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On-line monitoring of heavy metals-related toxicity with a microbial fuel cell biosensor

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ABSTRACT

This study describes an environmental biosensor for real time toxicity monitoring, which exploits high sensitivity of a microbial fuel cell (MFC) to variations in concentrations of electron donors and acceptors. Fast biosensor response to changes in total heavy metal concentration of a mining rock drainage was observed in laboratory tests with acceptable repeatability and a coefficient of determination (R^2) of 0.95–0.97. The biosensor response is attributed to interference of heavy metals with the activity of electroactive microorganisms. Biomolecular analysis of the anodic electroactive biofilms showed significant population differences between microbial populations of the biosensor exposed to heavy metals and a non-exposed (control) biosensor. Furthermore, the biosensor outputs were highly correlated ($R^2 = 0.92$) with the results of the Microtox toxicity assay. The results of this study contribute towards the development of a simple MFC-based biosensor capable of detecting, in real-time, changes in environmental conditions and providing a tool for on-site toxicity monitoring.

1. Introduction

Natural resources exploitation, such as mining, creates significant disturbances to ecosystems with a risk of residual materials propagating outside designated exploitation areas. Heavy metals may leach from mining sites affecting downstream water quality and causing acute and chronic toxicity in living organisms (Colmer and Hinkle, 1947; Maree et al., 1987). Accordingly, extensive environmental monitoring is of great importance during resource exploitation and after mining site closure; sometimes in perpetuity (Rathore and Wright, 1993). Frequent measurements are crucial for monitoring toxicity progression and timely detection of contaminant propagation. Unfortunately, existing environmental monitoring methods that employ microorganisms, bioluminescent bacteria, daphnia, algae or fish for in-vitro or in-vivo assays (Farré and Barceló, 2003; Reemtsma, 2001; Tothill and Turner, 1996) are laborious and require several hours to several days of laboratory analysis. The development of on-line (e.g. real-time measurements) toxicity monitoring sensors, which can be deployed in a field for continuous toxicity measurements, is important for early detection of contaminants. Microbial fuel cell (MFC) technology provides an approach for developing inexpensive, low maintenance autonomous biosensor that can be used for real-time monitoring of environmental conditions, including the presence of toxic compounds.

MFC is a bioelectrochemical system, which is actively studied for production of electricity from organic wastes and, more recently, for biosensing (Adekunle et al., 2019; Dávila et al., 2011; Di Lorenzo et al., 2014; Feng and Harper Jr, 2013; Kim et al., 2007; Yi et al., 2019; Yu et al., 2017; Zhao et al., 2019). In an MFC, electroactive microorganisms (electrogens) oxidize organic matter under anaerobic conditions through electron transfer to the anode, while oxygen reduction occurs at the cathode, leading to an electron flow between the electrodes. Current production in an MFC will depend on the presence of electroactive microorganisms, as well as on organic and inorganic compounds, which interfere with the anodic electron transfer by acting as electron acceptors (e.g. metals) or donors (e.g. organic materials) (Rabaey, 2005). This sensitivity of current generation to various compounds in the MFC environment forms the basis for MFC application in biosensing, as it is possible to correlate current with changes in analyte composition (Adekunle et al., 2019; Feng et al., 2013; Gurung et al., 2012; Kim et al., 2010; Yu et al., 2017; Zhang and Angelidaki, 2012; Zhou et al., 2018). Notably, single-chamber membraneless MFCs are simple and inexpensive in construction, since a proton exchange membrane is not used. These MFCs can operate for several years with little or no maintenance (Adekunle et al., 2017) giving this bioelectrochemical system an edge over conventional environmental monitoring methods.

In this study, the applicability of MFC-based biosensors for long-term (years) on-line (e.g. 5–10 min measurement interval) monitoring

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of toxicity related to the presence of heavy metals in mining rock drainage is investigated. This work builds on our recent study, which demonstrated higher sensitivity and stability of MFCs as compared to microbial electrolysis cells (MECs) for on-line toxicity monitoring (Adekunle et al., 2019). Here, we develop and test a simple algorithm for enabling toxicity estimations during biosensor exposure to varying levels of heavy metals and compare MFC - based biosensing results to toxicity results using the Microtox toxicity assay (Bulich, 1979). Finally, we examine the effect of heavy metals on the microbial community of an electroactive biofilm.

2. Materials and methods

2.1. Analytical methods and stock solutions

Heavy metal concentrations were measured by inductive coupled plasma mass spectrophotometry (ICP-MS) as described elsewhere (Beauchemin, 2010). Acetate concentration was measured by gas chromatography according to a previously described method (Tartakovsky et al., 2008). Total chemical oxygen demand (COD) concentration was measured according to Standard Methods (APHA et al., 1995). Water sample conductivity and pH were measured with Accumet AP75 conductivity meter and Orion Star A211 pH meter (ThermoFisher Scientific, Waltham, MA, USA), respectively.

A stock solution of an acetate-based carbon source consisting of sodium acetate, microelements and nutrients, is described elsewhere (Pinto et al., 2011). Mining rock drainage was obtained by percolating deionized water through a bed of mine tailings collected at the Canadian Malarctic mine (Quebec, Canada). Approximately 20 kg of crushed rocks obtained from mine tailings were loaded into a 20 L bucket and watered from the top at a flow rate of approximately 225 mL d^{-1} using perforated tubing wound in concentric circles. The leachate was recovered at the bottom of the bucket and collected in a glass container. To accelerate metal leaching, the pile was continuously aerated at the bottom at a flow rate of 0.002 $m^3\ s^{-1}$. The aerator was buried in a coarse silica sand to ensure uniform distribution of the injected air in the rock placed above. Fig. 1A shows a schematic diagram of the setup for obtaining the mining rock drainage. To obtain various concentrations of dissolved heavy metals, the leachate collected after 12 weeks of operating the setup shown in Fig. 1A was diluted with deionized water or a top soil leachate. The soil leachate was obtained by mixing 20 L of tap water with 5 kg of top soil collected at the Morgan Arboretum forest reserve (McGill University, Macdonald Campus, QC, Canada). After one day, the liquid was decanted and filtered.

