

Oxygen-reducing microbial cathodes monitoring toxic shocks in tap water

Antonin PrévotEAU*, Peter Clauwaert, Frederiek-Maarten Kerckhof, Korneel Rabaey*

Center for Microbial Ecology and Technology (CMET), Campus Coupure - Ghent University, Coupure Links 653, 9000 Ghent, Belgium

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ABSTRACT

Electroactive biofilms (EABs) have recently attracted considerable research interest for their possible use as amperometric biosensors in environmental or bioprocess monitoring, for example for in situ detection of toxic compounds. Almost exclusively, corresponding research has focused on heterotrophic, anodic EABs. These biofilms require sufficiently high organic loads and anoxic conditions to deliver a stable baseline current. Conversely, electroautotrophic O_2 -reducing EABs have recently been proposed to monitor toxic shocks in oxic solutions that are poor or devoid of organic substrate. This was done in optimal media and only assessed for formaldehyde as a model toxic compound. Here we show that O_2 -reducing EABs can grow in unamended tap water on carbon electrodes at + 0.2 V vs. Ag/AgCl. They retained substantial electroactivity for at least eight months without adding exogenous compounds. The most represented operational taxonomic units were assigned to the phylum Gammaproteobacteria ($25 \pm 15\%$, $n = 5$ electrodes). Cyclic voltammograms showed a reproducible Nernstian behavior for O_2 reduction with a mid-wave potential at + 0.27 V and variable plateau current densities ranging from -1 to $-22 \mu A cm^{-2}$ ($n = 10$ electrodes). The biocatalytic current was substantially impacted by the addition of either of three tested heavy metals (Hg(II), Cr(VI) or Pb(II)) or by organic pollutants (formaldehyde, 2,4-dichlorophenol, benzalkonium chloride), with limits of detection ranging from 0.5 to $10 mg L^{-1}$ ($2.5\text{--}61 \mu mol L^{-1}$). Response times were typically around 1 min. Comparison with previous reports suggests that O_2 -reducing microbial cathodes may be more sensitive to toxic shocks than anodic, heterotrophic EABs.

1. Introduction

Electroactive biofilms (EABs) can directly exchange electrons with a conductive surface and perform so-called microbial electrocatalysis (Guo et al., 2015). The corresponding microbial electrodes have been predominantly investigated for applications such as electricity production (microbial fuel cell – MFC), bioremediation and bioproduction (microbial electrosynthesis) (Rabaey and Rozendal, 2010). More recently, they have inspired considerable research for their possible use as biosensors for environmental or bioprocess monitoring (PrévotEAU and Rabaey, 2017). These EABs possess unique characteristics making them attractive as low cost biosensors. They can be considered as ‘self-assembled’ bioelectrodes because of their colonization and growth on electrodes, acting simultaneously as biological recognition element and transducer. As living systems, EABs are self-sustaining and can regenerate themselves if their environment allows cell replication, granting them much higher stability than non-living bioelectrodes, for example those using purified enzymes. The sensing EAB can be integrated in an MFC, allowing in some cases the sensor to be self-powered, which is attractive for off-the-grid applications in remote areas

(Chouler and Di Lorenzo, 2015). A distinct feature of EABs is their ability to respond to a broad spectrum of compounds, rather than being specific to a single one, which is even more evident when the biofilm is composed of a microbial community. For instance, heterotrophic EABs can produce anodic current by catabolizing a broad range of organic substrates via different reaction pathways (Pant et al., 2010). Hence, EABs have mostly been proposed for two non-selective biosensing of water quality: measuring the biological oxygen demand (BOD) in water (Di Lorenzo et al., 2014), and monitoring the presence of toxic compounds (Zhou et al., 2017). In the latter case, the presence of toxicants is detected by monitoring a concomitant decrease in electrical signal, most of the time a current (amperometric biosensors). This decrease occurs when the inhibition of the microbial metabolic activity becomes high enough to limit the current production of the EAB. A large spectrum of heavy metals or organic toxic compounds have been shown to decrease the current generated by EABs either integrated in an MFC, or in a so called microbial three-electrode cells i.e. with the bioelectrode poised at constant potential with the help of a reference electrode. The response time to the presence of toxicant typically ranges from seconds to hours (PrévotEAU and Rabaey, 2017). The early on-line detection of a

* Corresponding authors.

E-mail addresses: Antonin.PrevotEAU@UGent.be (A. PrévotEAU), Korneel.Rabaey@UGent.be (K. Rabaey).

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toxic shock in a watercourse, waterbody or along a bioprocess could allow immediate decision-making and intervention. The identity of the toxic compound(s) could be later determined off-line with traditional physicochemical methods, which are time-consuming and involve expensive apparatuses.

The large majority of studies on toxic shock monitoring have been performed on heterotrophic anodic EABs, which implies several limitations. These EABs require the presence of biodegradable organic substrate to produce current. Any variation of this substrate concentration below the saturation level leads to an alteration of current, which can imply a false positive of toxic shock. Molecular oxygen also decreases the current either by damaging obligate anaerobes of the EAB, or by competing for the electrons “generated” by aerobic microorganisms. This restricts the applicability of these heterotrophic EABs to anoxic waters with high BOD levels, such as some industrial or domestic wastewaters before treatment.

