Biosensors and Bioelectronics 173 (2021) 112822

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

An electroactive biofilm-based biosensor for water safety: Pollutants detection and early-warning

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ARTICLE INFO

Keywords:
Electroactive biofilm
Comprehensive biosensor
Water quality
BOD detection
Early-warning

ABSTRACT

Besides serving in wastewater treatment and energy generation fields, electroactive biofilm (EAB) has been employed as a sensitive bio-elements in a biosensor to monitor water quality by delivering electrical signals without additional mediators. Increasing studies have applied EAB-based biosensor in specific pollutant detection, typically biochemical oxygen demand (BOD) detection, as well as in early-warning of composite pollutants. Based on a comprehensive review of literatures, this study reveals how EAB outputs electrical signal, how we can evaluate and improve this performance, and what information we can expect from EAB-based biosensor. Since BOD detection and early-warning are normally confusing, this study manages to differentiate these two applications through distinguished purposes and metrics. Based on the introductions of progresses and applications of EAB-based biosensors so far, several novel strategies toward the future development of EAB-based biosensors are proposed.

1. Introduction of EAB-based biosensor

Water quality monitoring technology based on biosensor has emerged as one of the essential measures to ensure the safety of water supply and ecological environment (Ejeian et al., 2018). Biosensor is a device for qualitative or quantitative measurement of target analytes by employing some biological recognition elements, e.g. nucleic acids, antibodies, enzymes/proteins, organelles, microorganisms, tissue, etc., and integrated into a transduction system (Antonacci and Scognamiglio, 2020). By employing certain biological recognition elements, the biosensor can qualitatively or quantitatively measure pollutants in water supply, including biochemical oxygen demand (BOD), heavy metal, organic toxicants, and even the nitrate, and generate electrical, optical, thermometric, magnetic, micromechanical or respirometric signals (Parkhey and Mohan, 2019). In practical, different biosensors have been widely used as promising tools to quantitatively detect the concentration of target analytes or to deliver comprehensive early-warning, depending on the requirements of water quality monitoring (Fig. 1A). When the biosensors are employed to quantify the concentration of specific pollutants, the bio-elements (e.g. nucleic acids, antibodies, enzymes) must recognize targeting pollutants by a physical-chemical reaction, and should be high-sensitive and selective. Previous studies have reviewed this process and investigated the factors on biosensors working with wastewater and fresh water (Biswas et al., 2017; Ejeian et al., 2018). Another application of biosensor known as early-warning is to warn composite pollutants and aims to provide a quick and accurate alert for any possible pollutant that presents in the monitored water influent. The bio-elements employed in early warning biosensors are microorganisms, including bacteria, microalgae, yeast and fungi (Antonacci and Scognamiglio, 2020; Ejeian et al., 2018; Hassan et al., 2016). Bacteria can also be used to detect the concentration of a class of pollutants in special circumstances, such as BOD. Among all microorganisms that serve as sensing elements, luminescent bacteria and electroactive bacteria are believed two most important

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https://doi.org/10.1016/j.bios.2020.112822
Received 10 September 2020; Received in revised form 9 November 2020; Accepted 12 November 2020
Available online 17 November 2020

ones as they can spontaneously generate detectable fluorescence and electricity as warning signals without any additional chemical-mediators. Biosensors with luminescent bacteria have been used to monitor the biotoxicity of eco-environment for decades, and several related international standards (ISO 11348) have been established since 1998 (Abbas et al., 2018). However, luminescent bacteria are normally presented as suspensions and are not adapted for real-time online water quality monitoring. In contrast, biosensors with electroactive biofilm (EAB) formed by electroactive bacteria are capable of delivering online early warning.

Microbial electrochemistry and technology (MET) that link EAB metabolism to an electrochemical system originated in 20th century. In 1999, researchers for the first time reported direct current generation (without any exogenous mediators) by *Shewanella putrefaciens* in a biosensor (Kim et al., 1999). With increasing research interests and more industrial applications, MET related technologies, such as microbial fuel cell (MFC), microbial electrolysis cell (MEC), microbial desalination cell (MDC), and microbial electrochemical synthesis (MES), have been experiencing rapid development since last century (Fig. 1B). They not only greatly facilitate electricity and biofuel production from wastewater treatment, but also are readily configurations (except MES) for biosensors to monitor water quality (Adekunle et al., 2019a; Jiang et al., 2018b; Jin et al., 2016). Among more than 140 pieces of EAB-based-biosensor-related research articles published during the last two decades, most of them were focused on detecting the toxicity and organic matters (Fig. 1C). Biochemical oxygen demand (BOD) and toxicity biosensors based on MFC were invented by Dr. Kim and colleagues between 1999 and 2007 (Chang et al., 2004, 2005; Kim et al., 1999, 2003a, 2003b, 2006, 2007; Moon et al., 2004, 2005), while Korbi company (Korea) released commercially available MFC biosensors during this period. Later, more studies on EAB-based biosensors followed up towards improved stability and sensitivity (Davila et al., 2011; Di Lorenzo et al., 2009; Jin et al., 2016; Modin and Wilen, 2012; Prevoteau and Rabaey, 2017; Qi et al., 2020; Stein et al., 2010, 2011).

An EAB-based biosensor uses whole cells as sensing element and is a typical early-warning biosensor for water quality which delivers little selectivity but timely alert for composite pollutants (Fig. 1D). Although it can directly detect BOD concentration in the polluted water based on bioelectrochemical conversion relationship between organic matter (e.g. glucose, acetate, and volatile fatty acids-VFAs) and electrons (Abrevaya et al., 2015a), it is unable to quantify the accurate concentration of one particular toxicity as there is no a quantitative relationship between the toxic substances and transferred charge. As an early-warning device, EAB-based biosensor can realize real-time online alert for comprehensive water environmental contaminants, including BOD, heavy metal, organic toxicants, and even the nitrate (Abrevaya et al., 2015b; Jiang et al., 2018b). Previous literatures have investigated the performance of biosensor towards different target analytes, including linear range, response time, etc. (Do et al., 2020; Jiang et al., 2018b; Sevda et al., 2020). Based on a comprehensive review of more than 140 literatures mostly related to EAB-based biosensor, this study focuses on electroactive biofilm where sensing and early-warning performances originate, and manages to reveal what we can expect from it. We will show how EAB output electrical signals and its relationship with target analytes, followed by an introduction of recent advances in EAB-based biosensors, and an outlook for this technology. Also in order to address a normal

