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A novel biosensing system for rapid and sensitive detection of heavy metal toxicity in water

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ABSTRACT

Toxicity biosensors have recently gained significant attention due to their potential use in online monitoring. However, the effects of toxicants and the influence of dose, exposure time, and type and concentration of respiration substrate (RS) on the performance of a bioreactor are species-specific. Although these factors need to be investigated case-by-case as they can lead either to damage or self-repair of the affected microorganisms, they have seldom been considered in previous studies. Therefore, this work examined, for the first time, the effects of resting time and RS concentration on the performance of the biosensing system for toxicity of Cr^{6+} in water. In addition, it is also the first time that a novel non-contact fluid delivery system was applied to a toxicity biosensing system to prevent unstable responses. By choosing the best RS concentration and balancing the resting and exposure times, the proposed procedure exhibits promising results in terms of minimum detectable concentration (MDC), limit of detection (LOD), detection range, linearity, sensitivity, reproducibility and accuracy. The recovery time was only a few hours and the coefficients of variation of inhibition and recovery were only 12% and 9.6%, respectively, during six times reuse over one month of storage.

1. Introduction

Access to a clean water supply and sanitation are basic human rights, as declared in Resolution A/RES/64/292 of the United Nations General Assembly. Worldwide water demand has expanded by a factor of six in the past century because of population growth and is expected to continue increasing by 1% every year (UN-Water, 2020). However, climate change is causing a decline in both the quality and quantity of drinking water (UN-Water, 2020). In addition, water resources worldwide are under growing threat from pollution, mainly caused by agricultural activities, industrial dumping, and mining, exacerbated by weak administration (Chaudhry and Malik, 2017). Thus, potential adaptation technologies to mitigate water-related issues and effective monitoring of water quality are essential to securing water supplies in the era of climate change.

Heavy metals are generally defined as metals with relatively high densities compared to water (Ferguson, 1990). Heavy metal pollution mainly comes from metal-based industrial operations, such as mining, foundries, and smelters and adversely affects human health, leading to neuropathy and kidney, liver, respiratory and skin diseases, even at low concentrations (Sall et al., 2020). Heavy metals can be qualitatively and quantitatively analysed using chemical or physical methods, such as chromatography (gas chromatography – GC or high-performance liquid chromatography – HPLC), inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS). However, the cumulative toxicity on a living system is impossible to determine by these methods. Moreover, these techniques are time-consuming and labour-intensive, require skilled personnel, and depend on harmful chemicals and expensive equipment. Therefore, there is increased interest in biological toxicity assays which allow the risk to living species associated with polluted water to be assessed (Hassan et al., 2016). Unfortunately, except for microbial bioassays, biological assays are

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complex, require specialised equipment and skilled operators, and may give rise to ethical issues if higher organisms used (Gunjan and Gargi, 2015). They also take from at least 24 h to up to seven days to complete due to long acclimatisation times (Vosylienė, 2007; Hassan et al., 2016).

Microbial bioassay measures include enzyme levels (Zhang et al., 2018), bacterial growth and mortality (Qiu et al., 2017), and microbial luminescence (Bergua et al., 2021; Mirjani et al., 2021). In addition, measuring respiration inhibition (Yu et al., 2017; Liu et al., 2020) using activated sludge is a standard method for assessing toxicity (ISO, 2007; OECD, 2010). Even though this method does not give rise to ethical issues and is less time-consuming than techniques utilising higher organisms (Gunjan and Gargi, 2015), it cannot be used in online monitoring of toxicity because of the incubation and preparation process. In this context, research on biosensors using microorganisms to assess toxicity has recently gained significant attention due to the advantages over other methods. This method is especially sensitive to different heavy metals (Kurvet et al., 2017), and so has been widely studied and developed. Besides being classified by the bioreceptor element, such as enzymes, oestrogen receptors, antibodies, DNA, or whole cells (Hassan et al., 2016), toxicity biosensors can also be classified according to the transduction element, which can be optical (Linlin et al., 2020; Mao et al., 2020; Bergua et al., 2021), thermal (Ramanathan and Danielsson, 2001), mass sentive (Nolan et al., 2020), or electrochemical (Yang et al., 2018; Adekunle et al., 2019; Liu et al., 2020) which have been widely studied and used due to their low cost, high sensitivity and portable design. However, the sensitivity, precision and accuracy of electrochemical biosensors need to be improved for applications in which interference problems related to the one-pot design and the stress response of microbes must be prevented (Kumar et al., 2017; Yang et al., 2018; Liu et al., 2020). Up to date, there are few solutions have been proposed to avoid such problems, mainly using an isolation or starvation pre-incubation process to inhibit direct contact between heavy metal ions and mediators or buffers as well as the stress response (Yang et al., 2018, 2019; He et al., 2020). As expected, the microbes become more sensitive with toxicants without the presence of a respiration substrate (RS) solution, resulting in an outstanding LOD (lower limit of detection) of 0.03 mg/L and IC_{50} (half maximal inhibitory concentration) of 2.48 mg/L for Cr^{6+} (Yang et al., 2019), of 0.39 mg/L (He et al., 2020) and 0.35 mg/L (Yang et al., 2019) for Cu^{2+}. However, microbial repair cannot be activated in the absence of a suitable RS, causing an irreparable cell damage (Hernández-Martínez et al., 2018), consequently, resulting in a very low upper limit of quantification (ULOQ) which is less than 1 mg/L for Cu^{2+} (He et al., 2020).

In our previous work, a simple packed-bed bioreactor (PBBR) was demonstrated to be easy to self-build and cost-effective and has appropriate sensitivity, detection range, precision, and accuracy for fast detection of biochemical oxygen demand (BOD) (Phuong et al., 2019; Ngoc et al., 2020). When assessing toxicity, the PBBR can be classified as an oxygen-type electrochemical biosensor with an oxygen-permeable membrane; thus, the interference problems related to the one-pot design concept can be completely avoided. In addition, a biosensor with immobilised microorganisms can be expected to overcome the disadvantages of suspended microorganisms, which are widely employed, given the associated complex and time-consuming culture process. With all these advantages, a biosensor based on a PBBR could be a good candidate for an assay of acute toxicity. However, the effects of toxic substances on the performance of bioreactors need to be studied since they can cause either damage or self-repair of immobilised microorganisms, leading to an overestimation or underestimation of the inhibition level, respectively. When operated in semi-continuous mode, the immobilised microbes may need adequate "resting time" between each exposure to recover and avoid a stress response. Therefore, this study set out to determine the optimum substrate concentration and resting time needed to enhance the sensitivity, reproducibility and stability of a biosensor used to assess toxicity. The recovery time and reusability of the PBBR were also investigated. Due to its widely uses as the model toxicant for water toxicity monitoring and water treatment (Dhiman et al., 2020; Kumar et al., 2020), Chromium (Cr^{6+}) was chosen in this study for being employed in both biosensors and conventional oxygen consumption methods (ISO, 2007).

2. Method

2.1. Preparation of stock solutions

The GGA (glucose glutamic acid) solution that is used as a standard in BOD assays (Karube et al., 1977) was employed in this study as an organic substrate to enhance microbial respiratory activity. Accordingly, GGA stock solution equivalent to a BOD_5 of 200 mg/L was prepared by adding 150 mg of D-glucose (HiMedia, India) and 150 mg of glutamic acid (HiMedia, India) to 1 L of distilled water. Unless otherwise stated, the stock RS solution was diluted using drinking water to the desired concentration since it has been proven to be a reasonable alternative to phosphate buffer saline (Liu et al., 2013). The desired toxic sample (TS) solution was prepared by dissolving an appropriate amount of $K_2Cr_2O_7$ (Merck, Germany) in the corresponding RS solution.