2.2. Biosensor design and operation

Two biosensors denoted as BIOS-A and BIOS-B were constructed following the previously described design of a membraneless air-breathing cathode MFC (Grondin et al., 2012). The anode compartments had a volume of 100 mL and contained 10 mm thick anodes made of two layers of 10 × 5 cm carbon felts (SGL Group, Charlotte, NC, USA). Each air cathode was made of a 10 × 5 cm E4 electrode containing manganese oxide (Electric Fuel Ltd, Bet Shemesh, Israel). Both biosensors had external recirculation loops to provide constant mixing of the anode compartment liquid (anolyte) at a rate of 2 L h^{-1}, but only BIOS-B was operated with the external recirculation loop throughout the test, while recirculation of BIOS-A anodic liquid was only performed during the second half of the test. Fig. 1B shows a schematic diagram of the experimental setup.

One biosensor (BIOS-A), was fed with different dilutions of the rock drainage containing heavy metals at a flow rate corresponding to a hydraulic retention time of 4 h. At the start of BIOS-A testing, the influent concentration of metal ions was varied by dilution with deionized water. The biosensor was operated with a periodic connection/disconnection of a 10 Ω external resistor ($R_{ext} = 10\ \Omega$) according to the following algorithm (Algorithm-1). First, the high and low voltage limits were defined and biosensor operation was initiated in open circuit (R_{ext} disconnected) until the preset high voltage limit (V_{max}) was attained. Thereafter, R_{ext} was connected leading to a drop of the

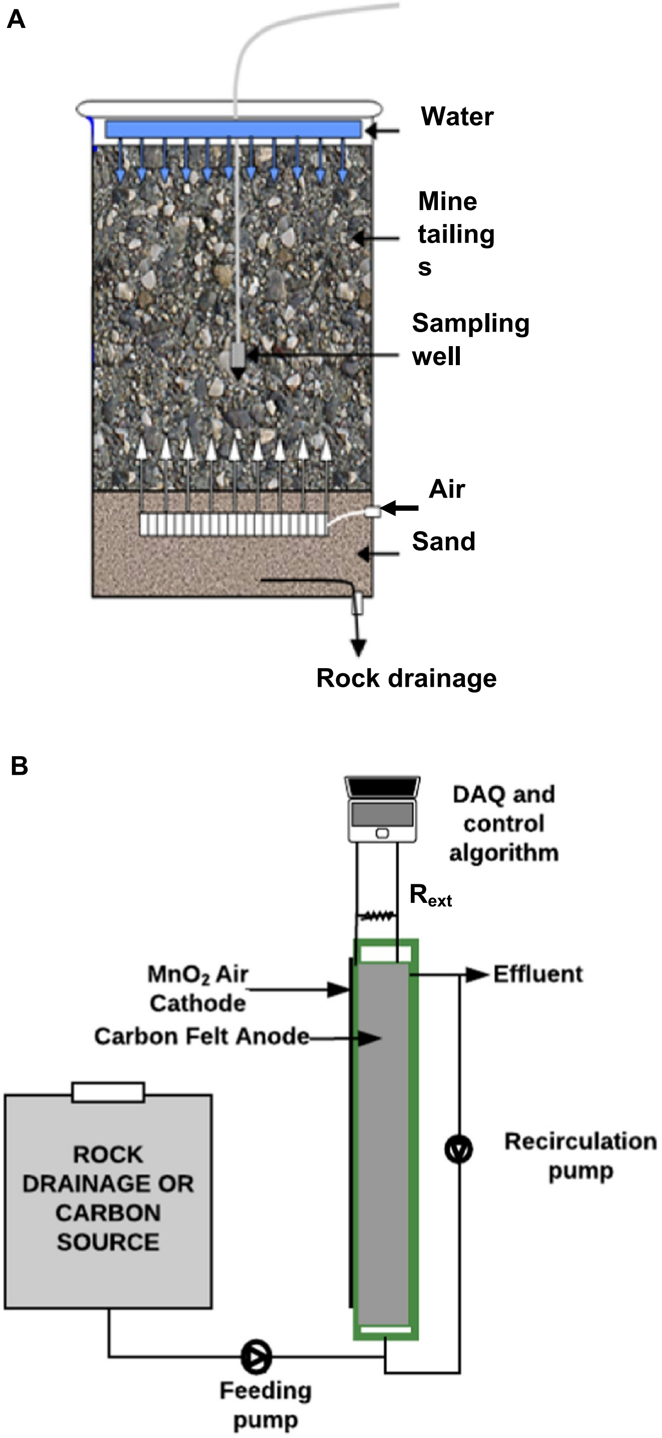

Fig. 1. Schematic diagram of (A) setup for obtaining mining rock drainage and (B) MFC biosensor (BIOS-A and BIOS-B).

biosensor voltage. Once the voltage decreased to the preset low voltage limit (V_{min}), R_{ext} was disconnected and the cycle of operation was repeated. The time to reach V_{max} after R_{ext} disconnection was used as a measure of influent stream toxicity. In the second part of biosensor testing, concentration of metal ions in the feed solution was varied by dilution of the leachate (rock drainage) with top soil leachate. In addition, to improve biosensor sensitivity the V_{max} and V_{min} values were also estimated in real time according to the modified algorithm (Algorithm-2) described later.

The second biosensor (BIOS-B) was operated at a fixed 126 Ω resistance and with a solution containing 150 $mg\,L^{-1}$ of acetate as a source of carbon, also at a hydraulic retention time of 4 h. To validate Algorithm-2, BIOS-B was operated at several influent acetate concentrations in a range of 120–470 $mg\,L^{-1}$.

The algorithms used to control R_{ext} connection/disconnection and estimate solution toxicity using BIOS-A were implemented in Matlab 2017a (Mathworks, Natick, MA, USA). A Labjack U3-LV (LabJack Corp., Lakewood, CO, USA) was used for data acquisition. External resistance connection/disconnection was achieved using a G6S-2F relay (Omron LLS, IL, USA). Data logging software written in Visual Basic (Microsoft Corp, Redmond, WA, USA) was used to record the voltage of BIOS-B.