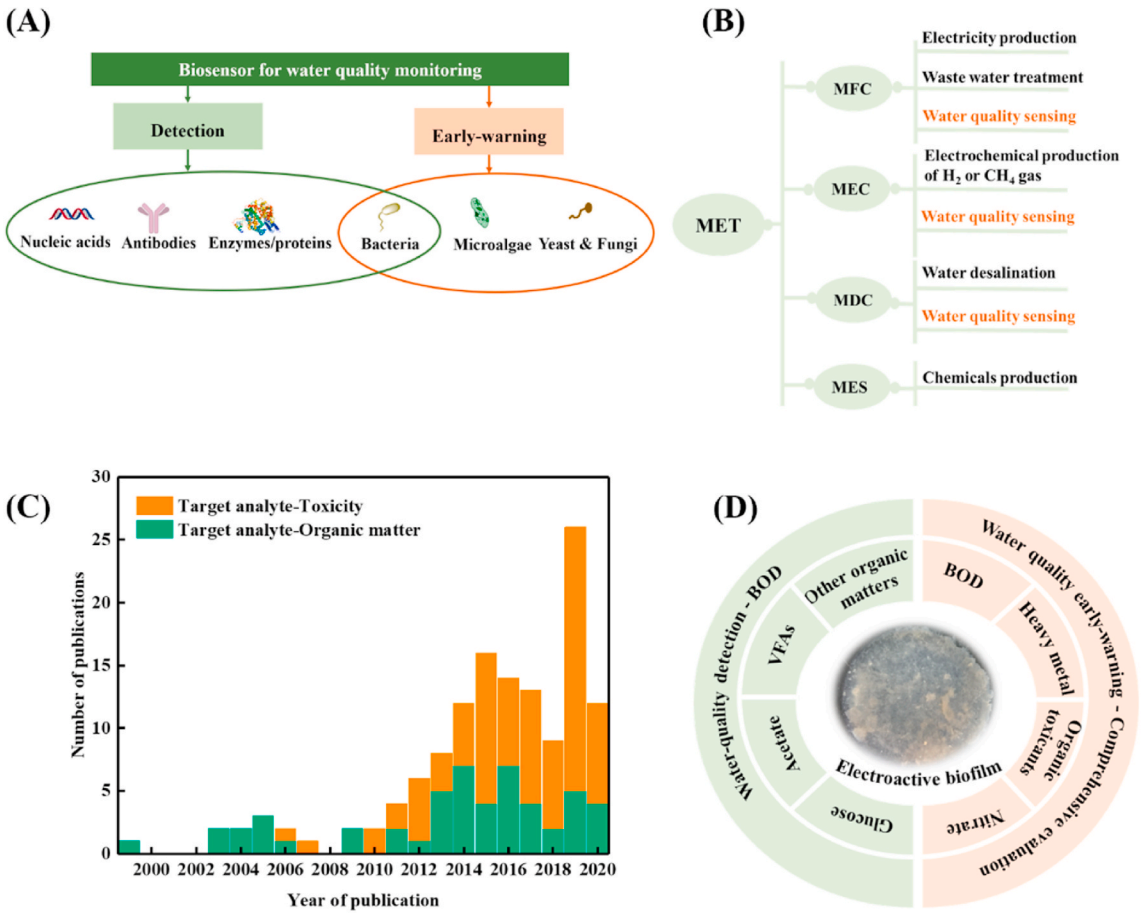

Fig. 1. An overall view EAB-based biosensor. (A) Different bio-elements in biosensor and their functions for water quality monitoring; (B) branches of microbial electrochemistry and technology (MET); (C) publications of EAB-based biosensor (key word: "microbial fuel cell biosensor" and "bioelectrochemical biosensor" and "microbial electrochemical biosensor") in last two decades from Web of Science; (D) EAB serves the biosensor.

confuse of BOD detection and early-warning, this study tries to differentiate these two applications of EAB-based biosensor through their distinguished purposes and metrics.

2. Electrical signal output of EAB

2.1. Electrons generation by EAB

The extracellular electron transfer (EET) of EAB closely relates to their metabolism and energy production. Electrons flowing to the dissimilatory respiration are finally observed as the "current signal", which accompanies with the synthesis of adenosine triphosphate (ATP) to support biological activity. This is the fundamental of EAB-based biosensors. Based on a phylogenetic analysis of the electroactive microorganisms that have been applied in MET, a recent research concluded that *Shewanella oneidensis* and *Geobacter sulfurreducens* were two model genuses to serve MET related devices (Logan et al., 2019). Organic matters (e.g. acetate, lactate) enter the cell as an electron donor and then be metabolized to release nicotinamide adenine dinucleotide (NADH), which would react with the enzymes and further release

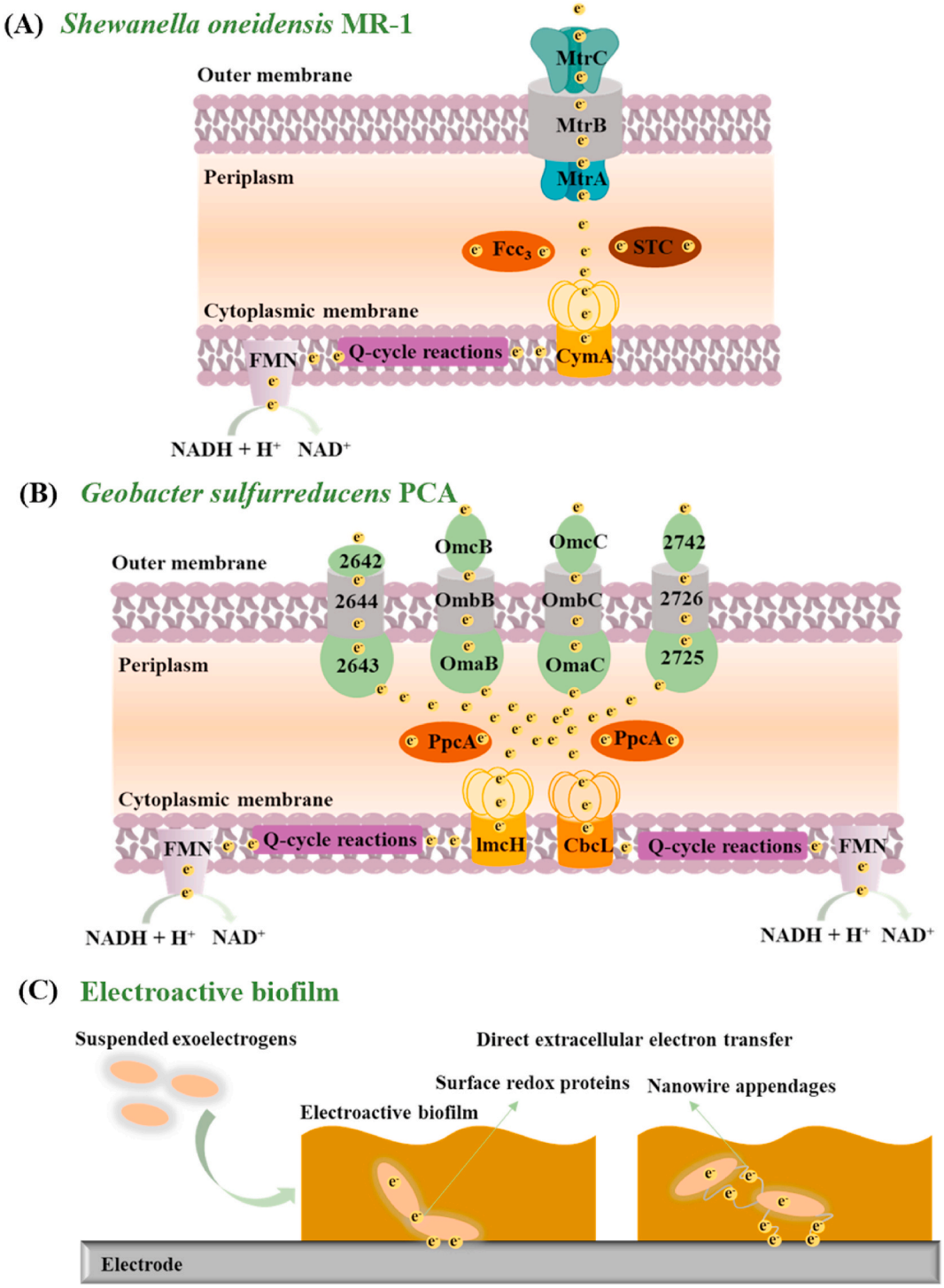

Fig. 2. The pathways from electron donor to acceptor in EAB. (A, B) Substance metabolism and extracellular electron transfer (EET) pathways of *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* PCA; (C) The formation of electroactive biofilm and direct extracellular electron transfer (DEET).