2.2. Preparation and operation of a simple toxicity biosensing system

The PBBR was prepared and the type of immobilised microorganisms was qualitatively determined according to the methods described in our recent work (Ngoc et al., 2020). Although the biosensor based on the PBBR was shown to have several advantages, the accumulation of air bubbles and bacteria inside the whole sensing system causes incorrect dissolved oxygen (DO) signals and responses, and this accumulation cannot be completely prevented with a peristaltic pump alone. Consequently, a complicated and time-consuming cleaning process is needed before each test. Working with toxicants can cause this problem to worsen; thus, in this study, the toxicity sensing system was modified by using a novel non-contact fluid delivery system (see Fig. 1A); three air cylinders driven by compressed air from a compressor were employed instead of two three-ways solenoid valves. The air-saturated RS or TS solution was first introduced into the PBBR by switching piston P1 to its outstroke position and keeping P2 and P3 in their instroke positions. The immobilised microorganisms were then exposed to the turbulent flow of the RS or TS solution until a steady-state DO response (DO_{out}) was reached. Next, piston P1 was switched to its instroke position while switching the others to their outstroke positions. The DO signal slightly increased until an initial DO value of the RS or TS solution (DO_{in}) was attained. Meanwhile, the sample was isolated inside the PBBR, and its organic compounds were degraded in the static condition by the immobilised microorganisms. After the desired resting time, the immobilised microorganisms were exposure to the turbulent flow again by switching piston P1 to its outstroke position and the others to their instroke position. The isolated RS or TS solution was released into the DO probe resulting in a decrease followed by an increase in DO response until the steady-state value (DO_{out}) was obtained again, with a peak in the DO profile (H_{peak}). The resting and exposure times were programmed though a self-made switching valve controller. It is clear that, from Fig. 1B, the responses of biosensing system equipped with a non-contact fluid delivery system are more stable across repeated measurements of a GGA solution, equivalent to a BOD_5 of 10 mg/L, than those of the former system (Phuong et al., 2019; Ngoc et al., 2020).

As shown in Fig. 2, unstable DO_{in} and DO_{out} values were unavoidably observed during the analysis of both RS or TS solutions due to the change in the dissolved oxygen level, which fluctuates with environmental temperature. However, the change in DO response (H_{peak}), which is used to determine the bioactivity of the immobilised microorganisms, was not affected by temperature change (Phuong et al., 2019). After changing from the RS to the TS solution, H_{peak} rapidly decreased for five consecutive peaks and then remained almost stable. Accordingly, the inhibition was calculated from the average H_{peak} values of the

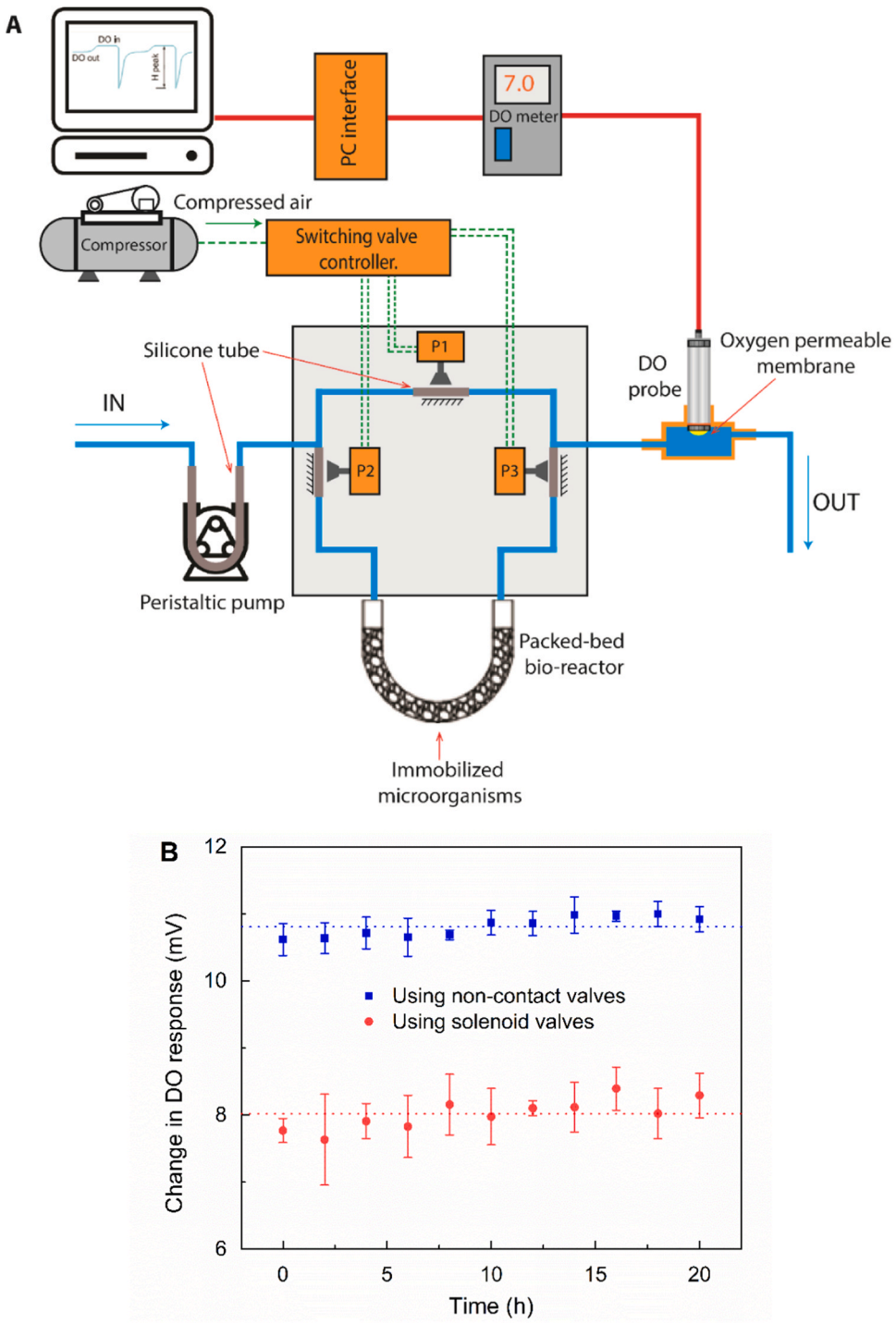

Fig. 1. (A) Schematic representation of the toxicity sensing system based on PBBR and non-contact delivery system; and (B) Reproducibilities of the current biosensing system and the former one (Phuong et al., 2019; Ngoc et al., 2020).

RS and TS solutions obtained from three consecutive measurements in the stable stages via Eq. (1):

$$\text{Inhibition}(\%) = \frac{H_{peak}^{RS} - H_{peak}^{TS}}{H_{peak}^{RS}} \times 100\% \quad (1)$$

The most important operating parameters affecting the performance of the toxicity biosensor, the effects of resting time, which ranged from 1 to 3 min, and RS concentration, which ranged from 5 to 30 mg/L, were studied carefully. Optimisation was carried out by changing one variable while fixing the other to obtain the lowest minimum detectable concentration (MDC) or limit of detection (LOD), and the widest detection range with acceptable linearity and sensitivity. All experiments were carried out in triplicate to determine the standard deviation.

