygen (DO) determination, and toxicity measurements.

2.3.1 Corrosion sensing

Biofilms are able to grow on almost all types of materials and metals, including copper alloys, despite the toxic effects that copper imposes on a large class of biological organisms [29]. The bacteria settlement is able to change the electrochemical characteristics of the conductive surfaces. The involved mechanism is the modification of the corrosion processes occurring at the metal–biofilm interface, mostly increasing the cathodic current, both in anaerobic and aerobic environments [30]. For passivable (non-corrodible) alloys like stainless steel, an increase of 300–

500 mV of free corrosion potential is induced by the first stage of biofilm growth [31,32]. Based on this effect, simple electrochemical devices named BIOX [33], composed of a stainless steel (or titanium) cathode and a zinc anode connected throw a high-value electrical resistance, were developed suitable for the combined on-line monitoring of (i) biofilm growth, (ii) microbial corrosion risk and (iii) antifouling treatments (e.g., chlorination) effectiveness [34]. Such types of sensors were the first application of a bioelectrochemical system at the industrial level [35,36]. Up to now, extensive use of these biosensors occurred mainly, but not exclusively, in cooling circuits of power plants and petrochemical sites, where a huge quantity of water (often seawater) crosses artificial canals or cooling towers. Indeed, biological growth and microbial corrosion are relevant issues for heat exchanger facilities cooled with natural water. Nonetheless, it was also demonstrated that the same type of biosensor can be applied to monitor bacterial activity in the soil (Figure 4.A) [37]. Indeed, cathodic polarization curves performed on stainless steel dipped in soil strongly increase when its surface is colonized by bacterial biofilm (Figure 4.B). Although not yet exploited in field applications, such MESs can be of particular interest for the on-line monitoring of the bioremediation processes of polluted soil, without the need for microbiological analysis.
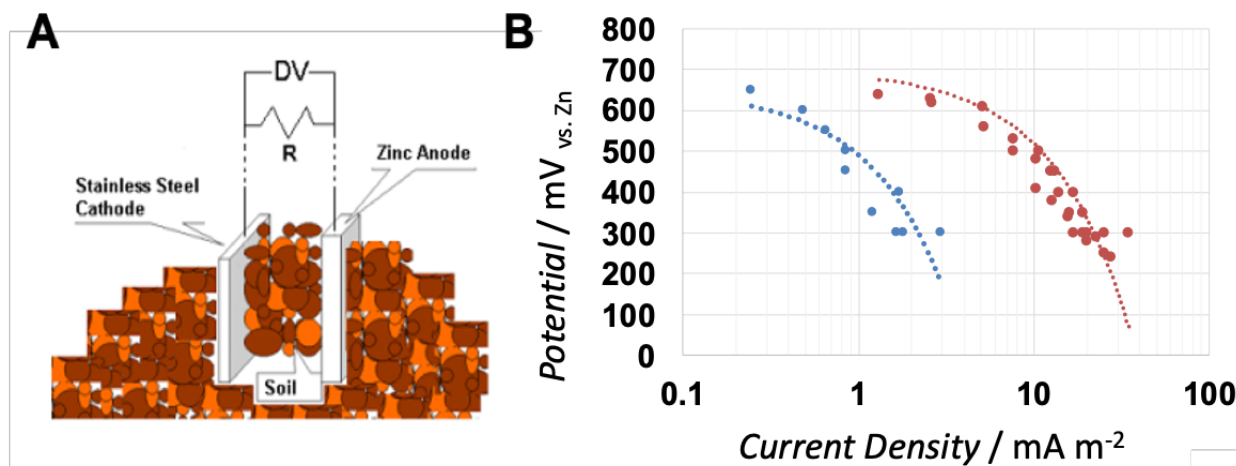
Figure 4. Schematic of soil bioprobe (A) and cathodic polarization curves drove on the stainless-steel working electrode of C10 (cell in sterilized soil) and C6 (cell in biotic soil) from (B). Figure adapted and modified with permission from Ref. [37]. Copyright (2008) Elsevier.

2.3.2 Dissolved oxygen (DO) determination

As mentioned above, the cathode can operate abiotically or with the presence of biofilm (biocathode). Supposing that the final electron acceptor (oxidant) is oxygen, the concentration of oxygen in dissolved or gaseous form should affect the thermodynamic and kinetics of the oxygen reduction reaction (ORR). Very recently, Gonzales Olias et al. [38] have correlated the DO concentration using ceramic-based soil microbial fuel cell (MFC). An increase in the DO concentration in the electrolyte resulted in a positive response of the overall MFC after approximately 3.3 minutes. It is important to underline that the anode biofilm operating mainly in anaerobic/anoxic conditions might be negatively affected by the presence of oxygen. Notably, the MFC design had a ceramic separator that protected the anaerobiosis occurring on the anode [38]. The effects of operating conditions such as temperature, pH, and solution conductivity were also evaluated. Additionally, another DO sensor was reported for measurements of the DO concentration as a function of the depth of the lake [39].