electrons that would go into a quinone pool. Finally, the extracellular electron transfer (EET) pathways would transfer electrons from quinone pool to extracellular electron receptors. Genetic studies of *Shewanella oneidensis* MR-1 suggested that six *c*-cytochromes (haem) involved in metal-reducing (Mtr) pathway-MtrC, MtrB, MtrA, Fcc_3, STC and CymA (Fig. 2A) (Shi et al., 2016). Compared with *Shewanella oneidensis* MR-1, *Geobacter sulfurreducens* PCA had a more complicated EET process involving porin–cytochrome (Pcc) pathways, which contain lmcH, CbcL, PpcA, 2642, 2644, 2643, OmcB, OmbB, OmaB, OmcC, OmbC, OmaC, 2742, 2726 and 2725 from genetic studies (Fig. 2B) (Shi et al., 2016).

Unlike luminescent bacteria that are mostly presented in the form of suspension, the exoelectrogens can form a biofilm (EAB as previously mentioned), which uses extracellular polymeric substance (EPS) to attach on the electrode surface. A mature EAB enables direct EET within the biofilm because it has surface redox proteins (e.g. cytochromes, flavins) and nanowire appendages (Fig. 2C) (Mccormick et al., 2015). Researchers found that nanowire appendages were conductive, but the whole electron transfer process needs further studies (Wang et al., 2019b). In conclusion, EAB can directly transfer electrons to terminal electron acceptor (e.g. electrode).

2.2. Sensitive elements and configurations of EAB-based biosensors

Being the sensitive element, EAB can serve as both bioanode and biocathode to monitor the water quality (Fig. 3A). So far, most studies have employed the bioanode as sensitive element, where EAB forms on the anode (bioanode) and organic carbon as the electron donor that was oxidated by EAB to produce electrons, carbon dioxide and protons in the bioanode. Once toxicants enter into bioanode, the electrons-producing process will be inhibited and early-warning for users. As previously mentioned, some chemicals (toxic substances) can be monitored by EAB-based biosensors, but this process is easily interfered by organic matters concentrations. It happens that when the influent contains both organic matters and toxic substances, the sensor may deliver a false-negative signal. This is why BOD and toxicity needs to be examined separately when the bioanode serves as the sensitive element. Being another way to avoid false-negative, some researchers employed biocathode as the sensitive element (Jiang et al., 2018a). With EAB on the cathode

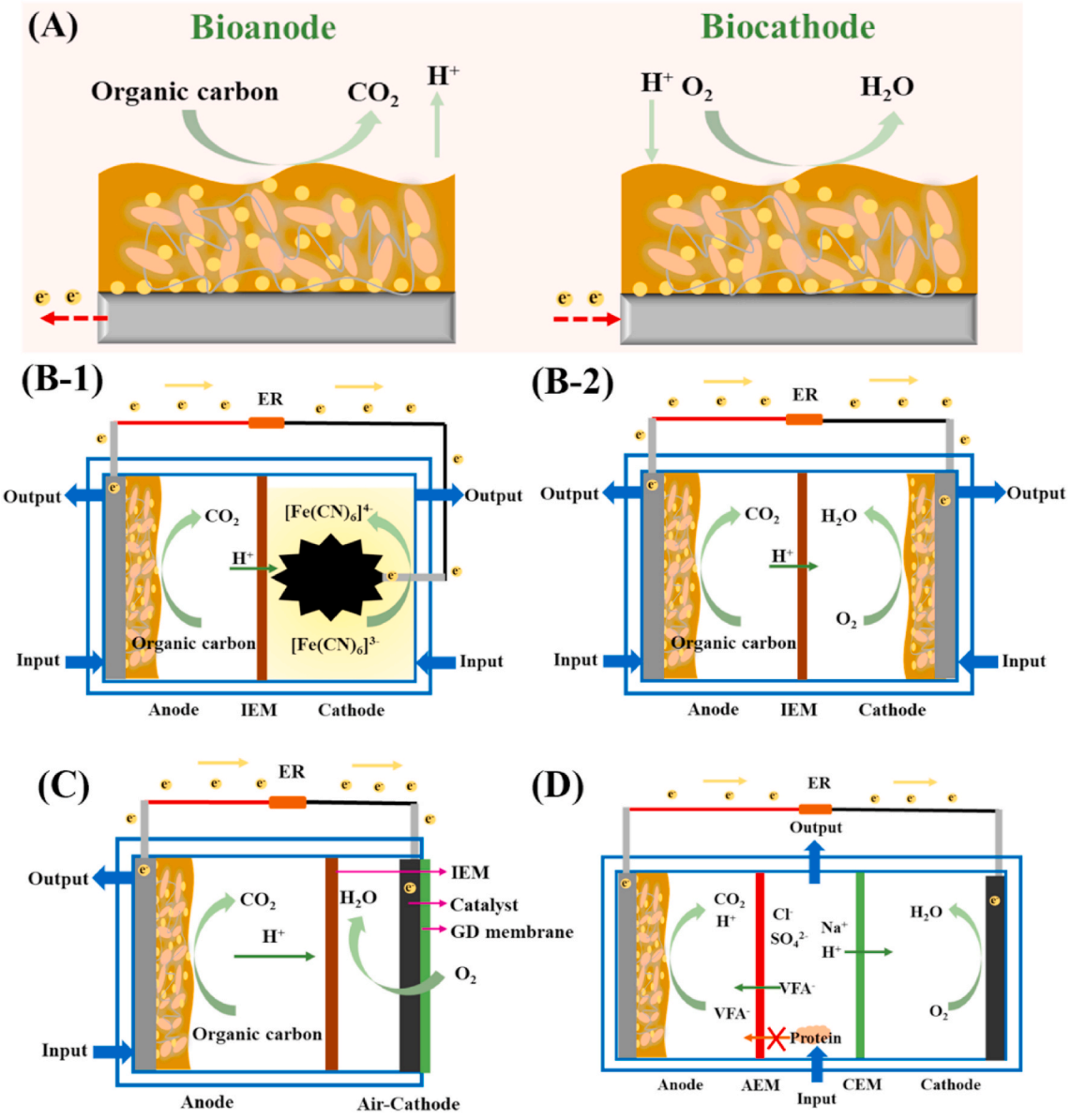

Fig. 3. Sensitive Elements and major configurations of EAB-based biosensor. (A) Bioanode and biocathode as sensitive elements; (B) double-chambers MFC as biosensor with chemical cathode (B-1) and biocathode (B-2); (C) single-chamber MFC as biosensor with air-cathode and (D) three-chambers MDC as biosensor.