MDC was experimentally determined as the concentration at which a toxicant first causes inhibition, while LOD was calculated according to Eq. (2):

$$LOD = \frac{3\delta_b}{S} \quad (2)$$

where σ_b is the standard deviation of blank measurements obtained from

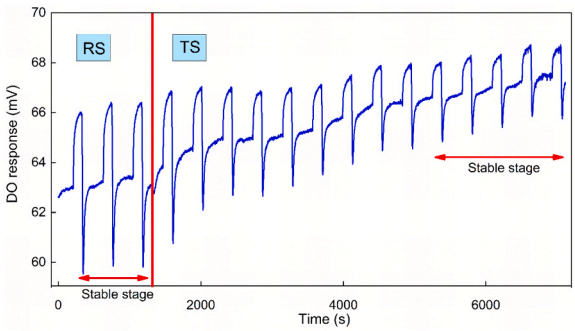

Fig. 2. The DO profiles of toxicity measurement with 10 mg/L Cr^{6+} (resting time t_{res} = 2 min, exposure time t_{exp} = 5 min, RS with BOD_5 = 10 mg/L).

11 blank samples which were prepared using the same procedure without adding chromate and S is the slope of the fitted regression line.

2.3. Determination of accuracy, reproducibility and stability

After obtaining the optimum operating parameters and determining the linear range, a five-level calibration curve with a regression equation and R-square coefficient was additionally generated by linear regression of the inhibition corresponding to the actual Cr^{6+} concentrations. To validate calibration curve accuracy, two quality control TS solutions were prepared and analysed in triplicate, after which the mean determined concentration was compared to the actual concentration and reported as per cent accuracy.

The reproducibility and stability of the developed toxicity biosensor were evaluated by the relative standard deviations (RSD), giving the coefficient of variation (CV). Reproducibility was determined by using three different bioreactors which were prepared using the same procedure, while the stability was assessed by reusing one PBBR which was stored in an air-saturated solution at room temperature for several days to measure the toxicity of the same TS solution. Recovery was calculated from the average H_{peak} values of the RS solutions before and after adding toxicant as shown in Eq. (3).

$$\text{Recovery}(\%) = \frac{H_{peak}^{RS-after}}{H_{peak}^{RS-before}} \times 100\% \quad (3)$$

2.4. Oxygen consumption rate inhibition method

The oxygen consumption rate inhibition method used in this study was based on ISO 8192 (ISO, 2007) and OECD 209 (OECD, 2010). OECD synthetic sewage which was proposed by the Organization for Economic Cooperation and Development (OECD) was used as RS and prepared by diluting 16.0 g peptone, 11.0 g beef extract, 3.0 g of urea, 0.7 g NaCl, 0.4 g $CaCl_2{\cdot}H_2O$, 2.8 g of K_2HPO_4, and 0.2 g $MgSO_4{\cdot}7H_2O$ in 1 L distilled water. A series of TS-OECD solutions with different concentrations of Cr^{6+} was prepared by dissolving appropriate amounts of Cr^{6+} in a solution, which contained 100 mL seeding solution in which total suspended solid content is 9 g/L, 20 mL of the above-prepared RS-OECD, and 200 mL of distilled water. Each bottle was stirred and aerated for 3 h at a constant temperature (20 °C) and then was immediately sealed. The dissolved oxygen content was continuously recorded at 5-min intervals until the DO value decreased to 2.0 mg/L. The rate of oxygen consumption was measured using a DO bench metre (HI5421, Hanna Instruments) and inhibition was calculated via Eq. (4):

$$\text{Inhibition}(\%) = \frac{R_b - R_s}{R_b} \times 100\% \quad (4)$$

where R_b and R_s is the respiration rates of the RS-OECD and TS-OECD, respectively.

3. Results and discussion

3.1. Isolation and identification of immobilised bacteria

After isolation, the bacterial colonies were identified as *Streptomyces, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Nitrosomonas*. There is a slight difference between the bacteria identified and their activity in this study (see Table 1) and our previous work (Ngoc et al., 2020) due to different sampling times. All six isolates can degrade starch while only three of them can additionally degrade protein. With collection during the porridge production stage, the microbial consortium is probably characterised by its use of starch, which is likely to be a major component of processing wastewater.

3.2. Effect of operating parameters

3.2.1. Respiration substrate concentration

It has been reported that an absence of an exogenous carbon source can prevent the stress response of microbes, resulting in enhanced measurement sensitivity (Barron, 1990; Catterall et al., 2010; He et al., 2020). However, the inhibition of heavy metals on endogenous respiration is irreversible and causes irreparable cell damage (Hernández-Martínez et al., 2018). Consequently, the microbes cannot be recovered and reused after toxicity tests in the absence of any RS. However, microbial repair can be activated in the presence of a suitable RS and may cause false-negative inhibition and low sensitivity (Barron, 1990; He et al., 2020; Liu et al., 2020). As summerized in Fig. 3, it is evident that the RS content should be correctly chosen to maximise the efficiency of any toxicity biosensor assay.

Fig. 4 shows the effect of the RS concentration on the inhibition of immobilised microorganisms for Cr^{6+} at different concentrations. It can be observed that inhibition increases gradually and then remains constant with increasing Cr^{6+} toxicant concentration in all RS solutions (see Fig. 4A). However, as can be seen in Table 2, linearity is very good for all cases as all R^2 values are above 0.95, which is one of the most important criteria for toxicity assay validation (Catterall et al., 2010), but sensitivity and linear range differ case-by-case. Working in the presence of higher concentrations of RS may result in problems of false-negative inhibition and low sensitivity as mentioned above. Consequently, the toxicity biosensing system showed the lowest sensitivity when operated at the highest RS content.

Although a plateau in inhibition occurred in all cases, the narrowest and widest linear ranges were observed when using RS solutions with BOD_5 values of 5 mg/L and 10 mg/L, respectively. It is widely accepted that the exogenous and endogenous respiration of microorganisms is inhibited by heavy metals at different levels and that the inhibition of endogenous respiration is much smaller than that of exogenous respiration (Dietrich and Burris, 1967; Stasinakis et al., 2003; Hernández-Martínez et al., 2018). Hernández-Martínez et al. (2018) observed three distinct zones, namely, no inhibition, inhibition of exogenous respiration only, and inhibition of both exogenous and endogenous respiration, corresponding to silver nanoparticle concentrations of less than 2, less than 4 and over 4 mg/L, respectively. This finding explains the plateau

Table 1
Identification and bioactivity of the isolated bacterial colonies.