For this reason, O_2 -reducing “electroautotrophic” EABs have recently been proposed as cathodic biosensors for toxic shock monitoring in O_2 -containing waters poor or devoid of biodegradable organic matter (Jiang et al., 2018, 2017; Liao et al., 2018b). Two of these studies investigated the response of biocathodes integrated within an MFC (Jiang et al., 2018, 2017). Although attractive for possible self-powering, these systems still require a non-limiting concentration of organic substrate in an anodic compartment separated by an ion exchange membrane. The absence of electrode potential control in these MFCs allows the microbial cathode to reach operating potentials below -0.2 V vs. Ag/AgCl (Jiang et al., 2018). At these potentials, abiotic O_2 reduction can typically occur on carbon-based electrodes at circumneutral pH, producing hydrogen peroxide possibly harmful to the biocatalyst (Milner et al., 2017; Prévotau and Mano, 2012). In all these studies, the cathodic EABs were grown and maintained in dedicated minimal media i.e. containing buffering salts, trace minerals, and vitamins. However, it seems unlikely that real applications would meet these optimal conditions. In an MFC, the O_2 -reducing microbial cathode was more sensitive to toxic shock than its anodic, heterotrophic counterparts (Jiang et al., 2017). However, the model toxicant formaldehyde have been the only compound used to investigate toxic shocks on O_2 -reducing microbial cathodes (Jiang et al., 2018, 2017; Liao et al., 2018b).

The primary aims of the present report are: (i) to evaluate the possibility to grow and maintain an O_2 -reducing microbial cathode in slightly amended and unamended tap water; (ii) to investigate the electrochemical properties and microbial community composition of these cathodic EABs grown under nutrient-limited conditions; and (iii) to monitor toxic shocks from six organic and inorganic pollutants on these EABs, and to compare these results with respect to the much broader literature available for anodic EABs.

2. Material and methods

2.1. Biofilm growth and electrochemical analysis

All electrochemical experiments were carried out with a potentiostat (VSP, Biologic, France). The cathodic biofilms were initially grown on 0.5 cm diameter graphite rods (~10 cm length) packed within graphite granules in the cathodic compartment of an electrochemical cell at $+0.2$ V vs. Ag/AgCl (3 M NaCl, ALS, Japan, $+0.210$ V vs. SHE at 28°C). The inoculum was obtained from a cathodic O_2 -reducing EABs previously grown from a mix of local soil (Coupure Links, Ghent University) and OLAND sludge (De Clippeleir et al., 2012). The growth medium for the graphite rods was Ghent (Belgium) tap water (pH = 7.8 and conductivity of $740 \pm 30 \mu\text{S cm}^{-1}$ at 23°C , chemical composition available in Table S1) amended with 5 vol% M9 medium to favor initial EAB development (see Supplementary Material for further details). Once the cathodic current produced in the growth reactor was stabilized, the graphite rods were extracted and one end of each rod (~1 cm) was cleaned from biological material and dried for electrical

connection. Each rod was then moved into a separated 3-electrode setup (100 mL open glass beakers) filled with unamended tap water and poised at $+0.1$ V vs. Ag/AgCl for reaching stable current before further testing. The counter electrodes were platinum spiral wires (~10 cm) and the references Ag/AgCl. The uncompensated resistances were assessed by current interrupt method (Bard and Faulkner, 2001) and their value was too small (~20–100 Ω , depending on surface of electrode immersed) to induce substantial ohmic drops considering the small currents recorded (<0.1 mA). A photo of a colonized graphite rod is displayed in Fig. S1. Microbial cathodes were also grown twice on glassy carbon rotating disc electrodes (RDE E6R1, 5 mm diameter, Pine) in tap water only to ensure that the EABs could grow without the addition of exogenous compounds except from the inoculum (scrapings of cathodic biofilms from graphite rods). The RDEs were polished before use as previously described (Prévotau et al., 2015), poised at $+0.2$ V and rotated at 160 rpm for biofilm growth.

All current densities are provided with respect to the geometric surface area of the microbial electrodes in solution. All experiments were performed under air, in absence of natural light and at $28 \pm 2^\circ\text{C}$ unless stated otherwise. The pH of the tap water did not evolve substantially during any experiment (7.8 ± 0.2 pH). Anoxic conditions were obtained by bubbling then sparging N_2/CO_2 (90:10, v/v) gas in the partially closed electrochemical cell.

2.2. Microbial community

Biofilms were scraped from five rods with a 70 vol% ethanol sterilized blade and their DNA extracted using the ZymoBIOMICS DNA Microprep Kit (Zymo Research, USA). The V3 and V4 hypervariable regions of the 16S rRNA gene amplicon libraries were amplified and sequenced by BaseClear BV (Leiden, the Netherlands). The sequencing runs were analyzed with the Illumina CASAVA pipeline (v1.8.3). Further details are provided in Supplementary Material. Raw sequencing data is publically available in the NCBI SRA under BioProject accession [PRJNA523591].

