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Artificial electrochemically active biofilm for improved sensing performance and quickly devising of water quality early warning biosensors

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ABSTRACT

A major challenge for devising an electrochemically active biofilm (EAB)-based biosensor for real-time water quality early-warning is the formation of EAB that requires several days to weeks. Besides the onerous and time-consuming preparation process, the naturally formed EABs are intensively concerned as they can hardly deliver repeatable electrical signals even at identical experimental conditions. To address these concerns, this study employed sodium alginate as immobilization agent to encapsulate *Shewanella oneidensis* MR-1 and prepared EAB for devising a biosensor in a short period of less than 1 h. The artificial EAB were found capable of delivering highly consistent electrical signals with each other when fed with the same samples. Morphology and bioelectrochemical properties of the artificial EAB were investigated to provide interpretations for these findings. Different concentrations of bacteria and alginate in forming the EAB were investigated for their effects on the biosensor's sensitivity. Results suggested that lower concentration of bacteria would be beneficial until it increased to 0.06 (OD_{660}). Concentration of sodium alginate affected the sensitivity as well and 1% was found an optimum amount to serve in the formation of EAB. A long-term operation of the biosensor with artificial EAB for 110 h was performed. Clear warning signals for incoming toxicants were observed over random signal fluctuations. All results suggested that the artificial EAB electrode would support a rapid devised and highly sensitivity biosensor.

1. Introduction

To address water environment pollution as a global challenge, water quality monitoring technologies emerge as a promising tool to detect pollutants presented in water and thus prevent further contamination (Horowitz, 2013). Biosensors that can directly reflect bioeffect of toxicity have been demonstrated advantageous over traditional chemical analytical technology in recent years (Antonacci and Scognamiglio, 2020). Most biosensors work through recognizing the concentration or effect of the toxicants. Those biosensors detecting the concentrations rely on some specific biomacromolecules including nucleic acids, antibodies, enzymes, or proteins, which can recognize specific target analytes (Dai et al., 2019). However, the preparation of this kind of biosensors is complicated, time consuming, and costly. On the contrary, cell-based biosensors, which monitor composite pollutants through their effect on selected microorganisms (e.g., bacteria, microalgae, and fungi) serving as sensitive elements, are able to alert early-warning for a variety of monitored water environments including urban rivers, lakes, and even sewage plants (Antonacci and Scognamiglio, 2020; Gupta et al., 2019).

Among all sensitive elements employed in early-warning biosensors, electrochemically active biofilm (EAB) formed by electroactive bacteria has been intensively studied as it can generate detectable bioelectricity as warning signals without any additional chemical-mediators (Chu et al., 2021; Jiang et al., 2018). A variety of organic matters including biochemical oxygen demand (BOD) and volatile fatty acids, especially toxicities like heavy metals and organic toxicants, can be detected by the changes of electrical signals generated by EAB (Do et al., 2019). The sensitivity that reflects the responding ability of biosensor towards the toxicities is a major matric for early-warning biosensor. In previous studies,

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improved sensitivity could be achieved when the transfer of organic matters and toxicity to biofilm was facilitated through optimizing flow rate, flow direction, and reactor scale (Shen et al., 2013; Xiao et al., 2020). Regulations of the electrode were also reported to increase the sensitivity, including employing constant external resistance, constant anode potential, constant anode current, and transient-state operation (Jiang et al., 2017; Stein et al., 2012; Yi et al., 2019). Modified EAB for more responsive performance was prepared through adding electroconductive or redox medium, quorum sensing effect, and extracellular polymeric substance (EPS), and regulating microbial community structure (Pan et al., 2020, Xiao et al., 2020; Yi et al., 2019). With a much improved sensitivity, there are still unaddressed concerns when applying in practical real-time early-warning. One major concern is time-consuming formation process of EAB. Previous studies reported that it would require several days to weeks to form an EAB in laboratorial culture, which greatly restrained timely early-warning process (Li et al., 2020; Liu et al., 2011; Qi et al., 2020a). In addition, to culture an EAB is too complicated for a non-professional to perform. Despite of these difficulties in forming an EAB, the process is easily affected by many culture conditions, leading to different biofilm status (e.g. thickness, density, and microbial community structure). This is why even feeding with identical culture in the same lab, the obtained EABs could hardly output repeatable electrical signals, which implies that the sensing process cannot be verifiable or convincing.

EAB mainly consist of electroactive bacteria and EPS including polysaccharides, proteins and humic acid (Li et al., 2020; Stöckl et al., 2019). An effective method to quickly form an EAB is to employ suspended electroactive bacteria and immobilized agents, particularly hydrogels that have been used in cell-based biosensor (Ishihara et al., 2020; Tucci et al., 2019). Researchers have found that hydrogels are capable of providing an extracellular matrix link and three-dimension (3D) environments that are biocompatible with cells and have applied them in electroactive bacteria immobilization (Hu et al., 2020; Li et al., 2016; Liu et al., 2015; Liu et al., 2014; McCuskey et al., 2020; Narayanaswamy Venkatesan and Dharmalingam, 2013; Uria et al., 2020). With the purpose of increasing bioelectricity generation, some studies used hydrogels (e.g. redox phospholipid polymer hydrogels, alginate/ redox mediator composites) to encapsulated electroactive bacteria (Lin et al., 2012; Szollosi et al., 2017). These previous studies suggest that the hydrogels may facilitate and regulate the formation of EAB.