No.	Isolation	Bioactivity				Identification
		Starch	Protein	Lipid	Cellulose	
1	S1	+	–	–	+	*Streptomyces*
2	B1	+	+	+	–	*Bacillus subtilis*
3	B2	+	–	–	+	*Bacillus cereus*
4	B3	+	+	–	+	*Pseudomonas aeruginosa*
5	B4	+	+	–	+	*Pseudomonas fluorescens*
6	B5	+	–	–	+	*Nitrosomonas*

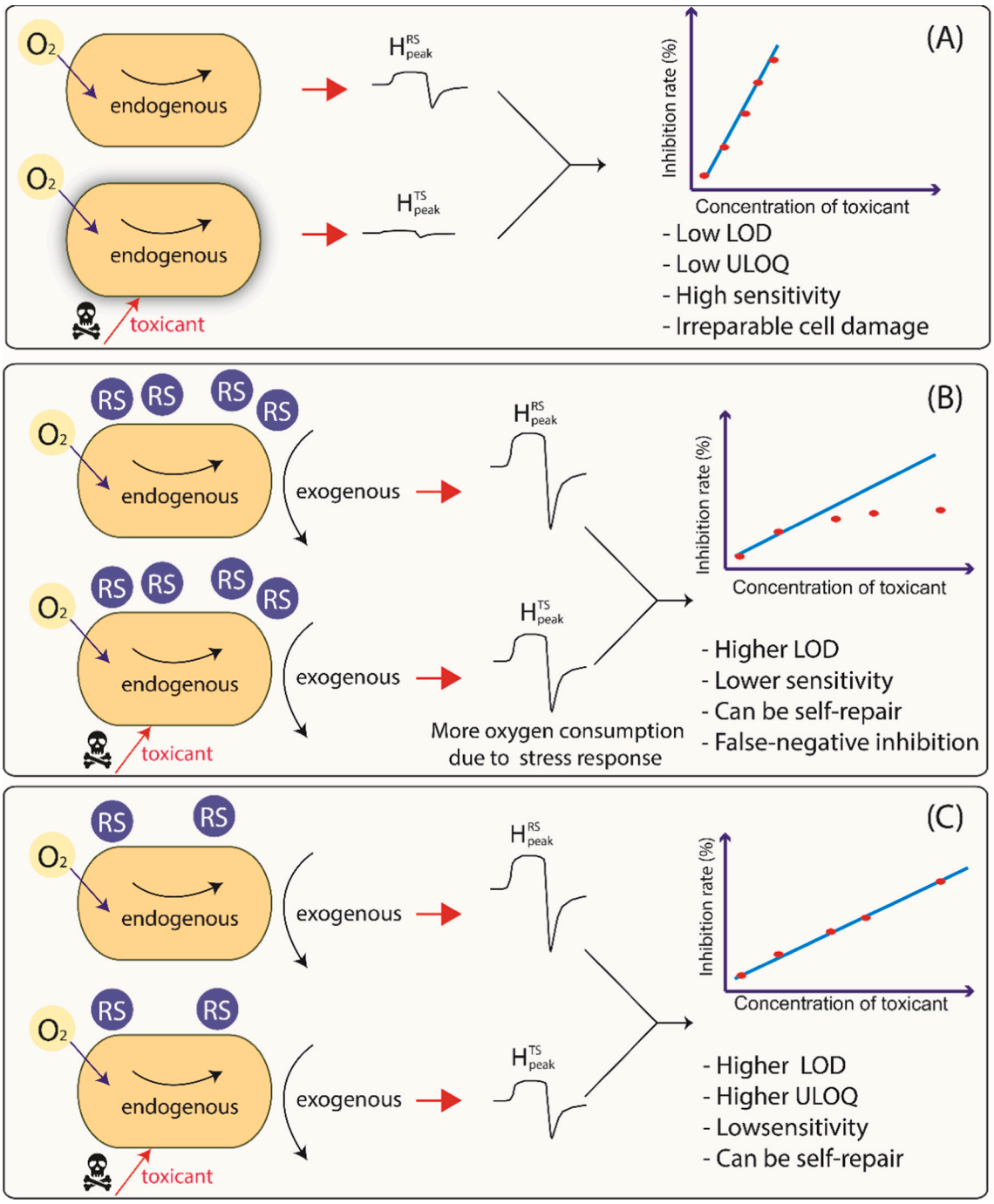

Fig. 3. Relationship of inhibition and toxicant concentration (A) in the absence of a RS, (B) in the presence of RS, and (C) in the presence of limited amount of RS.

in inhibition that was observed at a Cr^{6+} concentration of 5 mg/L when using an RS solution with a BOD_5 value of 5 mg/L. In contrast, the presence of a higher trophic level may activate microbial repair, resulting in a plateau in inhibition, as can be seen in both other cases. In addition, the membrane topology of the plasmid-encoding ChrA protein is known to be able to efflux chromate ions and reduce chromate accumulation and thus is responsible for the chromate-resistance of *Pseudomonas aeruginosa* and *Bacillus cereus*, which were predominant among the immobilised microorganisms (Cervantes et al., 1990; Jiménez-Mejía et al., 2006; Xia et al., 2021). Cr^{6+} resistance of L. *fusiformis* ZC1 has been determined by incubation in the presence of different amounts of K_2CrO_4 (He et al., 2011). Cell density gradually decreases to about 70% with increasing concentrations of K_2CrO_4 up to 10 mM, then reaches a plateau and slightly declines thereafter. The MIC of L. *fusiformis* ZC1 to Cr^{6+} was found to be 60 mM, which is six times higher than the value at which a plateau occurs. From these results, it is clear that the chromate-resistance of particular species in the immobilised microorganisms can explain the plateau in inhibition obtained from the toxicity biosensing system with all RS solutions.

Two well-known transport processes occur in a PBBR containing immobilised bacteria. The first is the transfer of RS, oxygen and toxicant from the bulk liquid phase to the bacterial surface, and the second is the simultaneous diffusion and RS consumption within the microbes. According to the film theory, there is a fictitious laminar film next to the boundary of any surface in contact with a flowing fluid (Kathiravan et al., 2010). The RS, oxygen and toxicant need to be transported to the

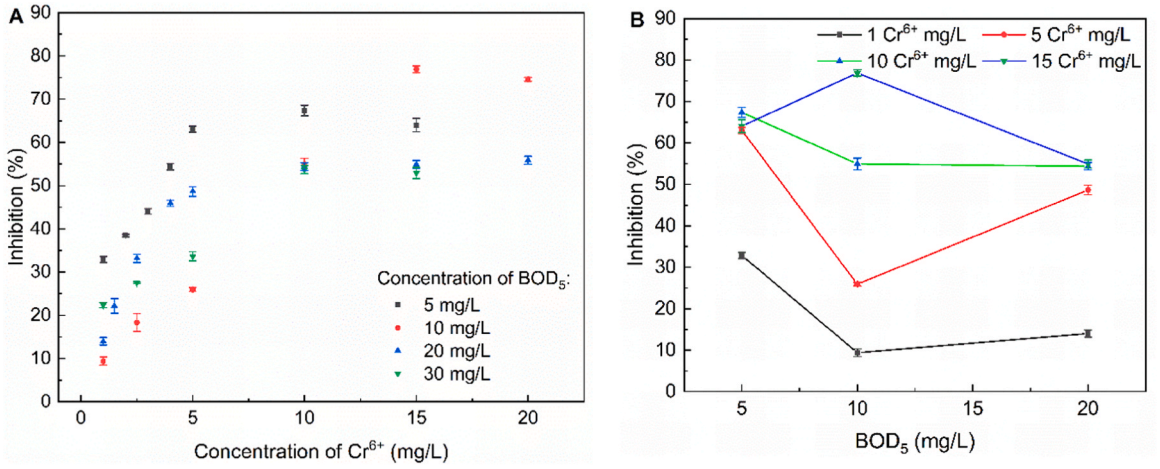

Fig. 4. Effect of substrate concentration on the inhibition of immobilised microorganisms for Cr^{6+} of different concentration in the form of (A) inhibition vs. concentration of Cr^{6+}, and (B) inhibition vs. BOD_5 (t_{res} = 2 min, t_{exp} = 5 min).

Table 2
Performance of toxicity biosensing system at different RS concentration.