2.3. Microtox toxicity assay

To confirm biosensor response, a Microtox assay (Bulich, 1979) was carried out at various heavy metal concentrations (up to 800 $\mu g\,L^{-1}$) obtained by diluting the rock drainage with top soil leachate. The assay is based on the luminescence of *Vibrio fischeri* exposed to water samples. 0.4 M NaCl was used to dilute the bacterial culture. Reconstitution solutions were supplied by Modern Water (Modern Water Inc, Newcastle, DE, USA). The luminescence at start (T0), after 30 min (T30) and 45 min (T45) was measured using a MicroBics M500 toxicity analyzer (Bio-Microbics Inc, KS, USA) and analyzed in Microsoft Excel 2017 (Microsoft Corporation, Redmond, WA, USA).

2.4. Microbial community analyses

To investigate the effect of heavy metals on the microbial community structure, biofilms developed at the BIOS-A and BIOS-B anodes were collected and analyzed. DNA extraction from carbon felt anodes (250 mg samples) collected at the end of biosensor experiments was carried out with a Powersoil® kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). Quantification and purity assessment of the genomic DNA was then done using the A260/A80 ratio determined by a NanoDrop™ 1000 Spectrophotometer (Thermofisher Scientific, Waltham, MA USA). Region V2 of the 16S rRNA gene was targeted for amplification with two sets of primers for eubacteria and archae, respectively. Amplification was performed with a HotStar polymerase master mix (Qiagen Inc, Toronto, ON, Canada). A specific forward primer-tag allowed identification of each sample. After verification of the amplified DNA quality by electrophoresis in a 2.0% agarose gel and visualization with UV illumination, gel bands containing the amplified DNA were selected and purified with an Illustra GFX PCR DNA purification kit (GE Healthcare, Little Chalfont, UK). Thereafter, quantification of the purified DNA with an Invitrogen kit (Thermofisher Scientific, Waltham, MA USA) was carried out using picogreen DNA stain and Lambda DNA for the standard curve. The fluorescence was then measured with a Tecan Safire microplate reader (Thermofisher Scientific, Waltham, MA USA). Archaeal and bacterial 16S rRNA genes were then sequenced using a Ion Torrent sequencing platform (Tremblay et al., 2015). Taxonomic analyses (sequence treatments and analyses, operational taxonomical units (OTUs) generation, taxonomic assignments) were carried out with an internal 16S/ITS pipeline (Tremblay et al., 2015; PMID:26300854) and were visualized in Microsoft Excel 2017 (Microsoft Corporation, Redmond, WA, USA).

Table 1
Influent and effluent concentrations of dissolved metals during BIOS-A sensor tests. Concentrations corresponding to the test with a total heavy metal concentration of 1095 $\mu g\,L^{-1}$ are shown.

Metal	Influent concentration ($\mu g\,L^{-1}$)	Effluent concentration ($\mu g\,L^{-1}$)
Aluminum	84	< 10*
Barium	28	21
Copper	15	7
Iron	224	< 70*
Manganese	719	619
Nickel	7	11
Lead	1	< 0.5*
Zinc	17	8

* Below detection limit.

3. Results and discussion

3.1. Biosensor response to heavy metals

The biosensor response to varying concentrations of dissolved heavy metals was studied using the mining rock drainage produced in the setup shown in Fig. 1A according to the procedure described above. The undiluted rock drainage had a pH of 6.5–8 and a conductivity of 2.9 $mS\,cm^{-1}$. With dilutions, pH remained unchanged but conductivity decreased to 1–2 $mS\,cm^{-1}$. On average, the rock drainage contained a total metal concentration of 5150 $\mu g\,L^{-1}$. Table 1 shows the concentrations of each metal in BIOS-A feed stream containing a total metal concentration of 1095 $\mu g\,L^{-1}$

The biosensor test was initiated by feeding diluted with deionized water rock drainage containing a total heavy metal ions concentration of 1095 $\mu g\,L^{-1}$ to BIOS-A. To estimate open circuit voltage, the biosensor was operated under open circuit conditions (disconnected R_{ext}) for 30 min at the start of the test. An open circuit voltage (V_{OCV}) of 0.35 V was recorded. Accordingly, V_{max} parameter of the algorithm described earlier was set to 0.3 V, while the V_{min} parameter was set to 0.03 V. As outlined in our previous study (Adekunle et al., 2019), the presence of dissolved heavy metals (e.g. Mg^{2+} and Fe^{2+}) results in decreased MFC current due to a combination of metal toxicity and a change in microbial metabolism. In particular, we showed that metal ions can be used as terminal electron acceptors by electroactive microorganisms. Therefore, with an increase in heavy metals concentration, an increase in the time to achieve the set V_{max} under open circuit conditions (t_{max}) can be expected. Subsequently, t_{max} was used as the biosensor response parameter in this test. During the test, total concentration of metals in the influent stream was changed to 2085, 2924, and finally to 5151 $\mu g\,L^{-1}$. At an influent total heavy metal concentration of 1095 $\mu g\,L^{-1}$ an average t_{max} value of 119 s was obtained. The subsequent changes in the metal concentration of the influent stream led to corresponding changes of t_{max}. Overall, regression analysis showed a linear correlation between t_{max} and metal concentration with a determination coefficient (R^2) of 0.96 with t_{max} increasing with increasing metal concentration, as can be seen from Fig. 2A.