This study employed hydrogels as immobilization agents to encapsulate electroactive bacteria and quickly formed an EAB that produced repeatable electrical signals. With this artificial EAB electrode, a biosensor was devised for early-warning and delivered timely responsive signals for toxicants introduced both in short-term and long-term experiments. Further investigations on morphology features and bioelectrochemical properties of the obtained EAB provided possible interpretations for the greatly reduced startup time and highly consistent repeatability. Different concentrations of immobilization agents and bacteria were tested for their effects on the sensitivity, with both experimental and modeling studies.

2. Materials and methods

2.1. Materials

This study employed a model strain *Shewanella oneidensis* MR-1 as electroactive bacteria, which was friendly supplied by Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University. The Luria Bertani (LB) medium (CM0996) applied in experimental studies was composed of 10 g/L pancreatic digest of casein, 5 g/L yeast extract, and 10 g/L sodium chloride. Sodium alginate, $CaCl_2$, and formaldehyde (wt. 40%) were provided by Macklin Co., Ltd. (Shanghai, China).

2.2. Preparation of artificial EAB electrode

The *Shewanella oneidensis* MR-1 was inoculated in LB medium, and was moved into shake cultivation for 24 h, at a temperature of 30 °C. The suspended bacteria were collected through centrifugation at 7000 rmp/min when *Shewanella oneidensis* MR-1 was growing at log phase, were then diluted to 200, 400, 800, 1200, and 1600 times by applying phosphate buffer. Correspondingly the OD_{660} was 0.25, 0.12, 0.06, 0.04, and 0.03 as measured by UV spectrophotometer (SOE-100, IQ-5). Sodium alginate (concentration: 1%, 1.5%, 2%, 2.5%) was employed as immobilization agent that was found biocompatible with the cell according to a previous study (Szollosi et al., 2017). The electrode with biofilm was prepared as follows (Fig. 1A). First, the collected bacteria and sodium alginate with identical volume were well mixed together. Then 0.4 mL of this mixture was added dropwise onto a piece of indium tin oxide (ITO) conductive glass (5 cm*5 cm) with a silicone pipette (center diameter: 4 cm) to make an ITO electrode, which was then spun at 350 rmp/min for 9 seconds on a spin coater (KW-4A, Beijing, China) to ensure the biofilm distributed uniformly on the electrode. A vacuum pump was employed in this process protect the biofilm. Next, the prepared ITO electrode with biofilm was sunk in 0.5% $CaCl_2$ solution for 10 seconds to allow cross-linking reaction. Finally, a light-passing plastic mesh was pressed over the prepared biofilm to keep a close attachment of the electrode and biofilm, in which *Shewanella oneidensis* MR-1 served to generate electrons and to transport them with surface redox proteins and nanowire appendages, while calcium alginate behaved as conjunctive agents of electroactive bacteria (Fig. 1B). For comparison, abiotic ITO electrodes without any substances and with only sodium alginates (1%, 1.5%, 2%, 2.5%) were also prepared and studied as control groups.

2.3. Device and operation of EAB sensor

A three-electrode reactor was employed as EAB sensor to alert an incoming toxicity from water environment in this study (Fig. 1A), where the prepared ITO electrode with biofilm served as working electrode to oxidate organic matters and to produce electrons, protons, and carbon dioxide. The produced electrons would be transferred to the counter electrode (CE), a titanium mesh that would reduce pollution from bacteria, through regulating the potential of working electrode with a saturated calomel electrode (SCE, +242 mV *vs.* standard hydrogen electrode, SHE, Leici Co. Ltd., China) as a reference electrode. During the whole operation, a constant working electrode potential of 0 V *vs.* SCE was applied by a potentiostat (CHI 1030B, Chenhua Instruments Co., Ltd., Shanghai, China) with eight-channels according to a previous study (Wang et al., 2013). LB medium of 25 g/L was employed as nutrient solution to startup the reactor, and the pH was 7. All tests were proceeded in a thermostatic incubator at a temperature of 32°C.

2.4. Morphology characterization

The morphology of biofilm was examined by employing a confocal laser scanning microscopy (CLSM, Leica TCS SP8, Germany). Before spin-coating, the molecular probes of live/dead (LIVE/DEAD® BacLight™ Bacterial Viability Kits, Invitrogen Detection Technologies, China Branch) were added in *Shewanella oneidensis* MR-1 and calcium alginate mixture to stain for 10 minutes. Then a clear 3D structure of EAB biofilm could be captured