RS concentration (as BOD_5, mg/L)	Sensitivity	Linearity (Adj-R^2)	Linear range (mg/L)
5	7.6313	0.9823	1–5
10	4.8555	0.9954	1–15
20	8.6895	0.9584	1–5
30	3.4711	0.9905	1–10

region by molecular diffusion to be consumed by immobilised bacteria and to affect their respiration. Practically, the toxic response depends on the exposure concentration $[Cr^{6+}]_{ex}$, which is lower than the toxicant concentration in the bulk liquid phase $[Cr^{6+}]_{liq}$ and depends on external mass transfer. However, exogenous oxygen uptake may induce the perturbation of dissolved oxygen, resulting in enhancement of this transfer (Vanrolleghem et al., 1992) and, consequently, to higher toxicant concentration at the reaction site of microorganisms. Accordingly, at the same $[Cr^{6+}]_{liq}$ value, $[Cr^{6+}]_{ex}$ increases with higher RS concentrations, increasing inhibition. However, microbial repair, which causes a false-negative IR, can be activated in the presence of high $[Cr^{6+}]_{ex}$ together with high RS concentrations, leading to more complex results, as can be seen in Fig. 4B. It can be concluded that the repair effect is unlikely to occur in a much lower trophic condition with the RS solution at a BOD_5 of 5 mg/L. At higher RS concentrations, these two effects conflict with each other, but the repair effect seems to be more dominant than the transfer effect with increases in both RS concentration and $[Cr^{6+}]_{liq}$. Consequently, the RS solution with a BOD_5 of 10 mg/L should be chosen as the optimum condition to obtain the lowest MDC and widest detection range with good linearity and acceptable sensitivity.

3.2.2. *Resting time*

The effect of resting time was investigated in the range of 1–3 min, while the exposure time was kept constant at 5 min; the results are shown in Fig. 5. Although inhibition increases gradually with increasing chromate concentration regardless of resting times, sensitivity and linear range are different case-by-case (see Fig. 5A and Table 3). As mentioned, inhibition depends on exposure concentration, which is

Table 3
Performance of toxicity biosensing system at different resting times.

Resting time (min)	Sensitivity	Linearity (Adj-R^2)	Linear range (mg/L)	LOD (mg/L)
1	38.4199	0.9882	0.1–1.0	0.0097
	1.1435	0.9941	1.0–10	0.3253
2	4.8886	0.9963	0.1–15	0.0762
3	4.0401	0.9886	0.1–15	0.0921

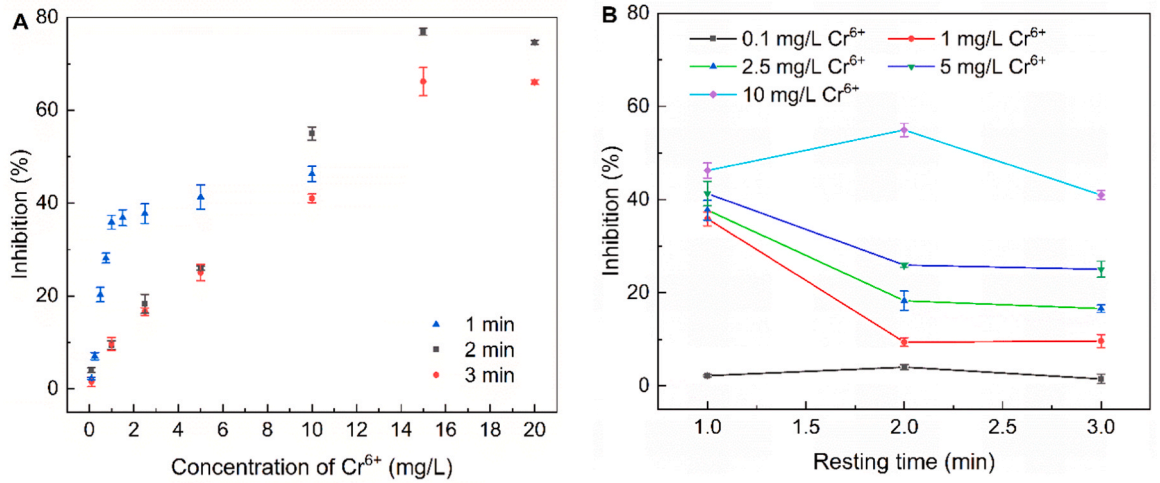

Fig. 5. Effect of resting time on the inhibition of immobilised microorganisms for Cr^{6+} of different concentration in the form of (A) inhibition vs. concentration of Cr^{6+}, and (B) inhibition vs. resting time (t_{exp} = 5 min, RS with BOD_5 = 10 mg/L).

affected by the toxicant concentration in the bulk liquid phase and external mass transfer. It is known that turbulent diffusion occurs much more rapidly than molecular diffusion, and so a shorter resting time leads to a higher mass transfer rate, resulting in a higher $[Cr^{6+}]_{ex}$ at the same $[Cr^{6+}]_{liq}$. Accordingly, as is shown in Fig. 5B, the inhibition of immobilised microorganisms generally decreases with increasing resting time. Very high sensitivity was observed at a resting time of 1 min, because an almost continuous mode at a high flow rate will lead to high turbulence, reduce boundary layer effects, and transport toxicants from the bulk liquid to the immobilised microorganisms. However, such a high $[Cr^{6+}]_{ex}$ can quickly activate microbial repair, causing a decrease in sensitivity above 1 mg/L Cr^{6+}. Meanwhile, increasing resting time to 2 min results in a decrease in sensitivity due to lower $[Cr^{6+}]_{ex}$ at the same $[Cr^{6+}]_{liq}$ but, in turn, the linear range is extended to 15 mg/L Cr^{6+}. Further increasing resting time to 3 min does not enhance the linear range but leads to a decrease in sensitivity. A prolonged resting time can increase the risk of insufficient dissolved oxygen supply for both endogenous and exogenous respiration.

3.3. Accuracy, reproducibility and stability

After determining the optimum conditions, the proposed toxicity assay was evaluated for accuracy, reproducibility and stability using the RS solution with BOD_5 of 10 mg/L and a resting time of 2 min. The traditional oxygen consumption rate inhibition method was also employed to make a comparison.

Fig. 6 shows that, in comparison with the traditional oxygen consumption rate inhibition method, the developed toxicity assay demonstrated higher precision, linearity, and wider linear range. In addition, the proposed method exhibits better reproducibility with RSD values of less than 10% compared to the value of 30% for the traditional method (OECD, 2010). Both traditional and developed methods offer comparable LOD values of 0.0839 and 0.0762 mg/L, respectively; however, a much lower MDC of 0.1 mg/L was experimentally confirmed compared to 1 mg/L for the traditional method. Therefore, although the traditional method exhibits higher sensitivity due to continuous and vigorous mixing, and consequently, a lower IC_{50} value compared to the proposed method, it cannot provide an early warning of the presence of toxic chromate in water at concentrations as low as 0.1 mg/L, which is the U. S. Environmental Protection Agency's standard MCL for chromium in drinking water (Higgins et al., 1998).

As can be seen in Fig. 7, the actual and calculated Cr^{6+} concentrations from the regression equation shown in Fig. 6A agree well, with the largest error at 0.23 mg/L. The higher the chromate concentration used, the better the accuracy. The results further demonstrate the applicability of our proposed approach for determining the toxicity of heavy metals in water.