Mixing and flow rate (retention time in the anodic compartment of the biosensor) are expected to influence t_{max}, since these factors affect concentrations of the carbon source and metal ions within the anode, which are vital to the biosensing process (Shen et al., 2013). It was hypothesized that with the increased mixing, the sensitivity of the

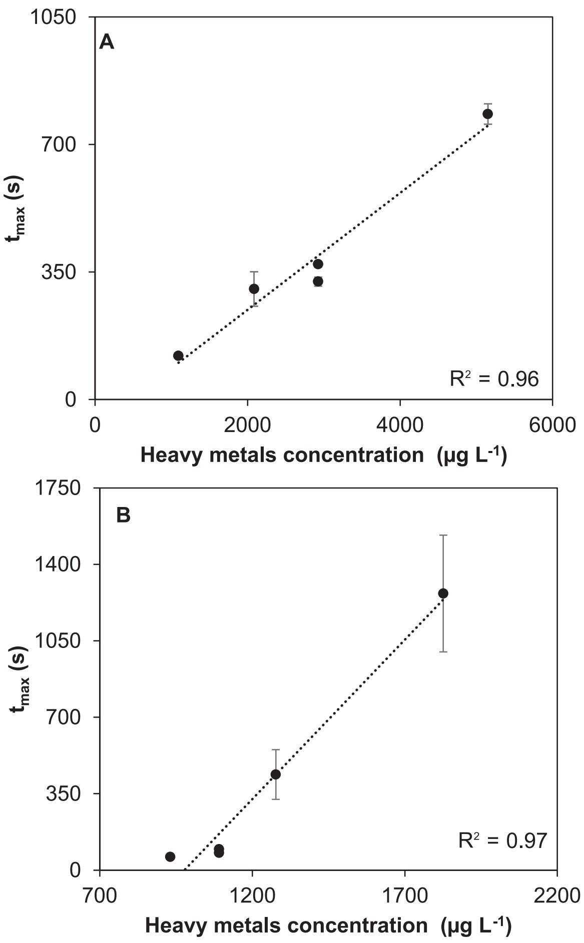

Fig. 2. (A) Dependence of MFC biosensor (BIOS-A) response (t_{max}) on heavy metals concentration in the absence of anodic liquid recirculation and (B) BIOS-A response to heavy metals during the anodic liquid recirculation test.

biosensor would increase. To confirm this hypothesis, the external recirculation loop of BIOS-A was activated and exposure to various concentrations of metal ions was repeated using the same rock drainage solution as in the previous test. The constant mixing increased the average $t_{\max}$ required to achieve the preset $V_{\max}$ value indicating an increased toxicity, i.e. biosensor's sensitivity to metal ions. In fact, voltage reversal was observed within a day of feeding a solution with a heavy metals concentration of 1826 $\mu g\,L^{-1}$ resulting in a sensor output of − 30 mV. The observed drop in biosensor voltage could be explained by increased delivery of heavy metals to the anodophilic microbial biofilm. Accordingly, instead of increasing the heavy metal concentration as in the previous test, it was decided to decrease the influent concentration to 1091.2 $\mu g\,L^{-1}$, and then to 931.2 $\mu g\,L^{-1}$, followed by a concentration increase to 1277 $\mu g\,L^{-1}$. Once the influent metal concentration was decreased to 1091.2 $\mu g\,L^{-1}$, from 1277 $\mu g\,L^{-}$ the biosensor V_{OCV} voltage recovered to 300 mV. Regression analysis of the resulting biosensor outputs confirmed linear correlation of $t_{\max}$ with total heavy metal concentrations ($R^2 = 0.97$), as can be seen from Fig. 2B. The increased toxicity of heavy metals due to anodic liquid recirculation (i.e. increased transport of dissolved metals to the biofilm) resulted in a higher standard deviation of t_{max} estimation at the highest concentration of heavy metals shown in Fig. 2B.

Based on the results of the tests described above, it can be concluded that the biosensor (BIOS-A) was able to track changes in the heavy metal concentrations of the rock drainage. Interestingly, a comparison of influent and effluent metal concentrations showed that some heavy metals were partially or entirely removed (concentrations reduced to levels below analytical detection limit). For example, at an influent total metal concentration of 1095 $\mu g\,L^{-1}$ (no recirculation) an effluent concentration of 666 $\mu g\,L^{-1}$ was measured. Table 1 summarizes changes in heavy metal concentrations after passing through the biosensor. Changes in heavy metal concentration could be explained by the reduction of metal ions and subsequent formation of elemental metals or insoluble metal sulfides at the cathode (Heijne et al., 2010; Tao et al., 2011). It therefore follows that accumulation of solids can be expected, unless the liquid flow rate is sufficient to remove the deposited solids. While at least partial removal of solids can be achieved by increasing the flow rate, the biosensor's sensitivity to the presence of metal ions will be increased, as discussed above. Therefore, the liquid flow rate should be optimized based on the expected range of dissolved metal concentrations. An improved biosensor design to facilitate removal of solids from the anodic compartment might be required for consistent long-term performance. It is important to highlight that $t_{\max}$, which was used as a toxicity indicator in this test, is a relative parameter that can depend on several other factors including the overall condition of the anode compartment, previous exposures, type of heavy metals dominant in the influent stream, and other factors. For example, carbon felt clogging with metal sediments can have an adverse effect on the sensor performance by increasing $t_{\max}$. Also, exposure to high levels of heavy metals may significantly affect anodophilic activity leading to prolonged recovery times.

Despite a slight conductivity increase with increasing heavy metal concentration in the influent stream (e.g. 1.3 $mS\,cm^{-1}$ vs 2.9 $mS\,cm^{-1}$ at metal concentrations of 1095 $\mu g\,L^{-1}$ and 5151 $\mu g\,L^{-1}$, respectively), anodophilic activity of the biosensor (BIOS-A) was reduced, giving credibility to an hypothesis of heavy metal toxicity towards anodophilic populations. Such impaired anodophilic activity can be hypothesized to decrease the biosensor internal capacitance, which is directly related to the anodophilic activity (Martin et al., 2013).

3.2. Enhanced algorithm for relative toxicity estimation

As stated earlier, several interconnected factors determine biosensor performance. For a biosensor deployed in the field, other parameters such as variations in carbon source concentration, temperature, the amount of precipitation and other environmental factors may affect anodophilic activity and, therefore, the open circuit voltage. Therefore, preset $V_{\max}$ and $V_{\min}$ values could be difficult to achieve. Continuous operation with poorly selected $V_{\max}$ and $V_{\min}$ boundaries may lead to either biosensor never achieving a pre-selected $V_{\max}$ value or in voltage reversal due to prolonged operation at an external resistance significantly below the biosensor internal resistance ($R_{ex} \ll R_{int}$). To optimize sensor performance, improve reproducibility of the measurements, and avoid its operation under conditions leading to voltage reversal, a real time algorithm for estimating $V_{\min}$ and $V_{\max}$ boundaries was developed.