(biocathode) and oxygen as electron acceptor, the biocathode can use electrons and protons to produce the water. The sensing process of biocathode depends on electro-autotrophic bacteria in EAB which (e.g. *Shewanella oneidensis* MR-1) have a bidirectional electron transport pathway (Jiang et al., 2017; Liao et al., 2018a, 2018b). Toxicants presented in biocathode would inhibit the electrons-accepting process and trigger early-warning. The sensing process was found unaffected by organic matters any more but still blocked by the fluctuation of dissolved oxygen (Jiang et al., 2018a). A recent study even suggested that without a membrane, both bioanode and biocathode could be used as sensitive elements to provide early-warning of toxicity (Zhao et al., 2019).

In order to address various practical requirements for water quality monitoring, three main configurations of EAB-based biosensors have been proposed including double-chambers, single-chamber and three-chambers. Double-chambers MFC with an ion exchange membrane (IEM), normally proton exchange membrane (PEM) and cation exchange membrane (CEM), is the most commonly used device of EAB-based biosensor. Its cathode can be either chemical (Figs. 3B-1) or biological (Figs. 3B-2). Single-chamber MFC with air-cathode (chemical catalysts) has a reduced size and increased electron transfer efficiency, as compared to other two configurations (Di Lorenzo et al., 2014) (Fig. 3C). A three-chambers MDC with anion exchange membrane (ACE) was found to deliver wider detection limit and achieve effective separation of large molecules like the protein and fat from interfering the sensing process when it was employed to detect volatile fatty acid (VFA) (Jin et al., 2016) (Fig. 3D).

3. Relationship between electrical signal and target analyte

3.1. BOD detection and early-warning

The traditional BOD detection aims to determine the amount of dissolved oxygen (DO) consumed by microbes in the biological metabolizing process of biodegradable organic matter (BOM) from water environment (Table 1). This is a time-consuming process (5 days, also called BOD_5) and delivers low repeatability. Researchers found that certain luminescent bacteria could also be employed to monitor BOD in a shorter period of processing (Table 1) (Sakaguchi et al., 2007). But the available species were very limited. Even though pure luminescent bacteria can be cultured and enriched into large amount, they do not have a wide utilization capacity for different types of organic matter due to its own metabolic pathways.

Electroactive biofilm (EAB) formed by exoelectrogens is also capable of monitoring the BOD, as BOM in the anode will serve as electron donor to generate electrons and O_2 in the cathode will work as electron acceptor to produce H_2O (Table 1) (Liu et al., 2018b). However, it should be noted that there is no quantitative relationship between the toxic substances and electron generation, which suggests that the concentration of specific substance is not yet determined.

Besides BOD detection, EAB-based biosensor greatly facilitates water quality early-warning technologies. Researchers have noticed that normal metabolism of electroactive bacteria can generate a stable electrical signal, regarding as a baseline. Then according to the requirement of water quality, a threshold showing a range around the baseline will be determined. When the EAB suffers from target analytes (e.g. heavy metal, organic toxicants) shock, a signal fluctuation from the baseline will arise to show the toxicity. A final alert will be made only when the fluctuation exceeds the threshold.

Aiming to set off timely and accurate alert for incoming toxicity, water quality early-warning devices provides the engineers of related industrial applications with important information to cut the potential risk of pollutants in water supply. And it is most likely that further examinations are required to identify the pollutants and to determine the concentrations afterwards. The sensitivity is an essential metric for early-warning technologies, which describes the effect of target analytes on current signal under real-time dynamic conditions. It is worth noting that early-warning process involves little selectivity or concentration issues.

Table 1
Reaction equations of different microbes as sensitive elements for BOD detection.

Sensitive elements	Reaction equations
Aerobic bacteria	$BOM + O_2 \xrightarrow{\text{Aerobic bacteria}} T_P + CO_2 + H_2O$
Luminescent bacteria	$BOM + O_2 \xrightarrow{\text{Luminescent bacteria}} T_P + CO_2 + H_2O$
EAB	Anode: $BOM + H_2O \xrightarrow{\text{Electroactive bacteria}} T_P + CO_2 + H^+ + e^-$
	Cathode: $O_2 + H^+ + e^- \xrightarrow[\text{Electroactive bacteria}]{\text{Catalyst}} H_2O$
	MFC: $BOM + O_2 \xrightarrow{\text{Catalyst}} T_P + CO_2 + H_2O$

Note: Where T_p is the final biodegradation products of BOM.

3.2. Kinetic model and response time

Derived from enzymatic kinetic model (Monod model) and electrochemical kinetic model (Butler–Volmer model), the Butler–Volmer–Monod (BVM) model has been employed to describe the relationship between substance concentration and electric current, in which BOD (namely BOM_Q in related literature) can be accurately calculated based on coulombic yield (Hamelers et al., 2011) (Table 2). Another study showed that BOD obtained by this model was consistent with traditional BOD_5 by employing a biosensor (Liu et al., 2018b). Some other modelling studies based on BVM model managed to describe the effects of toxic substances with different properties on electron transfer process and to simulate the responding process (Stein et al., 2011, 2012a, 2012b; Su et al., 2019) (Table 2). But the time period of the process was neglected. To simulate the real-time dynamic early-warning process, an empirical model was proposed by fitting experimental data (Wang et al., 2013) (Table 2). It provided a simple review on how current signals changed over the time under toxic shock. A recent work on the mass transfer of toxic substances in biofilms proposed a dynamic model to understand the early-warning process with a visible result (Qi et al., 2020) (Table 2).

Several studies have noticed that the detection time of BOD with EAB-based biosensor varies from 5 to 20 h, depending on different target analytes, microbial community structure of EAB, and electrode materials (Corbella et al., 2019; Gao et al., 2020; Kim et al., 2003a; Modin and Wilen, 2012) (Fig. 4). As mentioned previously, early-warning by EAB-based biosensor aims to deliver timely and accurate alert for pollutants. With significant efforts of many studies, the response time of early-warning can be as short as less than 3 h (Chang et al., 2004; Chen et al., 2016; Chouler et al., 2018; Khan et al., 2020; Li et al., 2020a; Liao et al., 2018b; Lu et al., 2020; Moon et al., 2004; Patil et al., 2010; Prevoteau et al., 2019; Qi et al., 2019; Yang et al., 2018; Yi et al., 2019; Yu et al., 2017, 2020; Zeng et al., 2017; Zhang and Angelidaki, 2011; Zhao et al., 2019) (Fig. 4). The early-warning for BOD would take longer time period than heavy metals and organic toxicants, because BOM is an electron donor and not a toxic substance. Obviously, it requires much more time to detect BOD than to perform early-warning regardless of the targeting pollutant, because early-warning is the dynamic change of current with the time, while BOD detection is an integral of current in a time period.

4. Performance advances in EAB-based biosensors

4.1. Recent progresses for BOD detection

In order to shorten the duration of BOD detection, a recent study managed to determine partial coulombic yield at the point of a

Table 2
Electrical signal analysis and kinetic model for BOD detection and early-warning.