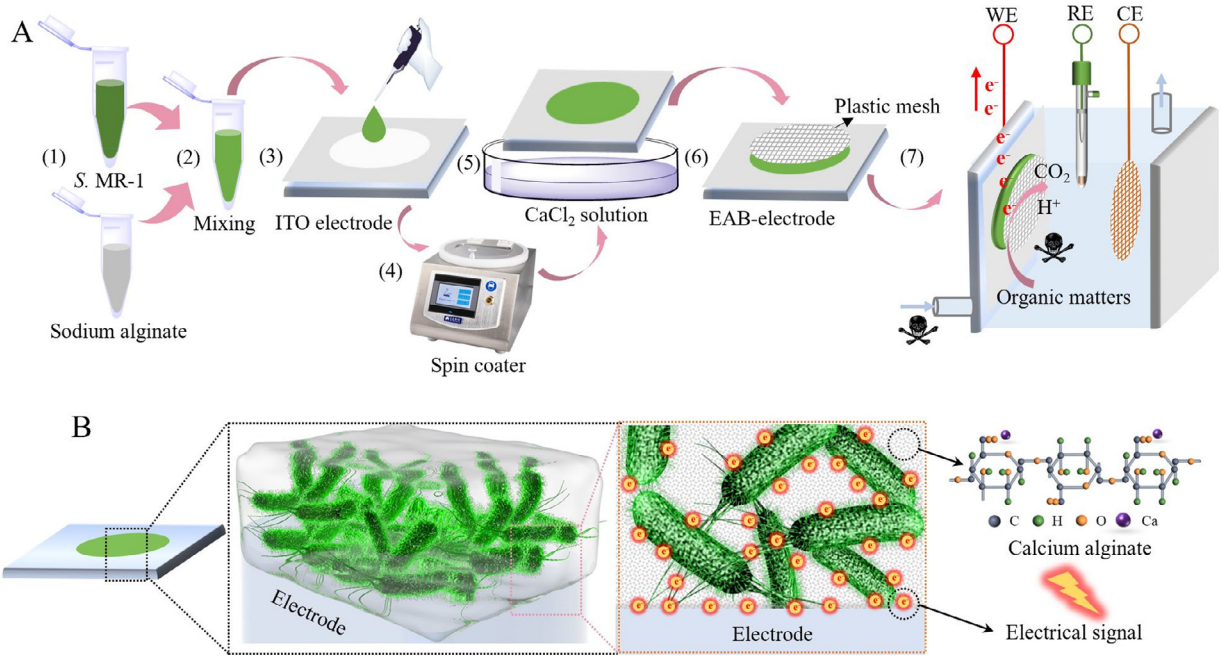

Fig. 1. (A) Preparation process of artificial EAB electrode and (B) schematic illustration of biofilm with mixed *Shewanella oneidensis* MR-1 and calcium alginate.

by CLSM with "Stack" model of LAS X software (Leica). The fluorescence intensity was analyzed by Image J software. Moreover, a scanning electron microscope (SEM, JEOL JSM 6301F, Japan) was employed to further observe micro-morphology. To obtain a sectional view, the biofilm sample was stripped down from ITO electrode and placed vertically on the sample table before scanning.

2.5. Bioelectrochemical tests and quantitative calculation of EAB biosensor

Before test, nitrogen gas was continuously pumped in nutrient solution for 15 minutes to remove dissolved oxygen. The current signals produced was recorded by a potentiostat every 4 seconds. Cyclic voltammetry (CV) and electrochemical impedance spectrum (EIS) measurements were performed through electrochemical workstation (Autolab, Metrohm Co. Ltd., Switzerland). For early-warning experiments, formaldehyde was employed as the toxicant to test the biosensor's sensitivity when EAB biosensor could generate a stable current signal. The inhibition ratio (*IR*) of current signal is calculated as

$$IR(\%)=(\Delta I/I)\times 100\% \quad (1)$$

where I is the stable current produced before toxic shock and ΔI is the current change after toxic shock (Qi et al., 2019).

To investigate the effect of bacteria concentration on early-warning performance, the current signals are normalized as (Qi et al., 2019)

$$NES=I_n/I \quad (2)$$

where *NES* is the normalized electrical signal and I_n is the recorded current every 4 seconds.

3. Results and discussion

3.1. Artificial biofilm for devising EAB based biosensor and its electrical signal output

Mixing *Shewanella oneidensis* MR-1 (OD_{660}=0.12) with 2% sodium alginate at volume ratio of 1:1 made a 3 D hydrogel to encapsulate the electroactive bacteria. After spin-coating and cross-linking reaction, the biofilm came in a round shape (Fig. 2A), which was more inerratic than a naturally formed biofilm (Sui et al., 2020). Further examination of the biofilm's morphology from front and top views of the biofilm by CLSM showed that it was homogeneous among the center, middle, and side area (Fig. 2B). This biofilm had a similar cell density with one of our previous studies (Qi et al., 2020a), and the biofilm thickness was about 100 μm. SEM images with side view revealed that electroactive bacteria and sodium alginate were evenly mixed together (Fig. 2C). Both CLSM and SEM images showed that cells were in contact with each other, providing easy pathways for extracellular electron transfer (EET) that was found mediated through surface redox proteins (e.g. cytochrome *c*), flavin that secreted by bacteria, even nanowires in EAB biofilm (Shi et al., 2016).

From the above-mentioned contents, the artificial EAB theoretically can transfer electrons to electrode. To investigate the performance of artificial biofilm for generating electrons to electrode, EIS and CV were performed. As a comparison, a control group did not attach any microorganism. As illustrated in EIS plots, the artificial EAB delivered much reduced diffusion resistance than the control at low frequency stage, suggesting that the EAB was in good attachment with ITO electrode (Fig. 2D). The results of CV also indicated that the constructed EAB was electrochemically active (Fig. 2E). With this rapidly formed EAB, the electrode served as working electrode in a three-electrode system for bioelectricity generation in the following experiments. When the nutrient solution was pumped into the reactor, an electrical signal was generated instantly, which achieved stable at around 5 μA after 2000 seconds (Fig. 2F). Compared to several similar previous studies that normally required several days or even weeks to form a mature biofilm for signal generation (Flexer et al., 2013; Liu et al., 2011; Xie et al., 2015), the EAB was all ready for toxicity alert in just 0.6 hour in this study.