In the proposed algorithm, the measurement cycle starts with biosensor connection to R_{ext}. Thereafter, biosensor voltage (V_s) is constantly monitored to detect pseudo steady state voltage corresponding to $V_{\min}$. Once steady state voltage is attained, R_{ext} is disconnected and the voltage is monitored again to detect steady state open circuit voltage (OCV) corresponding to a new $V_{\max}$ value. The voltage is considered at a steady state once its variation is lower than a predefined

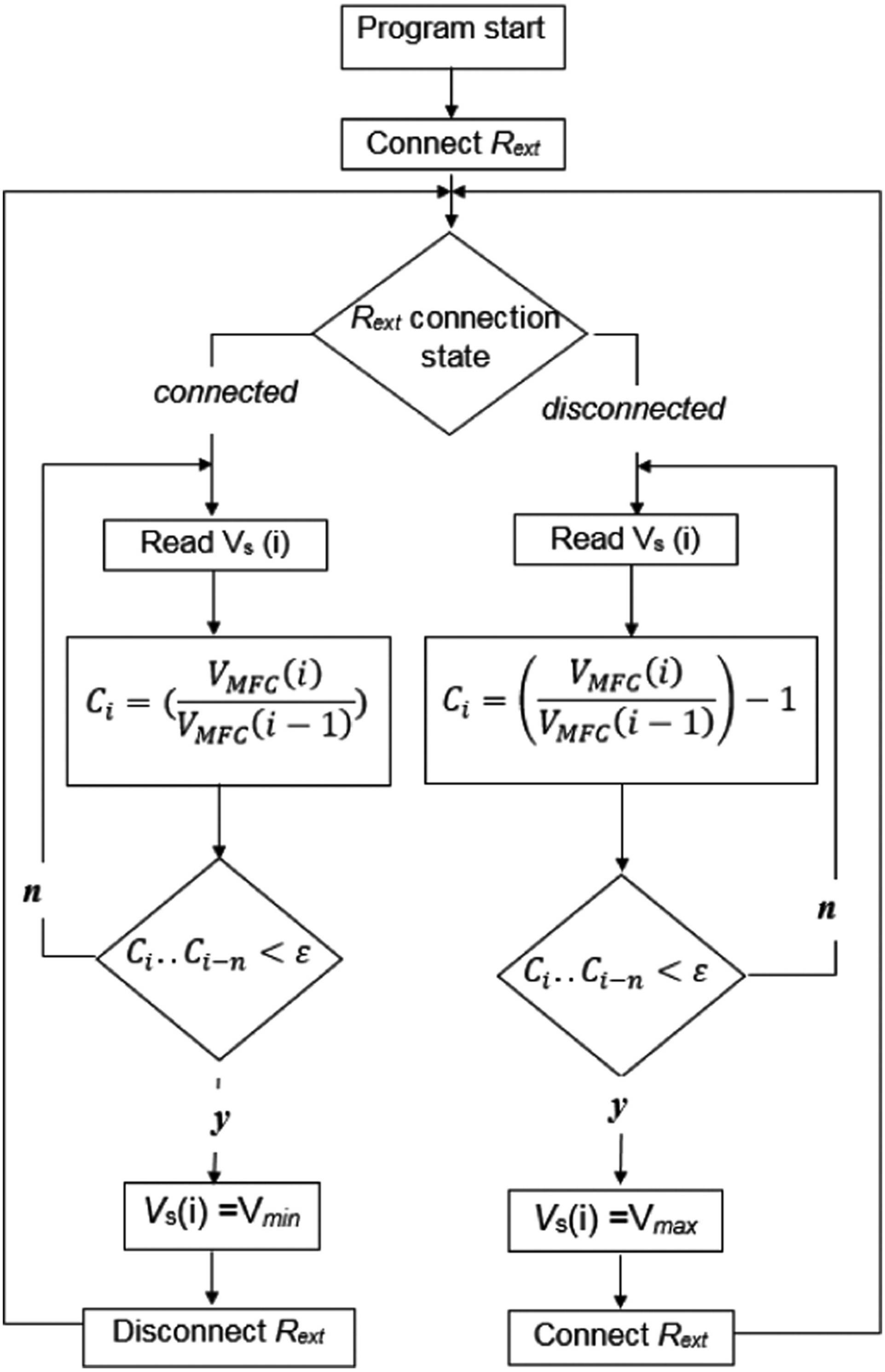

Fig. 3. Enhanced algorithm of biosensor operation. *n* is the predefined number of consecutive data acquisition points used to determine pseudo steady state, where the change of biosensor voltage (V*s*) is less than a preset small value ε (ε = 0.01).

small value (ε = 0.01) for several (e.g. *n*) consecutive data acquisitions. Thus, in this mode of operation, the connection and disconnection times depended on achieving stable voltage value, as detailed in Fig. 3.

Prior to applying the proposed algorithm for evaluating BIOS-A response to changes in metal concentrations, it was tested by changing carbon source concentrations of BIOS-B sensor and monitoring the resulting outputs. Before this test, BIOS-B was operated at an influent acetate concentration of 150 mg L^{-1} with a corresponding conductivity of 19 mS cm^{-1} and an average effluent acetate concentration of 70 mg L^{-1}. In the test, influent acetate concentrations were changed to 237.2, 468.8, 119.3 and 353.6 mg L^{-1}. At each level of influent acetate concentration, $V_{\min}$ and $V_{\max}$ values were estimated according to the developed algorithm. As shown in Fig. 4, changes of acetate concentration in the anode compartment (sampled in the recirculation