Items	BOD detection	Early-warning
Electrical signal analysis	$BOD = BOM_Q = (8000 \times \int_0^t Idt)/(FV)$ Where t is the detection duration; I is the current; F is the Faraday constant; V is the volume of solution.	$Sensitivity = \Delta I/(\Delta C \times A \times \Delta t)$ Where ΔI is the current change; ΔC is the change in toxicant concentration; A is the surface area of electrode; Δt is response time.
Kinetic model	**Monod model** $\mu = \mu_{max}\frac{S}{K_s + S}$ Where μ is the growth rate of microorganisms; μ_{max} is the maximum growth rate of microorganisms; S is the concentration of limiting substrate for growth; K_s is the value of S when $\mu/\mu_{max} = 0.5$. **Butler–Volmer model** $i = i_0\left\{\exp\left[\frac{(1-\alpha)nF}{RT}(E_{Anode} - E_{eq})\right] - \exp\left[\frac{-\alpha nF}{RT}(E_{Anode} - E_{eq})\right]\right\}$ Where i is current density; i_0 is exchange current density; α is charge transfer coefficient; n is number of electrons involved in the electrode reaction; F is Faraday constant; R is universal gas constant; T is temperature; E_{Anode} is anode potential; E_{eq} is equilibrium potential. **Butler–Volmer–Monod (BVM) model** $f = \frac{F}{RT}$ $\eta = E_{Anode} - E_{S/P}$ $I = I_{max}\frac{1-\exp(-f\eta)}{k_1\cdot\exp(-(1-\alpha)f\eta) + k_2\cdot\exp(-f\eta) + \left(\frac{K_s}{S}\right) + 1}$ Where I is current; I_{max} is the maximum current determined by maximum enzymatic rates of microorganisms; F is Faraday constant; R is universal gas constant; T is temperature; E_{Anode} is anode potential; $E_{s/p}$ is thermodynamical anode potential; α is charge transfer coefficient; K_1 and K_2 are two lumped parameters (the former is a ratio constant between biochemical rate constant and electrochemical rate constant, and the latter is a ratio constant between the forward reaction rate constant and backward reaction rate constant).	**Toxicity-BVM model** (1) When there is an noncompetitive toxicant (e.g. Cu^{2+}), $I = I_{max}\frac{1-\exp(-f\eta)}{k_1\cdot\exp(-(1-\alpha)f\eta) + k_2\cdot\exp(-f\eta) + \left(\frac{K_s}{S}\right) + 1}\bullet\frac{K_i}{K_i + C_i}$ (2) When the toxicant is an electron acceptor (e.g. NO_3^-), $I = I_{max}\frac{1-\exp(-f\eta)}{k_1\cdot\frac{K_i}{K_i + C_i}\cdot\exp(-(1-\alpha)f\eta) + k_2\cdot\exp(-f\eta) + \left(\frac{K_s}{S}\right) + 1}$ (3) When the toxicant is an acid, $I = I_{max}\frac{1-\exp(-f\eta)}{k_1\cdot\exp(-(1-\alpha)f\eta) + k_2\cdot\frac{K_i}{K_i + C_i}\cdot\exp(-f\eta) + \left(\frac{K_s}{S}\right) + 1}$ (4) When there is a competitive toxicant (e.g. formaldehyde) $I = I_{max}\frac{1-\exp(-f\eta)}{k_1\cdot\exp(-(1-\alpha)f\eta) + k_2\cdot\exp(-f\eta) + \left(\frac{K_s}{S}\right)\cdot\frac{K_i}{K_i + C_i} + 1}$ Where C_i is the concentration of toxicant; K_i is the affinity constant of toxicant. **Toxicity-Experience model** $I = \alpha \times e^{-bt/3600}$ Where α is the magnification factor; b is the toxic factor; t (s) is the contact time after toxicant shock; 3600 s/h is the scaling factor. **Toxicity-Mass transfer model** $IR_{X-t} = \frac{X(t)}{X} \times 100\% = \frac{erfinv\left(1 - \frac{C_{TV}}{C_0}\right) \times 2 \times \sqrt{D^* t/R_f}}{X} \times 100\%$ $IR_{X-t} \approx \frac{I(t)}{I} \times 100\%$ Where IR_{X-t} is the inhibition ratio of biofilm thickness; X is biofilm thickness; $X(t)$ is the inhibition biofilm thickness at t time; C_{TV} is the relative threshold value of toxicant concentration of biofilm inhibition; C_0 is the initial concentration; D^* is an effective diffusion coefficient of toxicant in biofilm; R_f is the restrictive factor.

Note: These models come from previous reports (Hamelers et al., 2011; Ortiz-Martínez et al., 2015; Qi et al., 2020; Stein et al., 2011; Wang et al., 2013).

maximum current drop rather than coulombic yield employed in traditional method (Gao et al., 2020). This progress of detection is based on an understanding that the endogenous respiration of electroactive bacteria can generate extra electrons at the last stage of BOD detection, which affects the accuracy of detection directly. It is notable that non-toxic domestic wastewater also contains nitrate, which would serve as electron acceptor to compete with the electrode and thus affect the accuracy of BOD detection. To address this, some denitrification inhibitors (e.g. sodium azide) would be added into the biofilm (Chang et al., 2005). However, these inhibitors are toxic and presents extra risk to the environment. A possible alternative is to make proper correction on current signals (Guo and Liu, 2020). In addition, dual detection of BOD and nitrate with *Shewanella loihica* biofilm can be realized by regulating the electrode potentials, as the potentials for organic matter oxidation and nitrate reduction are known to be distinct (Yi et al., 2020b). Besides the nitrate, some macromolecules (e.g. protein and lipid) may influence the detection of VFA in the anaerobic digestion process, which could be well addressed by employing an IEM membrane (Jiang et al., 2019; Jin et al., 2016, 2017).

4.2. Improving the sensitivity for early-warning

Various factors will influence the sensitivity of EAB-based biosensors, most of which lie in the major components of the device, i.e., bulk solution, boundary layer, biofilm and electrode (Fig. 5), and two important interfaces for mass and electron transfer (one exists between bulk solution and biofilm, and the other is between biofilm and electrode).

4.2.1. Mass transfer in bulk solution and boundary layer

Increasing the mass transfer on biofilm surface will greatly improve the sensitivity. By simply regulating the flow and shear rate while optimizing the biofilm structure like EPS, porosity, and density, the mass transfer was stimulated, leading to increased sensitivity (Shen et al., 2013). Employing flow-through electrodes could also facilitate the mass transfer and improve the sensitivity as compared with flow-by electrodes (Jiang et al., 2015).

It is generally believed that the EAB-based biosensor with smaller size had an increased ratio of biofilm area over bulk solution volume, thus would benefit the mass transfer (Ahn and Schröder, 2014; Davila et al., 2011; Di Lorenzo et al., 2009; Lee et al., 2015). In the same time, a fluid dynamics model using Fluent software was employed in some studies to help pick out materials for proper configuration design (Quaglio et al., 2018; Yi et al., 2020a). These efforts have greatly reduced the size of the biosensor reactor from centimeter to micron level (Fig. 6A). Although the micron-level reactor have shown advantages in sensitivity and integrated design (Chouler et al., 2018; Xiao et al., 2020a, 2020b, 2020c), pretreatments of the influent are required to exclude large particles from damaging the biosensor when it is applied to monitor real wastewater warning.