2.3.3 Toxicity at the cathode

Similar to the behavior at the anode, at the cathode (with or without bacteria) toxic compounds might also affect bacterial activity. In parallel, these bacteria are also susceptible to other polluting molecules and/or ions in which the producing electricity decreases due to the death of the electroactive biofilm. Cathodic biosensors have been proposed to broaden the applicability of the traditional heterotrophic anodic sensors to O_2-containing water and autotrophic conditions, without the requirement of organic carbon amendment [40]. At the biocathode, the operating microbial populations promote the reduction of different electron acceptors, (e.g., oxygen) [40]. The release or a sharp increase in the concentration of toxicants leads to reduced metabolic rates of electroactive bacteria and, consequently, to a change in the electric signal output. Recent studies have demonstrated that cathodic biofilms are more sensitive than anodic biofilms with regard to toxicants on both heavy metals (e.g., Hg(II), Cr(VI) or Pb(II)) and organic pollutants (formaldehyde, 2,4-dichlorophenol, benzalkonium chloride) [40-42]. Among the aforementioned studies, Liao et al. and Prévoteau et al. also investigated the composition of microbial communities

at the biocathode, identifying some putative electroautotrophic bacterial populations in the *Proteobacteria* and *Bacteroidetes* phyla.

Concluding remarks and outlook

An overview of MES technologies with various suitable environmental monitoring applications has been provided, and the advantages and disadvantages of MESs are summarized in Table 1 in terms of sensor type, applications, cost, suitability, analytical figures of merit, response times, and reliability. MESs have been developed for water toxicity monitoring and the successful detection of various toxicants, as well as pathogenic microbes. While these sensors offer attractive characteristics, they typically suffer from lower specificity. To overcome this challenge, future work should focus on genetically engineering bacteria to express specific enzymes of interest. Expressing and/or activating preferred metabolic pathways while suppressing unwanted ones has the potential to improve sensor specificity. Additional research is also necessary to evaluate the performances and analytical figures of merit for MESs in complex, biologically relevant environments where the electrode surface is exposed to numerous large molecules that can easily adsorb on the sensor surface, thereby impacting the sensor sensitivity. Future work needs to evaluate the performance of MESs for field monitoring and practical applications. Further studies need to methodically examine not only the preparation but also the operating conditions of sensing elements. In terms of the practical applications of MESs for monitoring water toxicity, future work needs to investigate how toxic stress changes (1) the biochemical responses and (2) the electrochemical signal responses at the electrode-microbe interface. Additionally, biofilm-based sensing elements and their interaction mechanism with electrode surfaces need to be examined further. Specifically, the spatial structure and distribution of biofilms as sensing elements need to be assessed with regard to the availability of biological electron donors and acceptors. Electrochemical engineering strategies could provide a means to improve the availability of biological toxicants and also biofilm composition, thereby resulting in improved sensor signal responses and overall performance.

Table 1. Summary of published work on microbial electrochemical sensing technologies examined and overviewed in this minireview.

Microbial Electrochemical Sensor Type	Application	Cost	Suitability	Analytical Figures of Merit	Response Times	Reliability	Ref.
Array of three MFCs linked hydraulically in series	Online sensor for BOD and COD measurements	Low cost	Correlates organic matter concentration in aqueous medium	Linear response up to 720 mg L^{-1} BOD_5 (1175 mg L^{-1} COD) with R^2 of 97%	5 days for BOD and 2.3 hours for COD	Stable current for reliable detection and quantification	[8]
MFC sensor in transient mode	Water monitoring, including organic matter and toxicity determination	Low cost	Good performance with high sensitivity for organic matter, acidic toxicity and heavy metal (Cu^{2+}) detection	Increased sensitivity by 50% to 81% for organic matter, 65% to 183% for acidic toxicity and 213% to 247% for heavy metal toxicity	Not reported	Performance needs to be evaluated in actual bodies of water where sensor is exposed to complex environmental parameters to evaluate reliability	[9]
Paper MFC based on screen-printed carbon electrodes onto single paper sheet	Rapid onsite shock sensor for bioactive formaldehyde in water	Cost-effective	Response to 0.1% v/v formaldehyde added	Rates of current decay of 0.011 µA min^{-1} for pMFC and 0.021 µA min^{-1} for fpMFC	165 min for pMFC and 200 min for fpMFC	Sensor performance might be susceptible to environmental factors, such as temperature, pH and conductivity	[10]
Microbial bioelectrochemical with *Escherichia coli* and supplemented redox mediator 2-HNQ, using functionalized CNT-SPE modified with –COOH and -NH₂ functional groups	Environmental Monitoring Applications	Not reported	Suitable for environmental sensing and detection of volatile organic contaminants	LOD of 0.4 mg L^{-1} for CHP	Not reported	Good reliability and reproducibility	[11]
Flat MMFC by compacting two filter membranes with carbon ink	"On-line sticker sensor" for real time *in situ* monitoring of wastewater quality	Not reported	Suitable for wastewater quality monitoring under shocks of toxic metals, Cr^{6+} and Ni^{2+}	Voltage of MMFC correlated with shock concentrations	Not reported	Exhibited good reliability, reusability and high stability of voltage signals	[12]
Portable self-powered microbial electrochemical sensor using a bio-inspired polymer mediating system	Online monitoring of Cr(VI); assessment of water quality	Cost-effective	Suitable for monitoring Cr(VI)	Linear range 4–18.5 4 mg L^{-1} ($R^2 = 0.983$); LOD of 2.4 mg L^{-1} Cr(VI); sensitivity of 0.31 ± 0.02 µA cm^{-2} mgCr(VI)$^{-1}$ L	Not reported	Reliability needs to be evaluated in real water samples	[13]
PMMFC integrated with power management system	"Disposable self-support shock sensor" for real time in situ monitoring of wastewater quality	Super low cost	Suitable to chromium, hypochlorite and acetate shocks in a batch-mode chamber	High signal sensitivity to a wide range of shocks of Cr^{6+}, NaClO and NaAc	Not reported	Good reliability for wastewater monitoring, long-term performance needs optimization	[14]