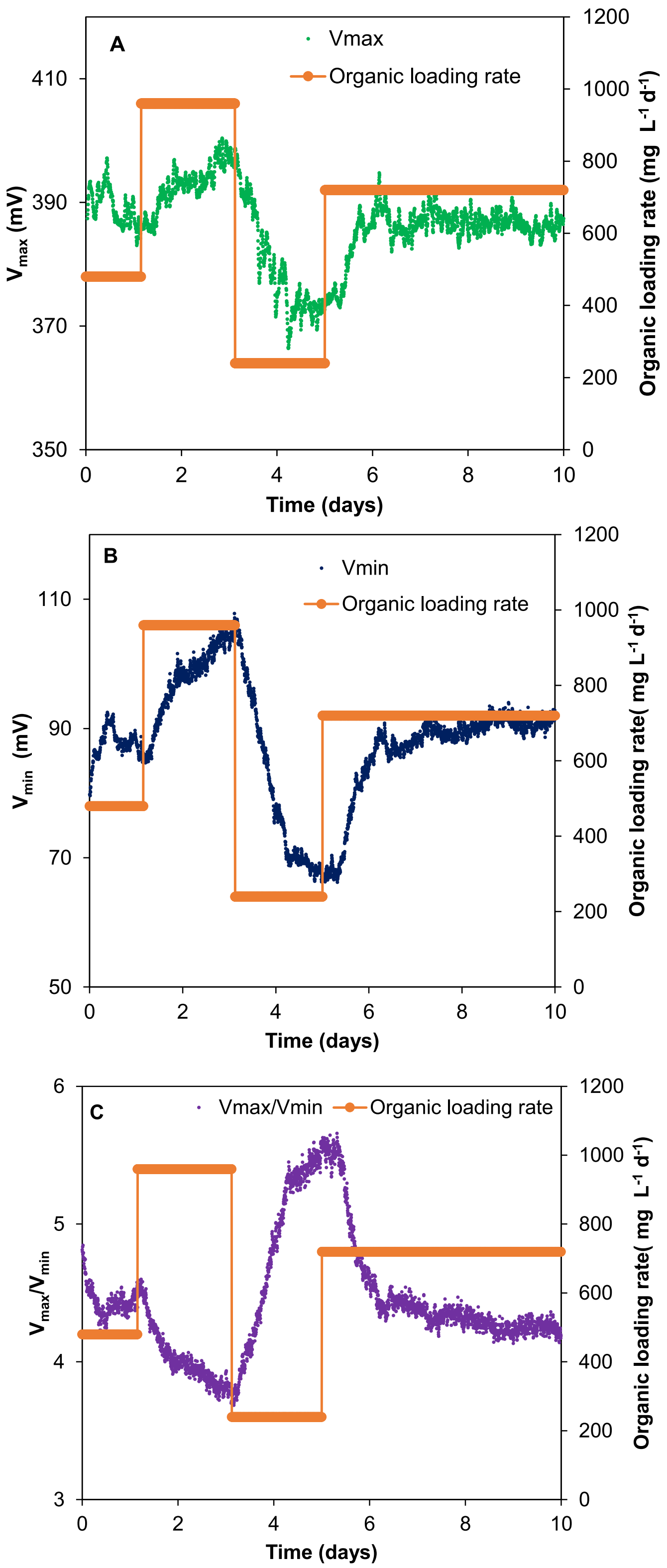

Fig. 4. Real time optimization of biosensor operation with changing organic matter availability. Transitions of V_{max} values (A), and V_{min} values (B) values. Transitions of the ratio (V_{max}/V_{min}) indicating the faster rate of V_{min} values change during biosensing.

loop) led to changes of both V_{max} (Fig. 4A) and V_{min} (Fig. 4B). Interestingly, the ratio (V_{max}/V_{min}) shows that although both parameters are affected by changes in the biosensor, the rate of V_{min} values change is greater than that of V_{max} values (Fig. 4C). V_{min} values, which correspond to the biosensor output with connected R_{ext}, directly reflect carbon source oxidation rate at the anode. Therefore, changes in the metabolic rate of the anodophilic population due to carbon source variations or anolyte toxicity might affect V_{min} to a greater extent. Overall, both voltages (V_{min} and V_{max}) and the V_{max}/V_{min} ratio can be considered as biosensor outputs in the regression analysis aimed at determining the best regression dependence.

Following the BIOS-B test, the algorithm was tested at several influent concentrations of heavy metals (43, 236, 408 and 600 μg $L^{-1)}$ in the influent stream of BIOS-A. In the tests, different concentrations of metals were obtained by diluting the rock drainage solution with top soil leachate in order to increase the organic matter availability to the electroactive microorganisms. The total COD in the top soil leachate was 27.7 mg L^{-1}. This biosensing test was carried out over a period of 2 months with real time V_{max} and V_{min} estimations according to the algorithm shown in Fig. 3. Changes in estimated values of V_{max} and V_{min} using this algorithm are shown in Fig. 5A, B. It can be seen that the V_{max} and V_{min} values of the biosensor change after exposure to heavy metals. Furthermore, it can be seen that the continuous and prolonged exposure to heavy metals prevented a recovery to voltage values before the test. However, there was a strong correlation between the ratio (V_{max}/V_{min}) and the heavy metals concentration in the rock drainage. In this case, the presence of heavy metals, which can act as electron acceptors, could be responsible for the reduction of V_{min} and V_{max} values. The correlation between the total heavy metal concentrations fed to the biosensor and the V_{max}/V_{min} ratio was high $R^2 = 0.96$, Fig. 5C), thus validating the applicability of the proposed algorithm for real-time continuous operation of the biosensor. On average, the measurement interval using this algorithm was approximately 5 min.

3.3. Biosensor comparison with the Microtox assay

Since an increasing concentration of heavy metals resulted in a decreased electrogenic activity (decreased power production), it can be suggested that BIOS-A output is proportional to the toxicity of the rock drainage solution. To verify this hypothesis, the biosensor (BIOS-A) response was compared to a common toxicity assay based on the activity of bioluminescent bacteria. The Microtox assay is a tool for rapid toxicity screening (Bulich, 1979). It is based on changes in the metabolic activity and inhibition of enzymatic activity catalyzing luminescence in bacteria *Vibrio fischeri*. As shown in Fig. 6A, the effect of increasing heavy metals concentration on the luminescence of *Vibrio fischeri* is evident with time (T) i.e. after 15 min (T15), 30 min (T30) and 45 min (T45) after the start of the experiment (T0). It is also clear from the results of the Microtox assay that increasing concentrations of heavy metals reduce *V. fischeri* luminescence. Importantly, the dose - response relationship observed from the Microtox assay is similar to the response observed with the MFC biosensor (Fig. 5D). In fact, the MFC biosensor appears to be more sensitive than the Microtox assay in its response to changing heavy metal concentration. This observation could be attributed to differences in exposure times, as the MFC biosensor was operated at a retention time of 4 h compared to a maximum exposure time of 45 min for the Microtox assay. Fig. 6B compares the results of the Microtox assay (T45 min) with the BIOS-A outputs at the corresponding metal concentrations showing a linear dependence with $R^2 = 0.92$. Overall, the results of the Microtox assay suggest that the proposed