In recent years, researchers have introduced microfluidic into EAB studies to further facilitate fluid performance (Fig. 6B) (Li et al., 2016; Yang et al., 2016). The main reason is that laminar flow involved in microfluidic is contributed to reduce the thickness of boundary layer. Yet just a few studies have followed up on this topic during this decade (Fig. 6C), the coupled technology with microfluidic and EAB biosensor may have more potential applications in the near future, with the progress of microfluidic related studies towards high-speed, low-consumption, miniaturization and automation.

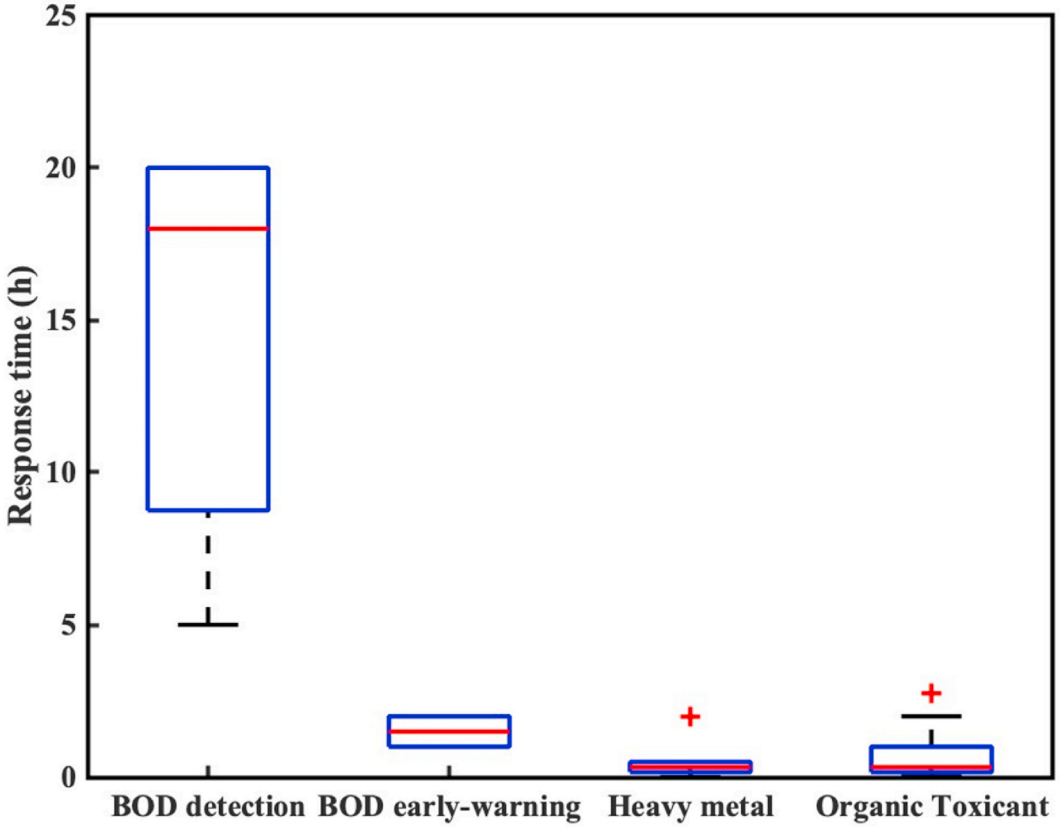

Fig. 4. Response time of BOD detection and early-warning using EAB-based biosensor. All data were obtained from literatures listed in manuscript.

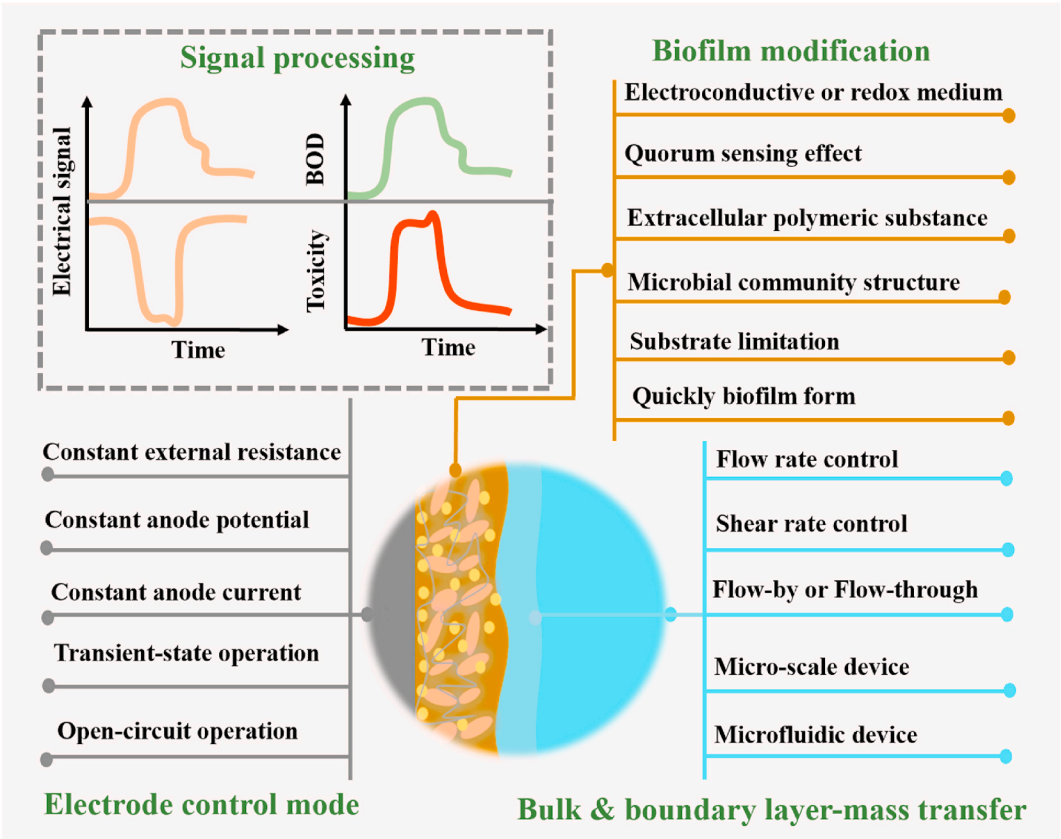

Fig. 5. Recent progresses of major components of EAB-based biosensor for improved early-warning performance.