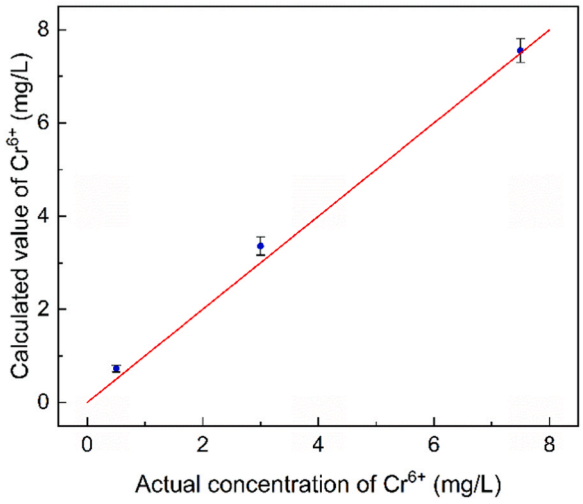

Fig. 7. Relationship between actual and calculated concentration of Cr^{6+} obtained from the proposed toxicity assay (t_{res} = 2 min, t_{exp} = 5 min, RS with BOD_5 = 10 mg/L).

Storage stability tests were conducted to determine the feasibility of reusing the PBBR after it was stored in an air-saturated solution at room temperature for several days to measure the toxicity of the same TS solution. Each run was performed in three steps. First, the PBBR was operated with an RS solution until a stable H_{peak} value was achieved. Then, the flow was switched to TS solution (point 1); as can be seen in Fig. 8A, the H_{peak} value decreases due to the inhibition effect of Cr^{6+} on the immobilised microorganisms until stability was again achieved. After switching back to the RS solution (point 2), the respiration of the immobilised microorganisms recovers, increasing the H_{peak} value. The recovery time is only a few hours, which is much shorter than the time reported in other studies (Adekunle et al., 2019; Liu et al., 2020). Similar trends were observed, although with slightly different results, as can be seen in Fig. 8B. However, the CVs of inhibition and recovery were only 12% and 9.6%, respectively, confirming the acceptable stability of the PBBR after toxicity measurements.

In addition, a systematic comparison between the results obtained from this work and others works is also presented in Table 4. It is clear that, the performance of the developed biosensing system is better than

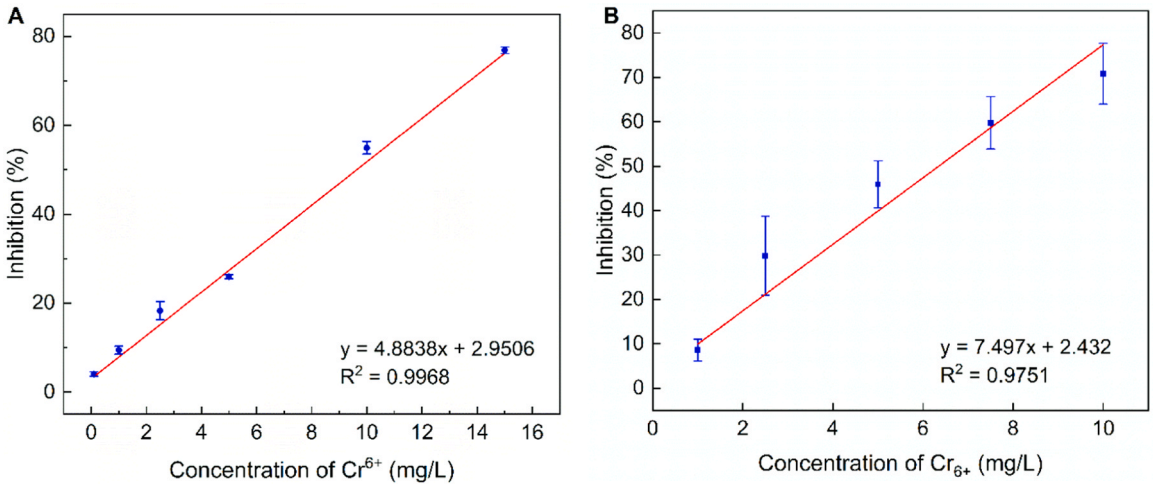

Fig. 6. Linear relationships between the inhibition and Cr^{6+} concentrations assessed by (A) the developed biosensing system (t_{res} = 2 min, t_{exp} = 5 min, RS with BOD_5 = 10 mg/L), and (B) the traditional oxygen consumption rate inhibition method.

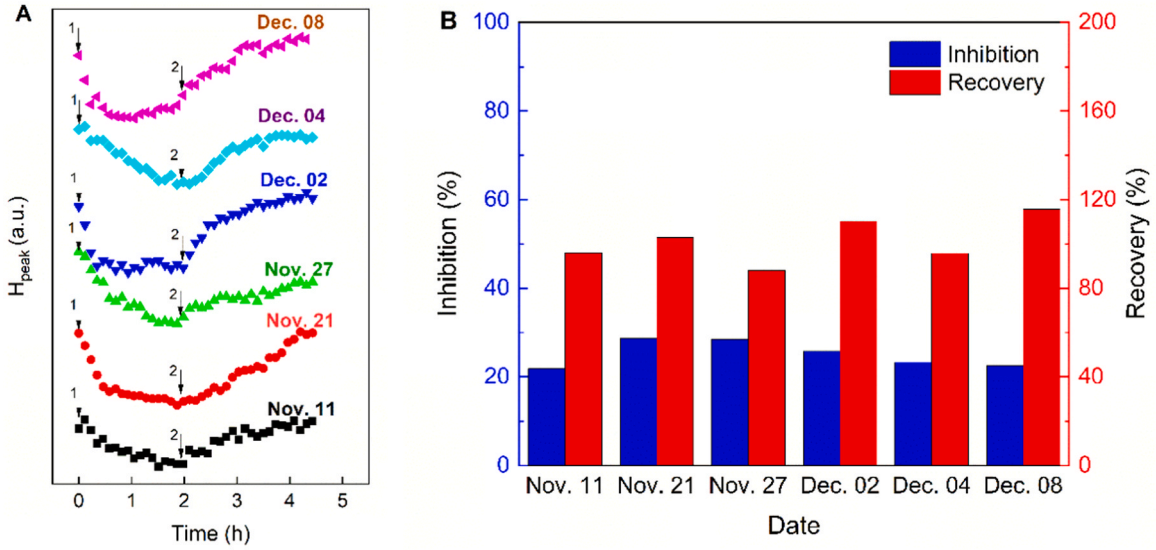

Fig. 8. The storage stability of the PBBR used for Cr^{6+} toxicity measurements: (A) H_{peak} values obtained from using TS solution with 5 mg/L Cr^{6+} (started at point 1) and RS solution with BOD_5 of 10 mg/L (started at point 2); (B) calculated inhibition and recovery for different testing day (t_{res} = 2 min, t_{exp} = 5 min, RS with BOD_5 = 10 mg/L).

Table 4
Performance of toxicity biosensing system for Cr^{6+} in this work and other works.