3.2. Outstanding repeatability of biosensor with artificial EAB

It is well acknowledged that naturally-formed biofilms are easily affected by culture conditions. This is why good repeatability

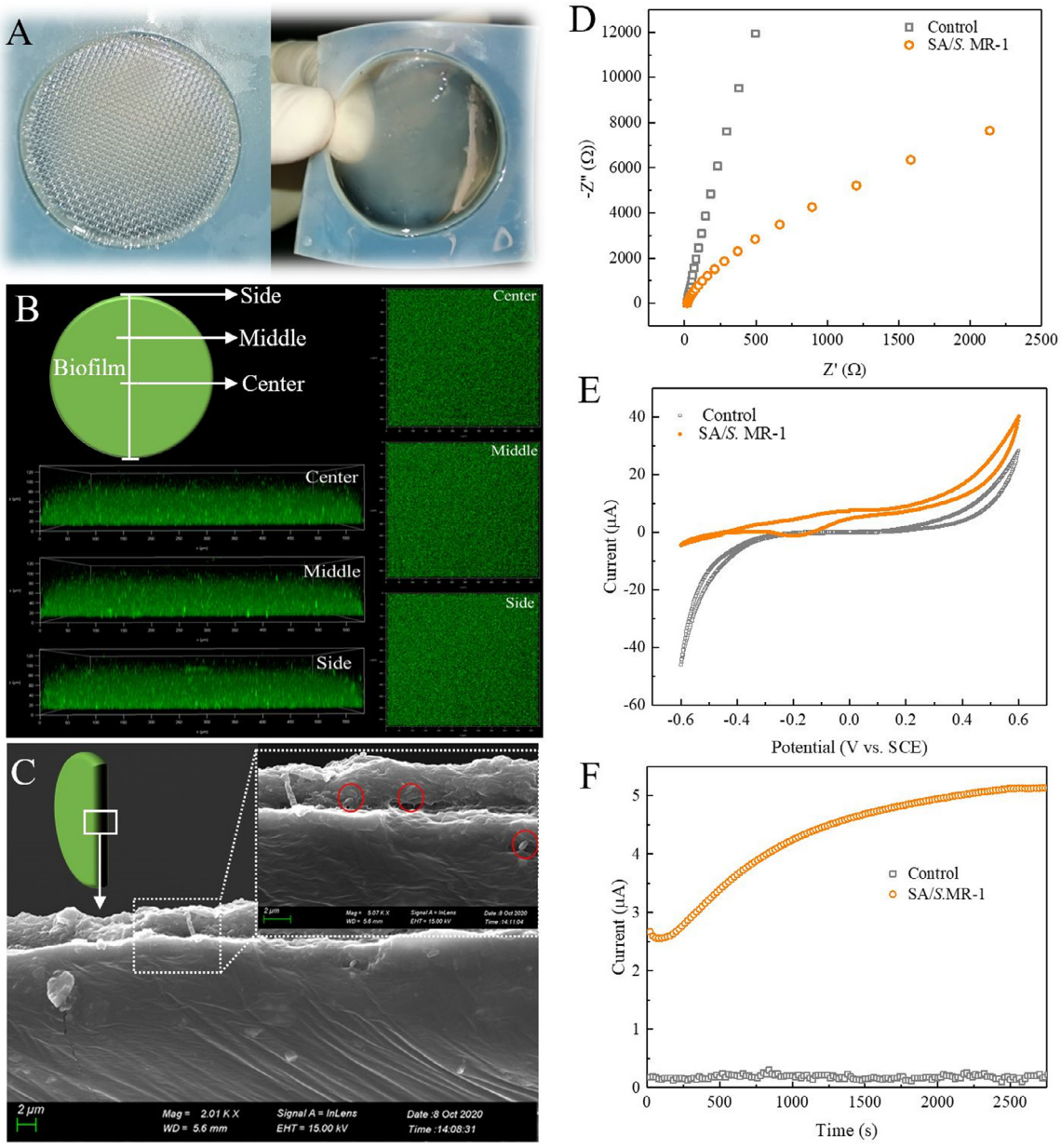

Fig. 2. Morphology and electrochemical characterizations of the artificial EAB. (A) a photograph of EAB electrode in this study, (B) CLSM images of center, middle and side areas of EAB, (C) an SEM cross-section image of EAB, (D) electrochemical impedance spectrum of EAB electrode and abiotic electrode (control), (E) Cyclic voltammetry curve for EAB electrode and control electrode (scan rate of 10 mV/s and applied potential between -0.6 and +0.6 V vs SCE), and (F) current signals output by EAB biosensor with artificial biofilm and abiotic electrode.

when changing the EAB electrode in a biosensor is a critical concern and challenge for biosensors that mostly rely on the biofilm to generate electrical signals for toxicity. To study the repeatability of biosensor with different EABs, besides artificial EAB prepared as described previously (Fig. 3A), traditional naturally formed EAB on the side of electrode with EPS secreted by themselves was also prepared according to a recent report (Fig. 3D) (Stöckl et al., 2019). Another tested EAB was obtained by gravity settling when the electrode was rotated 90 degrees which was reported as a fast accessed method to form an EAB electrode (Fig. 3H) (Li et al., 2017). All these EABs were prepared in duplicates to investigate their electricity generations under the same experimental conditions.