Microbial amperometric biosensor based on cyanobacteria *Anabaena variabilis*, carbon felt electrodes, alginate as entrapping polymer and BQ as redox mediator	Online herbicide detection through inhibition of generated photocurrent	Cost-effective	Suitable for detection of atrazine and diuron as model photosynthesis-inhibiting herbicides	Sensitivity of $-24.6\ \mu A\ \mu M^{-1}\ cm^{-2}$ towards atrazine up to 0.56 µM; turn on/off detection for strong inhibitor diuron; With encapsulation of BQ, sensitivity of $-7.7\ \mu A\ \mu M^{-1}\ cm^{-2}$ towards atrazine up to 131 µM (lower limit of detection 64 nM)	~20 min	Good reliability for environmental analysis; long-term operational stability and selectivity to class of contaminants needs to be evaluated	[15]
Carbon ultramicroelectrode arrays	Detection of pathogenic bacteria via electrochemical detection of redox-active phenazine metabolites	Low-cost	Suitable for detection *of Pseudomonas aeruginosa* phenazine metabolites (pyocyanin) produced during early infection stages	LOD of 1.0 µM and linear dynamic range of 1–250 µM for pyocyanin	105 µs	Great reliability for *P. aeruginosa* pathogen detection in complex biological media, synthetic cystic fibrosis media and wound fluid stimulant	[23] [24] [25] [27]
Inkjet-Printed Carbon Nanotube Electrodes	Detection of pathogenic bacteria via electrochemical detection of redox-active bacterial toxin pyocyanin	Low-cost	Suitable for detection *of P. aeruginosa* toxin pyocyanin	Linear range 0.10–100 µmol/L and LOD of 0.10 µmol/L for pyocyanin	Not reported	Good reliability for sensing in wound-like environment and growth media	[26]
Simple implementation of BIOX probe	On-line monitoring of biofilm growth in soli	Not reported	Suitable for monitoring development of biofilm in soil matrix	N/A	Not reported	Good reliability when analyzed with humidity and nutrient factors of soil	[37]
Ceramic soil MFC	Continuous, in situ monitoring of dissolved oxygen in water	Affordable	Sensor signal in terms of output voltage correlates with dissolved oxygen	Maximum voltage output of 321 ± 29 mV with a sensitivity in the linear range of 53.3 ± 22.6 mV L mg^{-1}	Not reported	Good reliability, sensor response mainly affected by temperature	[38]
Multi-cathode, single-anode system integrating a sediment microbial fuel cell -based biosensor	In-situ, continuous, and online monitoring of dissolved oxygen concentrations along various depths of lake water	Affordable	Signal feedback mechanism based on relationship between voltage and dissolved oxygen concentration at corresponding depths	Linear range of 0-9 mg L^{-1} for dissolved oxygen	Not reported	Good reliability and long-term performance during a 67-day period in a lake environment	[39]
Gas diffusion-biocathode MFC sensor	Toxicity monitoring in both aerobic and anaerobic water bodies	Low-cost	Suitable for detection of formaldehyde and monitoring air pollution	LOD of 20 ppm for formaldehyde	Not reported	Good reliability for formaldehyde detection and as a generic biosensor for monitoring gaseous pollutants	[42]

Table acronyms*:* MFC – microbial fuel cell, BOD − biological oxygen demand, COD – chemical oxygen demand, pMFC – paper-based microbial fuel cell, fpMFC – folded paper-based microbial fuel cell, CHP – 1-cyclohexyl-2-pyrrolidone, 2-HNQ – 2-hydroxy-1,4-naphthoquinone, CNT-SPE – carbon nanotube-coated screen-printed electrode; MMFC – membrane-based microbial fuel cell; PMMFC – paper-based multi-anode microbial fuel cell; NaAc – sodium acetate; BQ – p-benzoquinone, LOD – limit of detection

Declaration of competing interest

The authors declare no competing interests that could have appeared to influence the work reported in this paper.

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