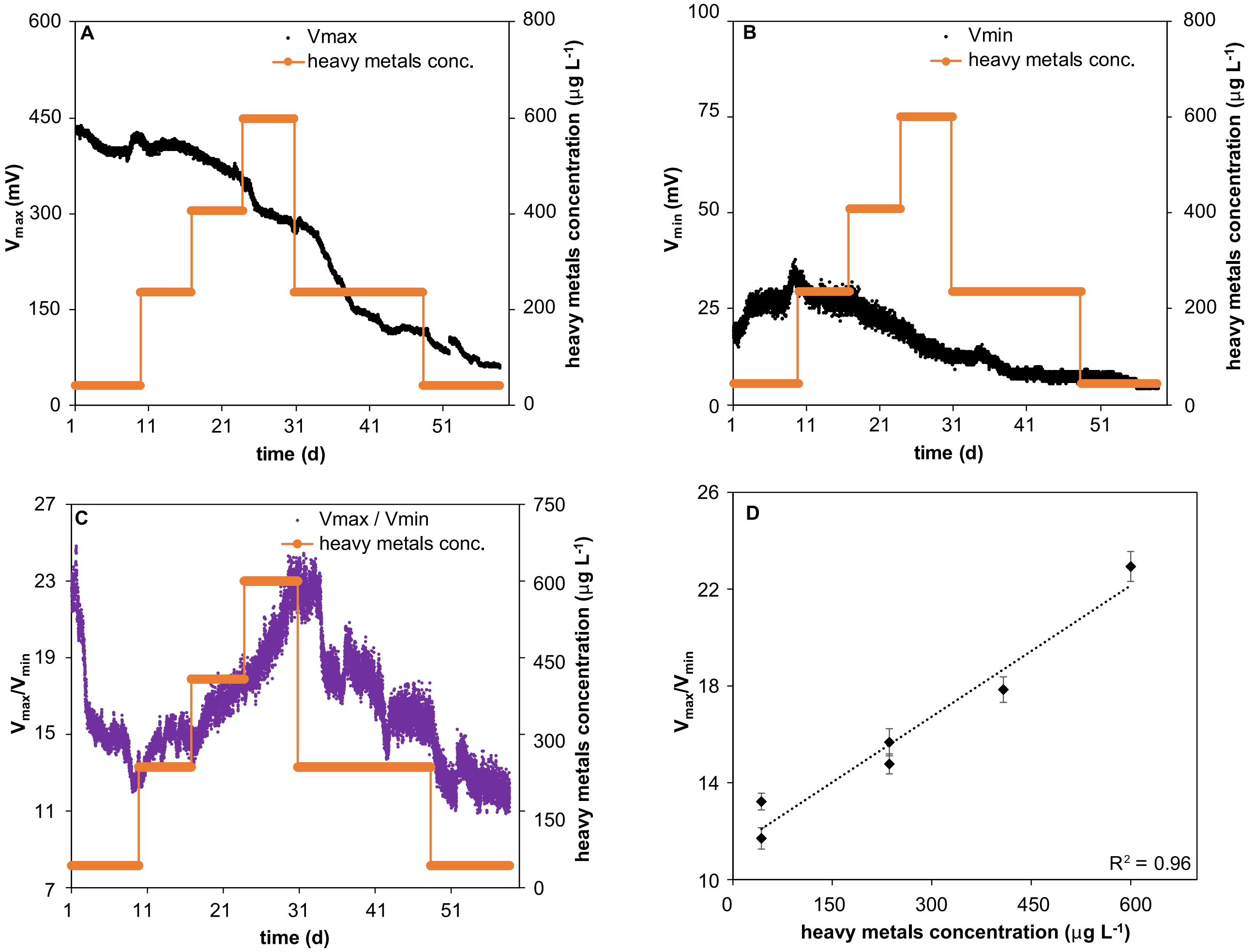

Fig. 5. Real time optimization of biosensor operation during exposure to heavy metal concentrations. Transitions of optimal V_{max} values (A), and V_{min} values (B). Transitions of the ratio (V_{max}/V_{min}) as a measure of biosensor response (C), linear correlations between steady state V_{max}/V_{min} values and total metals concentration (D).

biosensor could be used for real-time toxicity monitoring. More importantly, variations in the composition of heavy metals, carbon source availability, dissolved oxygen concentration, anodophilic microbial populations present at a particular site, and other external factors might affect biosensor outputs thus at times limiting its ability for direct measurements of dissolved metal concentrations. Nevertheless, qualitative changes in the sensor outputs can be successfully used for real-time toxicity monitoring, e.g. for detection of toxic spills or mining rock drainage propagation.

3.4. Microbial community analysis

Analysis of bacterial and archaeal 16S rRNA gene sequences from both biosensors (BIOS-A and BIOS-B) showed considerable phylogenetic diversity in the anode microbial communities (Table 2), particularly for bacteria. The bacterial population from the anode of the acetate-fed biosensor BIOS-B was dominated by one genus, *Desulfuromonas* (40.56% relative abundance), which are known anaerobic reducing and electroactive bacteria (Bond et al., 2002). The bacterial population of the anode of biosensor BIOS-A, which was fed with heavy metals, showed higher heterogeneity. The dominant genera (above a selected 2% threshold) by relative abundance included *Treponema* (11.3%), *Diaphorobacter* (5.1%), *Desulfotignum* (4.6%), *Ferritophicum* (4.5%), *Desulfococcus* (4.3%), *Ferrovum* (2.5%), *Smithella* (2.3%), and *Methylogaea* (2.1%). *Diaphorobacter* are denitrifiers (Khan and Hiraishi, 2002), *Desulfotignum and Smithella* are phosphite and propionate oxidizing microorganisms, respectively (Liu et al., 1999; Schink et al., 2002), *Ferritophicum and Ferrovum* are iron oxidizing bacteria (Johnson et al., 2014), and *Desulfococcus* are sulphate reducing microorganisms. The presence of the *Ferritophicum* and *Ferrovum* genera may explain the removal of iron observed in BIOS-A effluent (Table 1). Contrary to bacteria, archaeal populations did not vary extensively and the dominant methanogens in both biosensors remained approximately the same, with the exception of a switch in the dominance of *Methanosarcina* and *Methanospirillum* (Table 2). Expectedly, the acetocalastic methanogen *Methanosarcina* was dominant in BIOS-B fed with acetate, while the hydrogentropic methanogen *Methanospirillum* was abundant in BIOS-A, highlighting the differences between the two environments.