The biosensors with these three EAB electrodes delivered distinct repeatabilities (Fig. 3). At beginning when feeding continuously for 0.8 h, the current signals output by the two biosensors with artificial EAB were quite similar (Fig. 3B). After that, water sample containing 0.06% of formaldehyde was fed in the reactor. Two current signals produced by two biosensors with artificial EAB declined almost at the same time and with the same rate (Fig. 3C). However, the generated current signals from two biosensors with naturally formed EAB were not repeatable in startup process with batch mode (Fig. 3E). Moreover, it required a long startup period up to 45 hours, which far exceeded the startup time of biosensor with artificial EAB. When these two biosensors were operated with continuous feed, two current signals immediately drop sharply, suggesting the repeatability of this type of biosensor was not promising (Fig. 3F). Further examination by CLSM revealed that no mature biofilm could be found on the electrode, indicating that high current signal in the batch startup process was mainly produced by suspended bacteria rather than a biofilm (Figure S1A). As for the biosensor with electrode rotated 90 degrees, its current signals took about 15 hours to arrive at stable under gravity

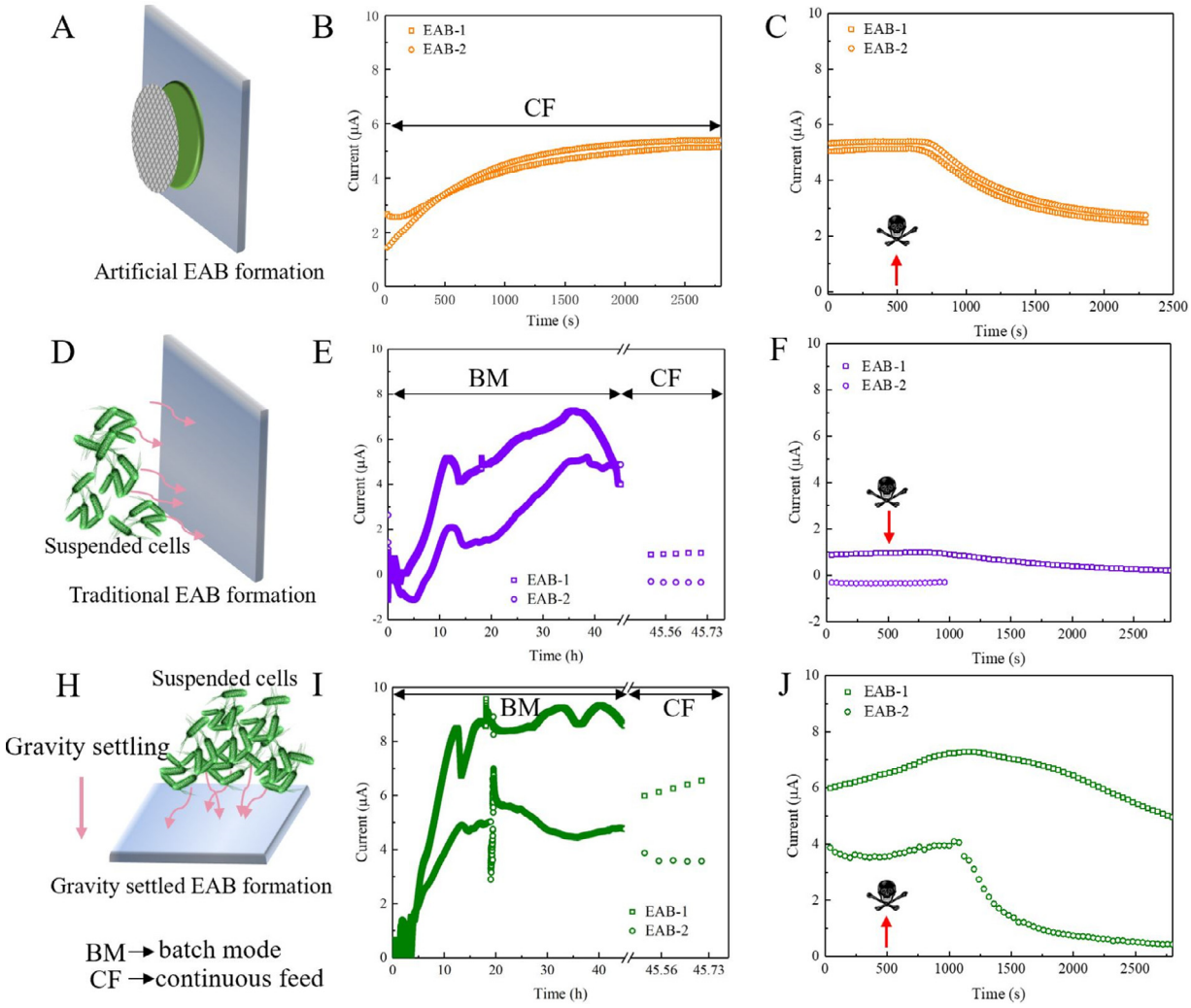

Fig. 3. The repeatability of three biosensors devised with artificial EAB formation (A, B, C), traditional EAB formation (D, E, F), and gravity settled EAB formation (H, I, J).

settling, still much longer than that of biosensor with artificial EAB (0.6 h) (Fig. 3H). Like biosensor with naturally formed EAB, two biosensors with rotated electrode failed to deliver repeatable current signals during the startup process. When switching to continuous feed, those currents dropped as well (Fig. 3H). The biofilm on rotated electrode appeared thick in the middle and thin at the edge in CLSM images (Figure S1B). In addition, the two biosensors with rotated electrode delivered much different inhibition rates of one reaching to 80% and the other less than 20% (Fig. 3I). In conclusion, ITO electrode with artificial EAB could be employed as a modulized electrode to devise the biosensor for fast startup and repeatable signal output.

3.3. Effects of electroactive bacteria concentration on sensitivity

Aiming to determine how the concentration of electroactive bacteria affects the biosensor's sensitivity, different concentrations of bacteria encapsulated in 3D hydrogel were investigated while the concentration of immobilization agents was fixed at 2%. Results showed that the current signals gradually increased with the bacteria concentration (OD_{660}) from 0.3 to 2.5 (Fig. 4A). Nevertheless, the abiotic control with only sodium alginates of 2% did not generate any current signals for early-warning of formaldehyde. Since high concentration of electron donors (organic matters) could diffuse to the whole EAB and ensure that electroactive bacteria would obtain enough electron donors for bioelectricity generation, the electron donors would be considered sufficient to support the early-warning process.