Toxicity assays	Time		Microbial sources	LOD	IC_{50}/EC_{50} (mg/L)	Linearity range	Estimated cost	Reusable	Refs.
	Incubation time	Response time							
Oxygen-type electrochemical biosensor	0	4000 s[a,b]	Activated sludge	0.3253	9.63	0.1 – 15	Investment 1000 USD Operating 10 USD/test	Yes	This study
A mediator-free whole-cell electrochemical biosensing system	60 min	3000 s[a,c]	*Shewanella oneidensis*	0.03	2.48	N/A[d]	N/A	No	(Yang et al., 2019)
Colorimetric biosensor	30 min	N/A	*E. coli* strain ATCC 25922	51	N/A	N/A	N/A	No	(Wasito et al., 2019)
Amperometric biosensor	90 min		*Psychrobacter* sp.	N/A	14	N/A	N/A	No	(Wang et al., 2013)
A batch-mode cube microbial fuel cell	0	>30 min	Fresh wastewater	1[e]	N/A	N/A	N/A	No	(Liu et al., 2014)
Flat microliter membrane-based microbial fuel cell	0	60–120 min	Electrogenic bacteria	>5[e]	N/A	N/A	N/A	Yes[f]	(Xu et al., 2015)
Nitrification inhibition	2 h	2 h	Nitrifying bacteria	N/A	35.6	N/A	Investment 921.4 € Operating 138.8 €/test	No	(Dalzell et al., 2002)
Respiration inhibition	3 h	1 h	Activated sludge	N/A	36.2	N/A	Investment 27,045.5 € Operating 21.6 €/test	No	
ATP luminescence	30 min	2 h	Activated sludge	N/A	37.5	N/A	Investment 39,834.0 € Operating 119.9 €/test	No	
In vivo L-alanine-aminopeptidase inhibition	15 min	2 h 15	Activated sludge	N/A	228.5	N/A	Investment 5112.9 € Operating 30.1 €/test	No	
Microtox	30 min	1 h 15	*Vibrio fischeri*	N/A	22.5	N/A	Investment 17,612.3 € Operating 48.9 €/test	No	

[a] The higher the concentration of toxicant, the longer the response time.
[b] Operated with 10 mg/L Cr^{6+}.
[c] Operated with 0.4 mg/L Cu^{2+}.
[d] Not available.
[e] Shock test.
[f] Requires 80 h to be recovered to the original level after shocks.

that of the others both in terms of technical and economical aspect. This result proved that the proposed assay has high potential for actual application.

4. Conclusion

In this work, a novel biosensing system based on a PBBR was developed for rapid and accurate measurement of toxicity of Cr^{6+} in water. Optimisation results showed that the lowest MDC (0.1 mg/L), lowest LOD (0.0762 mg/L), widest detection range (0.1–15 mg/L), highest linearity (Adj-$R^2 = 0.9963$) and acceptable sensitivity (4.8886) were achieved with an RS concentration with BOD_5 of 10 mg/L at a resting time of 2 min. In addition, a better reproducibility was obtained from the proposed biosensing method (RSD < 10%) compared to the traditional one (RSD > 30%). The developed biosensing system also exhibits a good accuracy which is up to 97% and the feasibility of reusing the PBBR after toxicity measurements. The reasonable investment cost of the whole system, which can be as low as 1000 US dollars for the standard version and minimal automation level, together with its low operating cost of 10 US dollars per test, make the developed biosensing system more applicable for toxicity early warning, especially in developing countries.

The predominant presence of *Pseudomonas aeruginosa* and *Bacillus cereus*, whose chromate-resistance is widely known, and the self-repair effect may both be responsible for the plateau in inhibition obtained from both the traditional and developed toxicity assays, regardless of the operating conditions. Thus, future work will focus on developing a PBBR from a specific microorganism that has no resistance ability against the target toxicants.

CRediT authorship contribution statement

Le Thi Bao Ngoc: Methodology, Investigation, Writing - original draft, Formal analysis, Funding acquisition. **Duong Nhat Linh:** Investigation, Writing - original draft. **Nguyen Van Minh:** Methodology, Investigation. **Nguyen Phuc Hoang Duy:** Conceptualization, Software, Investigation. **Pham Thi Thuy Phuong:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Funding acquisition.

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.