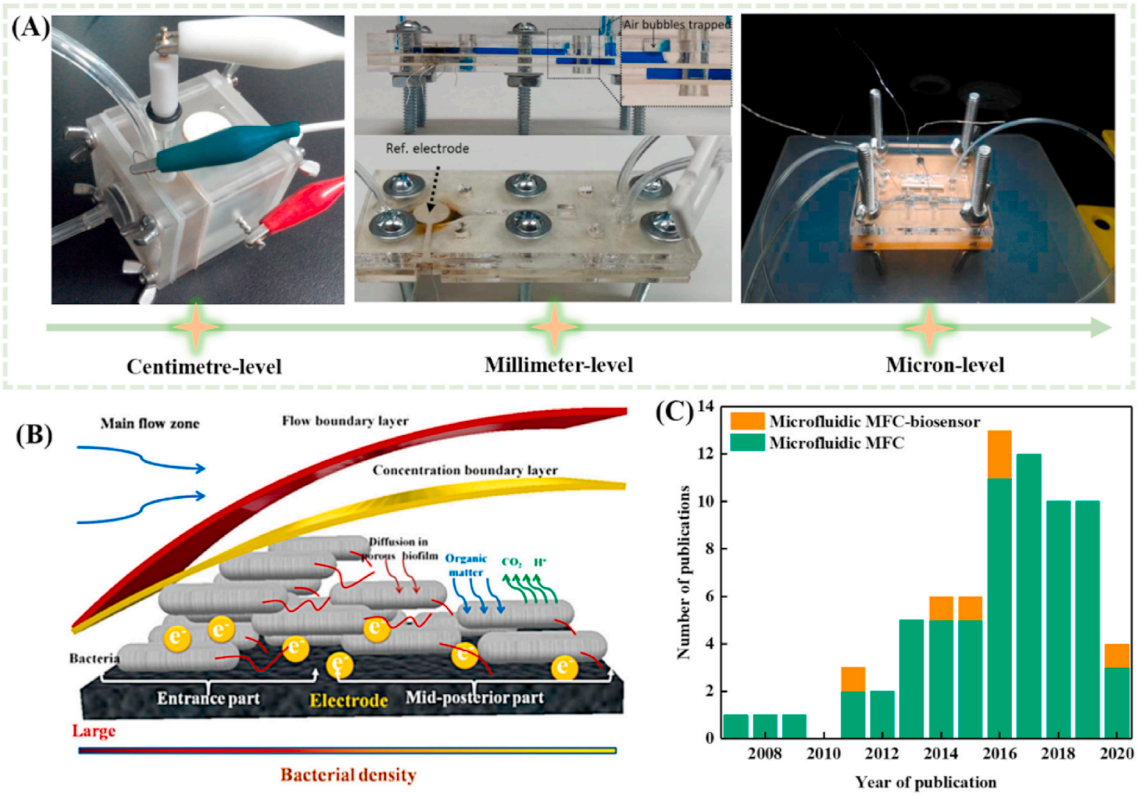

Fig. 6. Reduced size of EAB-based biosensor device. (A) Three biosensor reactors from centimeter to micron-level. (B) Microfluidic processing. (C) Published research papers related to key word of microfluidic MFC and microfluidic MFC-biosensor in Web of Science during the year 2008–2020.

4.2.2. *Biofilm modification*

The characteristics of biofilm are highly related to the sensitivity and stability of sensors. Previous studies revealed that adding conductive nanoparticles (e.g. conjugated polyelectrolyte) and soluble redox substances (e.g. magnetite) into EAB could accelerate EET and optimize the microbial community (Liu et al., 2018a; Ren et al., 2019). This would benefit to the detection of biosensor operated in sequential batch, but is hard to be applied to real-time monitoring in terms of increasing costs and the stability. An alternative is to add biological signal molecules to modify EAB, as these molecules can facilitate cell-to-cell communications (Pan et al., 2020). In particular, the signal molecules were found to mediate intercellular communication, help to maintain a high sensitivity of EAB, and facilitate the recovery of exoelectrogens after a sensing process.

Extracellular polymeric substance (EPS) positively affects the formation of biofilm as well. However, the presence of toxic substances would stimulate the EPS production of microorganisms, and EPS could absorb a large amount of toxic substances, especially heavy metals (Yi et al., 2019). This finding suggests that EPS would lead to inaccurate sensing results and needs regulations. A recent study used different anode potentials to regulate EPS and their responses to toxic substances (Hou et al., 2020). Results showed that the appropriate anode potential could protect EAB from metal ion shock by regulating EPS. Conversely, the appropriate anode potential can also make EAB more vulnerable to suffer from metal ion shock by regulating EPS. When the EAB was acclimated at an acetate limited condition (0.1 g/L), the insulative polysaccharides is found to be reduced, and therefore the EAB was thin and highly electroactive with more *Geobacter* in the microbial community (Li et al., 2020b).

Other studies on improving the sensitivity through biofilm modification includes facilitating the growth of electroactive bacteria as dominant in microbial community of EAB (Xiao et al., 2020a), reducing the biofilm thickness by scraping to accelerate the mass transfer (Qi et al., 2020), and decreasing the time period of the formation of EAB by gravity settling (Li et al., 2017).

4.2.3. *Working mode of electrodes*

Like many other MET devices, the working mode of electrodes affects the performance of biosensors through regulating the metabolic activity of EAB and the current. However, this has not been a general understanding until 2010 when researchers found that the baseline current under nontoxic conditions can be stabilized by controlling the overpotential (Stein et al., 2010). After a detailed investigation of two generally applied working modes of electrodes, i.e., constant external resistance and constant anode potential, a following study revealed that an increased sensitivity can be acquired by applying a low external resistance to obtain a large current output, which resulted in a relatively high anode potential in the meantime (Stein et al., 2012c). . Similarly, the sensitivity can be also increased by applying high anode potential or large current output using a potentiostat (or a galvanostat). These findings were attributed to the decrease of EPS production in EAB at a more positive anode potential, which was believed to introduce high-efficiency mass transfer of target analytes (e.g. Cd^{2+}, Ag^{+}) and finally improve the sensitivity (Hou et al., 2020; Yi et al., 2019).

Besides these two working modes, some particular operations were proposed to increase the sensitivity. A study employed transient state operation to provide intermittent high anode potential, allowing more electron export (larger current output) and leading to higher sensitivity (Tian et al., 2017). Particularly in nitrate early-warning, a more positive anode potential restricted the reaction rate of the nitrate, impeding the sensing process. In this case, researchers proposed that the anode could be operated in open circuit to maintain a more negative anode potential, and thus obtained improved sensitivity for nitrate early-warning (Wang et al., 2018).

5. Application cases

Researchers have employed EAB-based biosensors in various water environments, including sewage treatment plants, river, underground water, and effluent from oil industry, to study their performances on early warning (Fig. 7A). When there is a biological treatment unit in sewage treatment plant, which is easily damaged by the incoming shock of toxic substances, EAB-based biosensor would be placed before it and provide real-time warning to allow emergency measures (Corbella et al., 2019; Feng and Harper, 2013; Feng et al., 2013). A small EAB-based sensor has been installed at the inlet of drainage pipeline to warn possible pollution caused by industrial wastewater leakage (Agostino et al., 2020; Prevoteau et al., 2019). EAB-based sensor even served to monitor the groundwater in a well of daily use in some African city (Velasquez-Orta et al., 2017). An MFC sensor with tailored configuration was applied in oil industry to detect oil leakage (Dai et al., 2019). EAB-based biosensor also applies in several particular water environments. It has been equipped in up-flow anaerobic sludge bed (UASB) reactor and macrophyte residues bioreactor to monitor the ongoing reactions by providing alert for abnormal products (Fig. 7B) (Jia et al., 2016; Liu et al., 2020; Wang et al., 2019a). It has also been placed in the rhizosphere of rice plants to detect the damage of acid rain to the plant (Fig. 7C) (Li et al., 2018).