All current signals generated by artificial EABs with any bacteria concentration tested declined once water sample containing 0.06% of formaldehyde was fed in the reactors, and the toxicity processes had good reproducibility under each concentration (Figure S2). These current signals were normalized by Eq. (2). Results showed that there was no significant difference among the toxicity processes when the bacterial concentration exceeded 0.06 (Fig. 4B). Nevertheless, the toxicity process was more obvious when the bacterial concentration was very small (0.04 and 0.03). The inhibition ratio slightly decreased with larger bacteria concentration (Fig. 4C). A normal understanding is that a reduced concentration of bacteria would benefit mass transfer, unless the bacteria is scarce to restrain electrons conduction and thus to affect the output of current signals. To further analyze the spatial distribution of biofilms with different bacterial concentrations, 3D CLSM images scanning was employed to observe these biofilms, and fluorescence intensity analysis was performed on the interfaces at the same Z-axis position. Images from CLSM (Figure S3) illustrated that average fluorescence intensity calculated by ImageJ software gradually increased with larger bacteria concentration employed (Figure S4). But this influence became weak when the bacterial concentrations exceeded 0.06, which was consistent with

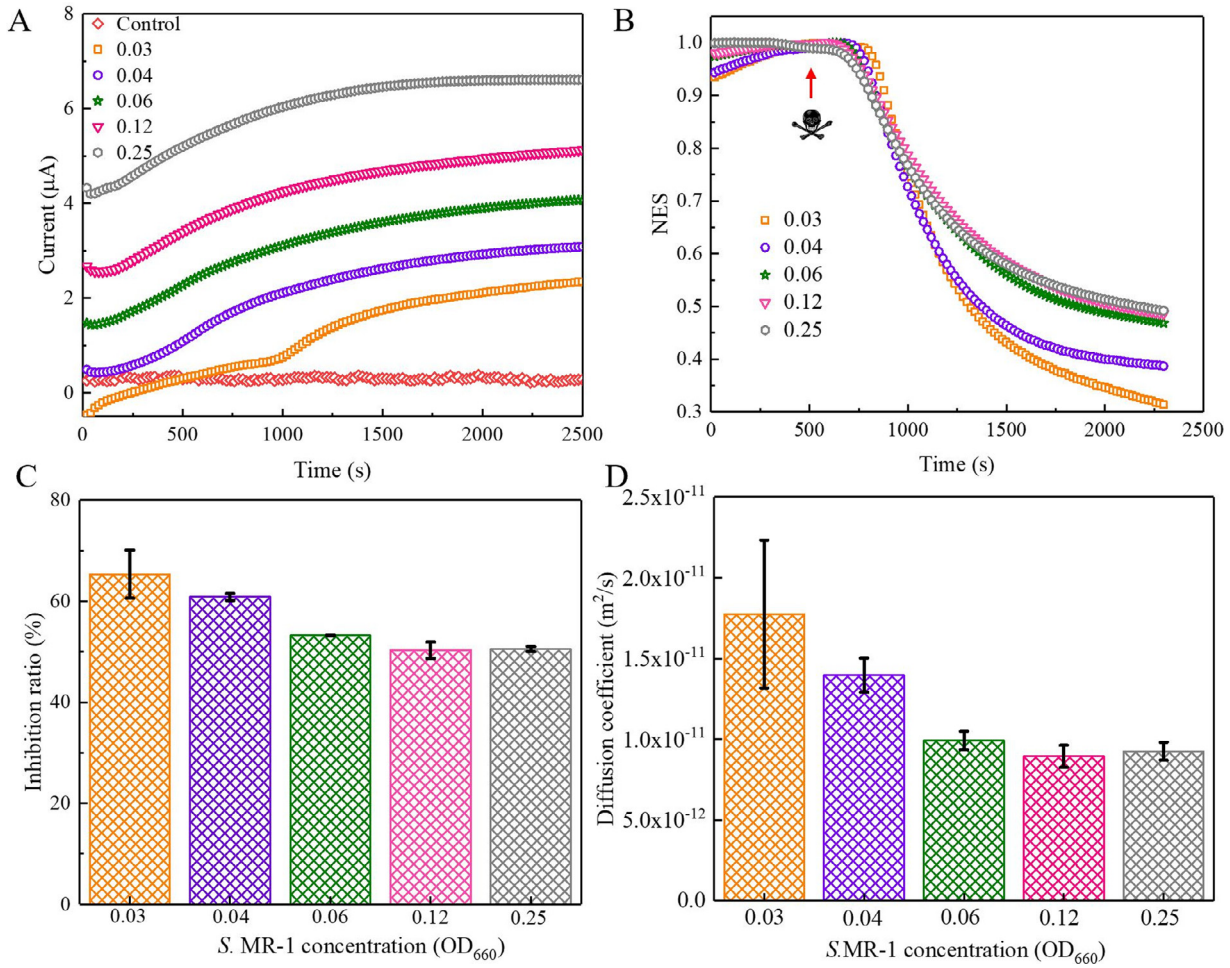

Fig. 4. Effects of electroactive bacteria concentration in artificial EAB on biosensor's sensitivity. (A) Current signals with different electroactive bacteria concentrations (OD_{660} = 0 (control), 0.03, 0.04, 0.06, 0.12, 0.25), (B) normalized electrical signal (NES) changes with different electroactive bacteria concentrations for 0.06% of formaldehyde shock within 30 min, (C) corresponded inhibition ratio, and (D) simulative diffusion coefficient.

the change of NES (sensitivity) with bacteria concentration. These findings suggested that the biosensor's sensitivity would not be much affected by mass transfer when the concentration of bacteria exceeded 0.06.