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References

Adekunle, A., Raghavan, V., Tartakovsky, B., 2019. On-line monitoring of heavy metals-related toxicity with a microbial fuel cell biosensor. Biosens. Bioelectron. 132, 382–390.
Barron, M.G., 1990. Bioconcentration. Will water-borne organic chemicals accumulate in aquatic animals? Environ. Sci. Technol. 24, 1612–1618.
Bergua, J.F., Álvarez-Diduk, R., Hu, L., Hassan, A.H.A., Merkoçi, A., 2021. Improved Aliivibrio fischeri based-toxicity assay: graphene-oxide as a sensitivity booster with a mobile-phone application. J. Hazard. Mater. 406, 124434.
Catterall, K., Robertson, D., Hudson, S., Teasdale, P.R., Welsh, D.T., John, R., 2010. A sensitive, rapid ferricyanide-mediated toxicity bioassay developed using Escherichia coli. Talanta 82, 751–757.
Cervantes, C., Ohtake, H., Chu, L., Misra, T.K., Silver, S., 1990. Cloning, nucleotide sequence, and expression of the chromate resistance determinant of Pseudomonas aeruginosa plasmid pUM505. Bacteriol 172, 287–291.
Chaudhry, F., Malik, M., 2017. Factors affecting water pollution: a review. Ecosyst. Ecography 7, 1–3.
Dalzell, D.J., Alte, S., Aspichueta, E., de la Sota, A., Etxebarria, J., Gutierrez, M., Hoffmann, C.C., Sales, D., Obst, U., Christofi, N., 2002. A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge. Chemosphere 47, 535–545.
Dhiman, P., Sharma, S., Kumar, A., Shekh, M., Sharma, G., Naushad, M., 2020. Rapid visible and solar photocatalytic Cr(VI) reduction and electrochemical sensing of dopamine using solution combustion synthesized ZnO–Fe_2O_3 nano heterojunctions: mechanism elucidation. Ceram. Int. 46, 12255–12268.
Dietrich, S.M., Burris, R.H., 1967. Effect of exogenous substrates on the endogenous respiration of bacteria. J. Bacteriol. 93, 1467–1470.
Ferguson, J.E., 1990. The Heavy Elements: Chemistry, Environmental Impact and Health Effects, New York.
Gunjan, D., Gargi, B., 2015. Toxicity tests to check water quality. Int. Res. J. Environ. Sci. 4, 87–90.
Hassan, S.H.A., Ginkel, Van, Hussein, S.W., Abskharon, M.A.M., Oh, S.-E, R., 2016. Toxicity assessment using different bioassays and microbial biosensors. Environ. Int. 92–93, 106–118.
He, J., Zhai, J., Yu, D., Fang, Y., Liu, C., Liu, L., Dong, S., 2020. A respiration substrate-less isolation method for acute toxicity assessment. Chemosphere 244, 125511.
He, M., Li, X., Liu, H., Miller, S.J., Wang, G., Rensing, C., 2011. Characterization and genomic analysis of a highly chromate resistant and reducing bacterial strain Lysinibacillus fusiformis ZC1. J. Hazard. Mater. 185, 682–688.
Hernández-Martínez, G.R., Zepeda, A., Ordaz, A., Sánchez-Catzin, L.A., Estrada-Díaz, Z. D., Thalasso, F., 2018. High-throughput microrespirometric characterization of activated sludge inhibition by silver nanoparticles. Environ. Sci. Water Res. Technol. 4, 721–730.
Higgins, T.E., Halloran, A.R., Dobbins, M.E., Pittignano, A.J., 1998. In situ reduction of hexavalent chromium in alkaline soils enriched with chromite ore processing residue. J. Air Waste Manag. Assoc. 48, 1100–1106.
ISO, 2007. Water Quality–Test for Inhibition of Oxygen Consumption by Activated Sludge for Carbonaceous and Ammonium Oxidation. International Organization for Standardization, Geneva.
Jiménez-Mejía, R., Campos-García, J., Cervantes, C., 2006. Membrane topology of the chromate transporter ChrA of Pseudomonas aeruginosa. FEMS Microbiol. Lett. 262, 178–184.
Karube, I., Matsunaga, T., Mitsuda, S., Suzuki, S., 1977. Microbial electrode BOD sensors. Biotechnol. Bioeng. 19, 1535–1547.
Kathiravan, M.N., Karthiga Rani, R., Karthick, R., Muthukumar, K., 2010. Mass transfer studies on the reduction of Cr(VI) using calcium alginate immobilized Bacillus sp. in packed bed reactor. Bioresour. Technol. 101, 853–858.
Kumar, A., Sharma, S.K., Sharma, G., Naushad, M., Stadler, F.J., 2020. CeO2/g-C3N4/V2O5 ternary nano hetero-structures decorated with CQDs for enhanced photo-reduction capabilities under different light sources: dual Z-scheme mechanism. J. Alloy. Compd. 838, 155692.
Kumar, R., Singh, L., Zularisam, A.W., Hai, F.I., 2017. Microbial fuel cell is emerging as a versatile technology: a review on its possible applications, challenges and strategies to improve the performances. Int. J. Energ. Res. 42, 369–394.
Kurvet, I., Juganson, K., Vija, H., Sihtmäe, M., Blinova, I., Syvertsen-Wiig, G., Kahru, A., 2017. Toxicity of nine (doped) rare earth metal oxides and respective individual metals to aquatic microorganisms Vibrio fischeri and Tetrahymena thermophila. Materials 10, 754.
Linlin, G. Yang, G., Yang, B., Gu, J., 2020. Development of the yeast Saccharomyces cerevisiae as a biosensor for the toxicity detection of toxic substances. bioRxiv.
Liu, B., Lei, Y., Li, B., 2014. A batch-mode cube microbial fuel cell based "shock" biosensor for wastewater quality monitoring. Biosens. Bioelectron. 62, 308–314.
Liu, C., Zhao, H., Gao, S., Jia, J., Zhao, L., Yong, D., Dong, S., 2013. A reagent-free tubular biofilm reactor for on-line determination of biochemical oxygen demand. Biosens. Bioelectron. 45, 213–218.
Liu, L., Zhang, H., He, J., Dong, S., 2020. Investigation on the stress response of microbes in acute toxicity assay. Anal. Chim. Acta 1099, 46–51.
Mao, Y., Gao, S., Yao, L., Wang, L., Qu, H., Wu, Y., Chen, Y., Zheng, L., 2021. Single-atom nanozyme enabled fast and highly sensitive colorimetric detection of Cr (VI). J. Hazard. Mater. 408, 124898.
Mirjani, M., Soleimani, M., Salari, V., 2021. Toxicity assessment of total petroleum hydrocarbons in aquatic environments using the bioluminescent bacterium Aliivibrio fischeri. Ecotoxicol. Environ. Saf. 207, 111554.
Ngoc, L.T.B., Tu, T.A., Hien, L.T.T., Linh, D.N., Tri, N., Duy, N.P.H., Cuong, H.T., Phuong, P.T.T., 2020. Simple approach for the rapid estimation of BOD_5 in food processing wastewater. Environ. Sci. Pollut. Res. 1–11.
Nolan, P., Auer, S., Spehar, A., Oplatowska-Stachowiak, M., Campbell, K., 2021. Evaluation of mass sensitive micro-array biosensors for their feasibility in multiplex detection of low molecular weight toxins using mycotoxins as model compounds. Talanta 222, 121521.
OECD, 2010. Test No. 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation).
Phuong, P.T.T., Duy, N.P.H., Van, N.T.T., Linh, D.H.T., 2019. Self-build packed-bed bioreactor for rapid and effective BOD estimation. Environ. Sci. Pollut. Res. 26, 25656–25667.
Qiu, T.A., Nguyen, T.H.T., Hudson-Smith, N.V., Clement, P.L., Forester, D.-C., Frew, H., Hang, M.N., Murphy, C.J., Hamers, R.J., Feng, Z.V.J.Ac, 2017. Growth-based

bacterial viability assay for interference-free and high-throughput toxicity screening of nanomaterials. Anal. Chem. 89, 2057–2064.
Ramanathan, K., Danielsson, B., 2001. Principles and applications of thermal biosensors. Biosens. Bioelectron. 16, 417–423.
Sall, M.L., Diaw, A.K.D., Gningue-Sall, D., Efremova Aaron, S., Aaron, J.-J., 2020. Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. Environ. Sci. Pollut. Res. 27, 29927–29942.
Stasinakis, A.S., Thomaidis, N.S., Giannes, A.S., Lekkas, T.D., 2003. Effect of arsenic and mercury speciation on inhibition of respiration rate in activated sludge systems. Environ. Sci. Pollut. Res. Int. 10, 177–182.
UN-Water, 2020. Water and Climate Change. World Water Development Report 2020.
Vanrolleghem, P., Van Impe, J., Vanderwalle, J., Verstraete, W., 1992. Advanced monitoring and control of the activated sludge process: on-line estimation of crucial biological variables in a structured model with the RODTOX biosensor. IFAC Proc. 25, 355–358.
Vosylienė, M.Z., 2007. Review of the methods for acute and chronic toxicity assessment of single substances, effluents and industrial waters. Acta Zool. Litu. 17, 3–15.
Wang, X., Liu, M., Wang, X., Wu, Z., Yang, L., Xia, S., Chen, L., Zhao, J., 2013. P-benzoquinone-mediated amperometric biosensor developed with Psychrobacter sp. for toxicity testing of heavy metals. Biosens. Bioelectron. 41, 557–562.
Wasito, H., Fatoni, A., Hermawan, D., Susilowati, S.S., 2019. Immobilized bacterial biosensor for rapid and effective monitoring of acute toxicity in water. Ecotoxicol. Environ. Saf. 170, 205–209.
Xia, X., Wu, S., Zhou, Z., Wang, G., 2021. Microbial Cd (II) and Cr (VI) resistance mechanisms and application in bioremediation. J. Hazard. Mater. 401, 123685.
Xu, Z., Liu, B., Dong, Q., Lei, Y., Li, Y., Ren, J., McCutcheon, J., Li, B., 2015. Flat microliter membrane-based microbial fuel cell as "on-line sticker sensor" for self-supported in situ monitoring of wastewater shocks. Bioresour. Technol. 197, 244–251.
Yang, Y., Fang, D., Liu, Y., Liu, R., Wang, X., Yu, Y., Zhi, J., 2018. Problems analysis and new fabrication strategies of mediated electrochemical biosensors for wastewater toxicity assessment. Biosens. Bioelectron. 108, 82–88.
Yang, Y., Fang, Z., Yu, Y.-Y., Wang, Y.-Z., Naraginti, S., Yong, Y.-C., 2019. A mediator-free whole-cell electrochemical biosensing system for sensitive assessment of heavy metal toxicity in water. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. 79, 1071–1080.
Yu, D., Bai, L., Zhai, J., Wang, Y., Dong, S., 2017. Toxicity detection in water containing heavy metal ions with a self-powered microbial fuel cell-based biosensor. Talanta 168, 210–216.
Zhang, Y., Ren, T., Tian, H., Jin, B., He, J., 2018. Hydrogel-encapsulated enzyme facilitates colorimetric acute toxicity assessment of heavy metal ions. ACS Appl. Mater. Interfaces 10, 26705–26712.