6. Conclusions and novel strategies

Based on the explanations of why EAB can serve as sensitive element to realize early-warning, as well as a review of biosensor-related studies, it is clear that early-warning is notably distinct from other sensing processes like BOD detection and COD detection, as early-warning aims

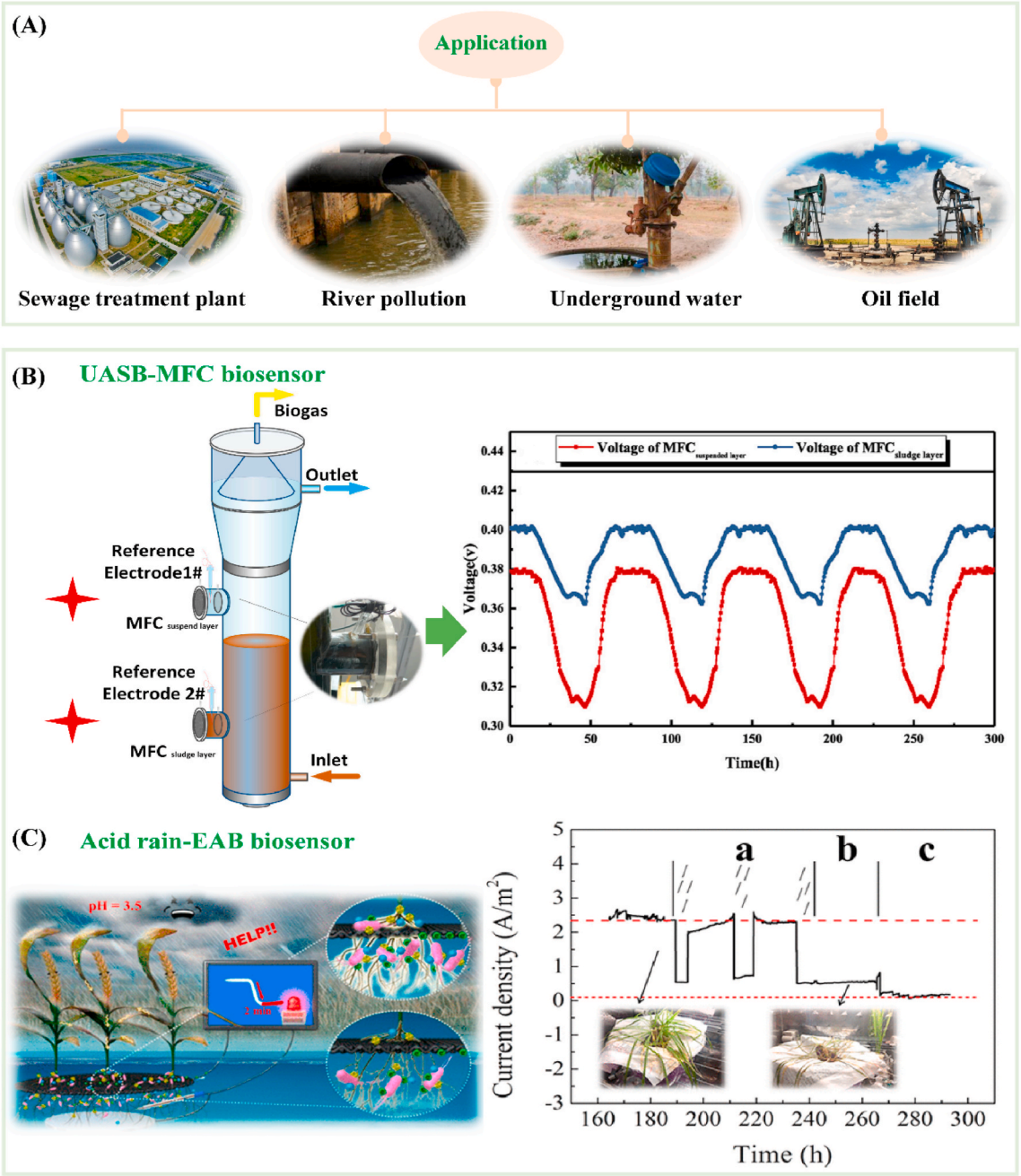

Fig. 7. Applications of EAB-based biosensors in water quality early-warning. (A) EAB-based biosensor can serve different water environments. (B) An MFC biosensor in UASB reactor to monitor abnormal products. (C) EAB-based biosensor in the earth for acid rain detection.

to deliver timely alert for composite pollutants while addresses little selectivity concern or seldom involves concentration determination. This alert signal should also be accurate so as to take proper measures to avoid further risk of exposing to pollutants. Great efforts and progress have been made in previous researches related to early-warning technology by EAB-based biosensor, including but not limit to employing optimized configuration and biofilm, suitable sensitive elements, and novel working modes to increase the sensitivity, reducing interference to decrease false signal, as well as modeling to investigate the sensing process. However, future study may need to address the following concerns.

(i) *Improving the accuracy of early-warning alert.* False-negatives or false-positives may appear when the EAB suffers from a shock of complex contaminants. It has been resolved that employing the biocathode as a sensitive element can avoid false-negatives when the sewage contains both organic matter and toxicants. However, the industrial waters may be far more complicated than this. Possible solutions may be tailored configurations or operations of biosensors, or particular pretreatments to exclude the distractors.

(ii)*Understanding how EAB delivers sensing performance.* Electrical signals are visible responses of electroactive bacteria to target analytes. However, it is still unclear how EAB and DEET components (e.g. surface redox proteins and nanowire appendages) are involved to deliver sensing performance. A very recent study managed to extract nanowires from *Geobacter sulfurreducens* and found them capable of detecting the ammonia at low humidity environment (Smith et al., 2020). To follow up, future studies may be able to reveal how ammonium ions affect key enzymes and proteins in these nanowires. Advanced molecular biotechnology also provides helpful tools to study how the toxicity affects the enzymes or proteins involved in DEET.

As for the formation of EAB, EPS is known as a contributor. But it would negatively affect the sensitivity in the meantime, and that needs to be regulated. Besides common measures like applying particular electrode potential and employing proper substance, genetic tools may provide a possible solution to regulate EPS, such as gene GSU1501 (*xapD*) in *Geobacter sulfurreducens*.

(iii)*To build reproducibility of EAB-based biosensor towards the standardization.* Unlike biosensors that employ luminescent bacteria, none standards related with the EAB-based biosensors have been issued, which considerably restrains its commercial applications. One of the main concerns about the performance of EAB-based biosensor is the weak reproducibility that can be attributed to the discrepancy of the morphology and microbial community of naturally formed EAB. Although it would be hard to obtain identical biofilms, some regulations on the thickness and density would contribute to deliver reproducible signals. In addition, employing standardized electronic donor and electroactive bacteria would also help to maintain consistent results among different studies.

(iv)*Construction of signal transmission system and improvement of readability.* Depending on cloud service system and internet technology, the biosensor's signals are now available from a distance. Researchers should develop responsive circuits and wireless transmission systems for different application environments, and the acquired signals also need data analysis. Currently, only a few studies focused on the readability of signals (Adekunle et al., 2019b). Future biosensor applications may require computing models to provide the users with more readable results.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC No. 51778324, 51422810).

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