To further verify the effect of bio-current generation and mass transfer on sensitivity, a kinetic model integrated Nernst-Monod model of bio-electrons and 1D mass transfer model of toxicants (Supplementary materials 1.2) was proposed to simulate the toxicity process of formaldehyde in the artificial biofilm with different electroactive bacteria concentrations. Nernst-Monod model of bioelectricity generation provided a relationship between current and electroactive bacteria concentrations, while 1D mass transfer model connected together the concentration of toxicants, diffusion coefficients, biofilm thickness, and the time. Our previous study revealed an intimate connection between the current signals and mass transfer of toxicants in EAB (Qi et al., 2020a). Herein, the diffusion coefficients of formaldehyde were approximately calculated by fitting the current signals of toxicity process, and the fitted results output by MATLAB software were shown in Figure S5. Obviously the fitted curve was more in line with experimental data when low concentration of electroactive bacteria was employed in readily cultured EAB. Fig. 4D illustrated that the diffusion coefficients significantly increased with larger bacteria concentration from 0.04 and 0.03, but presented no obvious difference when the concentration exceeded 0.06, which was consistent with the change of inhibition rate with bacteria concentration.

3.4. Effects of immobilization agent concentration on sensitivity

This study employed sodium alginate as immobilization agent to encapsulate electroactive bacteria, because several studies succeeded in extracting alginate polysaccharide from EPS, a substance secreted by electroactive bacteria and critical to form the biofilm (Jiang et al., 2019; Pronk et al., 2017). The concentration of EPS was found to affect the biosensor's sensitivity (Yi et al., 2019). This implied that the concentration of sodium alginate as immobilization agent might have potential effect on early-warning process.

With different concentration of sodium alginate hydrogel applied (1%, 1.5%, 2% and 2.5%), current signals changed with the time when the working electrode potential was fixed at 0 V against a SCE reference electrode (Fig. 5A). Current signals with different concentration of immobilization agents appeared similar before toxicity, because high concentration of organic matters provided enough electron donors for the whole biofilm. Then the toxicants were introduced into the biosensors. With increased concentration of sodium alginate, the biosensor delivered slower response to toxic substances with a lower inhibition ratio (Fig. 5A). The abiotic ITO electrodes with only sodium alginates did not produce any current signals, or respond to any toxicants (Figure S6). However, this effect was not obvious when the concentration of sodium alginate was lower than 1.5% (Fig. 5B), which could be interpreted as low concentration of sodium alginate had little impact on the transfer of formaldehyde in biofilm. Herein, the biofilm could not