The results of this microbial community analysis show the adaptability of an MFC-based biosensor to its environment, as indigenous electroactive microorganisms become established on the anode. Such adaptability demonstrates that biosensing is possible with diverse

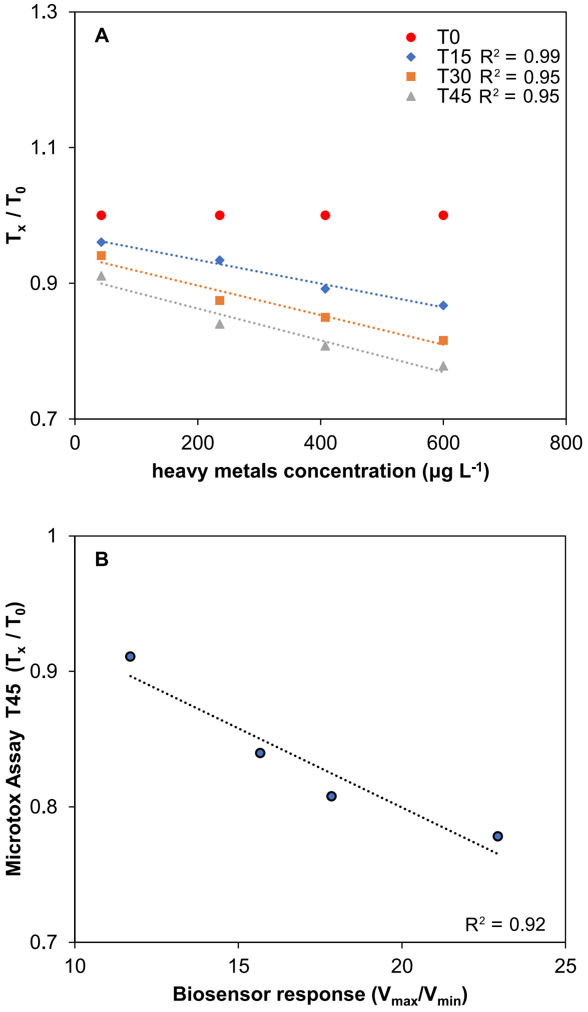

Fig. 6. (A) Bioluminescence of *Vibrio fisheri* (T_x/T_o) as a function of heavy metal concentration in Microtox assay and (B) a comparison of V_{max}/V_{min} values of BIOS-A with the corresponding bioluminescence values obtained in the Microtox assay (T = 45 min).

indigenous microorganisms thus eliminating the need for maintaining pure microbial cultures. If a biosensor is exposed to high concentrations of heavy metals for a prolonged period of time, adaptation of the electroactive microbial populations can be expected. Nevertheless, such adaptation does not necessarily lead to complete recovery of the biosensor, since electroactive bacteria are capable of utilizing dissolved metals as electron acceptors, resulting in reduced current of the biosensor.

4. Conclusion

This study evaluated the possibility of on-line monitoring of heavy metals related toxicity at short (e.g. 5 min) measurement intervals using an MFC-based biosensor. Furthermore, high correlation of the biosensor outputs with total heavy metal concentrations was observed. An algorithm for enhanced measurement accuracy was proposed and successfully demonstrated in a series of laboratory tests. The response of the biosensor to changing heavy metal concentrations was compared with the results of the Microtox assay showing linear correlation between the two methods, thus suggesting that the biosensor could be used for real-time toxicity monitoring. Finally, it was shown that exposure to heavy metals led to a shift in the dominant microbial communities of the biosensor. Overall, the results of this study provide a basis for developing a novel biomonitoring device suitable for long-term on-line monitoring (quantitative or qualitative) of heavy-metals related toxicity in remote locations.

Table 2
Taxonomic summary of the rock drainage-fed BIOS-A and acetate-fed BIOS-B biosensors. Percentages represent the relative abundance of all OTUs that were assigned their corresponding taxon.

EUBACTERIA DOMAIN		
Genus	**BIOS-A**	**BIOS-B**
< 2% of population	14.49%	8.22%
unclassified	40.65%	46.99%
Helicobacteraceae	1.29%	0.07%
Thiomonas	1.67%	0.01%
Cupriavidus	0.67%	0.02%
Kozakia	1.43%	0.02%
Yaniella	1.77%	0.02%
Ferrovum	2.45%	0.61%
Methylogaea	2.08%	0.03%
Smithella	2.25%	0.19%
Desulfococcus	4.26%	0.01%
Desulfotignum	4.60%	0.01%
Diaphorobacter	5.11%	0.03%
Ferritrophicum	4.53%	0.06%
Treponema	11.26%	3.15%
Desulfuromonas	1.48%	40.56%
ARCHAE DOMAIN		
Genus	**BIOS-A**	**BIOS-B**
< 2% of population	0%	2%
unclassified	12%	9%
Methermicoccus	0%	1%
Methanomethylovorans	0%	0%
Methanosaeta	0%	1%
Methanosarcina	1%	12%
Methanospirillum	10%	2%
Methanobacterium	13%	10%
Methanoregula	63%	64%

CRediT authorship contribution statement

Ademola Adekunle: Conceptualization, Methodology, Software, Investigation, Data curation, Formal analysis, Validation, Writing - original draft, Writing - review & editing. **Vijaya Raghavan:** Conceptualization, Supervision. **Boris Tartakovsky:** Conceptualization, Methodology, Writing - review & editing, Supervision.

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Declaration of interests

None.

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