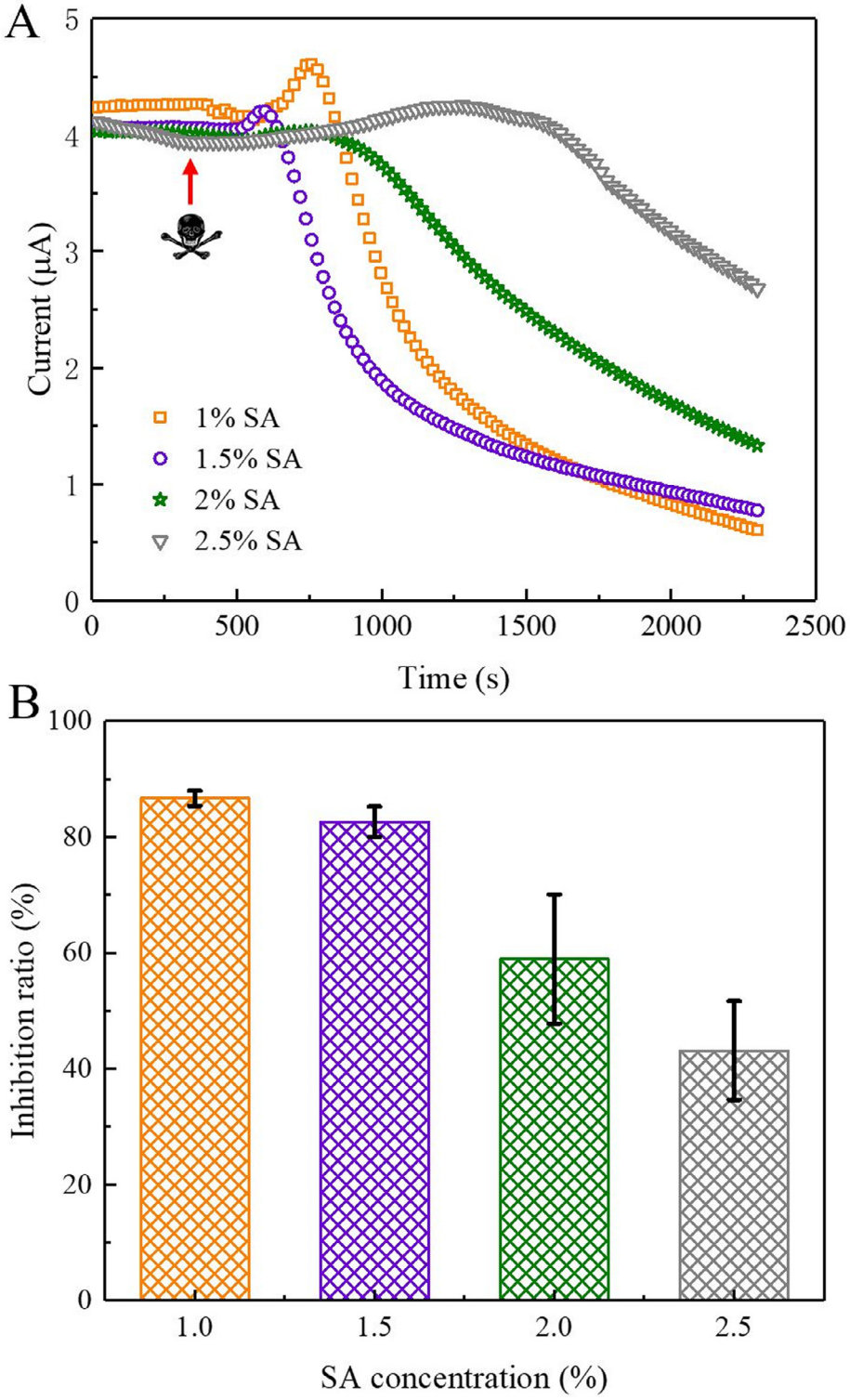

Fig.5. Effects of immobilization agent concentration on sensitivity. (A) Current signals with different sodium alginate concentrations of 1%, 1.5%, 2%, and 2.5% when exposed to 0.06% formaldehyde shocks within 30 min. (B) Inhibition ratios.

form with the immobilization technology when the sodium alginate concentration is lower than 1% in this study. Thus 1% of sodium alginate was optimum for artificial EAB formation. A previous study reported that biofilm thickness would impact the transportation of toxicants in biofilm and further affect the sensitivity (Qi et al., 2020a). However, 3D CLSM images of the biofilms with different concentration of immobilization agents showed no obvious difference in the thickness and similar morphology structures (Figure S7 and S8). By excluding the biofilm thickness, high concentration of sodium alginate would be considered a major contributor for the limited mass transfer of toxicants in biofilm. This interpretation was evidenced by the calculations of diffusion coefficients of formaldehyde in biofilms with different concentration of sodium alginate, which concluded that higher sodium alginate

concentration would lead to lower diffusion coefficient (Figure S9). In addition, the dose-effect curves with different concentration of formaldehyde were illustrated in Figure S10, revealing again that low concentration of sodium alginate would come with improved sensitivity. The artificial EAB with 1% of sodium alginate had an inhibition ratio of up to 45% when it exposed to 0.02% formaldehyde. An earlier study suggested that electroactive bacteria would generate more EPS to protect itself from further toxic impacts when it suffered toxicants shock (Patil et al., 2010). On the contrary, the artificial EAB may keep sensitive toward toxic impacts rather than act against them as natural biofilms do.

3.5. Comprehensiveness and real-time online operation

In practical application, EAB-based biosensor is expected to deliver early-warning signals for incoming toxicants in monitored water environment (Qi et al., 2020b). To investigate the availability of the biosensor with artificial EAB for composite toxicants, the phenol, which was also presented in water as a pollutant, was employed to study the sensitivity of the biosensor. Results showed that phenol with a concentration of 0.06% could be alerted by the biosensor with a 35% inhibition ratio (Figure S11). This finding suggested that the biosensor with artificial biofilm was able to alert various pollutants.

Biosensors employed during long-term operation may encounter environmental interferences, and thus deliver unstable signals in this process. To investigate long-term performance of the biosensor with artificial EAB, it was operated for 110 hours. Despite of random fluctuations, its electrical signal output showed clear changes soon after feeding water sample containing 0.06% and 0.1% of formaldehyde during 90~100 h (Figure S12A). The corresponding inhibition ratios were 55% and 76%, respectively (Figure S12B), suggesting a weakly-reduced sensitivity than in short-term monitoring. Moreover, the biosensor could recover 87% after toxicity shock (Figure S12B). At the end of 110 h operation, the biofilm had developed into a mature EAB (Figure S13). The above studies were conducted under laboratory simulation conditions. It's noted that a pre-treatment process will be provided against contamination issue during the practical application. In addition, if pH is not considered an early-warning target, the effect of pH on calcium alginate can be eliminated by adding buffer solution in real water samples.

4. Conclusions

By employing sodium alginate as immobilization agent, this study proposed a simple and fast way to form an artificial EAB electrode enriched with *Shewanella oneidensis* MR-1 for highly sensitive biosensor to monitor incoming toxicants in water environment. The artificial EABs were found to deliver repeatable and effective electrical signals under identical early-warning experimental conditions. Different ratios of bacteria and alginate concentrations in forming the EAB were investigated for their effects on the biosensor's sensitivity. Results indicated that low concentration of electroactive bacteria would lead to higher sensitivity, but extreme concentration upper 0.06 (OD_{660}) would not affect the sensitivity anymore. In addition, 1% sodium alginate was found an optimum amount to serve in EAB. Besides short-term early-warning experiments, long-time operation for 110 h was also performed with the biosensor with artificial EAB. Despite of random fluctuations caused by the surrounding, the tested biosensor delivered clear signals for incoming toxicants. All results suggested that the artificial EAB electrode could serve as modularized electrode to devise and regulate an early-warning biosensor. Significantly, this work is expected to push EAB-based biosensor into a national or international standard of water quality early-warning in the future.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117164.

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