2.3. Toxic shock monitoring

The set-up is depicted in Fig. 1. Current of single microbial cathodes were continuously recorded at $+0.1$ V vs. Ag/AgCl (plateau current) and the 0.1 L electrochemical cell connected to a 2 L open recirculation glass bottle containing 2 L of tap water. A homemade peristaltic pump

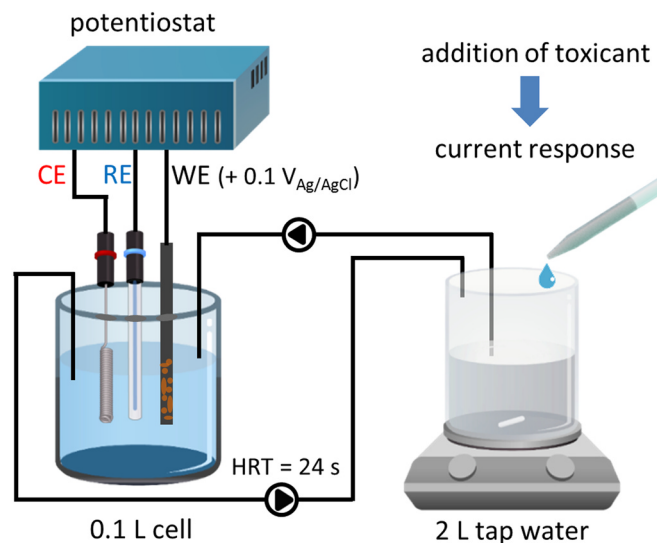


Fig. 1. Principle of the recirculating setup for assessing EABs responses to toxic shocks. CE, RE and WE stands for counter, reference and working electrode, respectively.

(mounted with a Watson Marlow 313D pump head) was used to recirculate the water in the cell at 4.1 mL s^{-1} (hydraulic retention time (HRT) of 24 s). The appropriate volume of concentrated toxicant was added in the recirculating bottle to reach the target concentration. The tap water in the recirculating bottle was continuously mixed at 800 rpm with a magnetic stirrer to allow a fast homogenization of the toxicant concentration. The mass concentrations for heavy metals (Hg, Cr, Pb) are provided with respect to the metallic element. Suppliers, purities and relations between mass and molar concentrations are provided in Table S2. When the impact of replacing the contaminated tap water by fresh tap water was investigated ('0 ppm' arrows in Fig. 4), two 2 L bottles were initially filled with fresh tap water to ensure they share the same physicochemical properties (T, pH, pO_2 , conductivity, chemical and microbial compositions). One bottle was first connected in the recirculation loop until a stable baseline in current was recorded, then used for monitoring the toxic shock associated with one single toxicant. Afterwards, it was replaced by the second bottle with uncontaminated tap water. The effluent of the cell was then discarded during at least 10 HRTs before reconnecting the recirculation loop. This setup was also used to assess the long term electroactivity of the biofilm in tap water, for which the tap water of the recirculating bottle was periodically replaced (every ~ 3 days) and no toxic compound added.

3. Results and discussion

3.1. Biofilm growth and electrochemical analysis

Fig. 2A displays a chronoamperogram corresponding to the growth of a cathodic biofilm on a glassy carbon RDE at $+0.2 \text{ V vs. Ag/AgCl}$ in Ghent tap water. This potential is far above the onset potential for abiotic O_2 reduction on carbon electrode in tap water (-0.15 V , see Fig. S2). The cathodic current was almost zero and with a flat profile for about 1 day after inoculation before increasing quasi-exponentially with time and stabilizing around $22 \mu\text{A cm}^{-2}$. Representative CVs recorded in anoxic conditions or under air are shown on Fig. 2B. The CV recorded under anoxic conditions (thin blue line) does not exhibit any significant faradaic current, an absence commonly observed for O_2 -reducing autotrophic EABs (Yates et al., 2016). This lack of measurable redox peaks under nonturnover conditions clearly contrasts with the plain peaks observed for anodic communities (Zhang et al., 2017). The catalytic current is only observed under air (thick black line), confirming that dissolved O_2 is the compound electroreduced by the EAB. The CV under air displays a sigmoid shape corresponding to a quasi-reversible heterogeneous electron transfer i.e. with a kinetics that does not limit the current density (Yates et al., 2016; Zacharoff and El-Naggar, 2017; Zhang et al., 2017).

The mid-wave potential of the CV and the peaks potential of its first derivative (Fig. S3) both provide a midpoint potential $E_{1/2}$ of 0.27 V . This suggests that this value may be the formal potential of the redox protein performing the heterogeneous electron transfer i.e. the primary electron acceptor of the electroautotrophic community at the considered pH (~ 7.8). Quasi-reversibility and similar $E_{1/2}$ are typically observed for O_2 -reducing EABs grown at electrode potentials high

enough to avoid abiotic O_2 reduction ($> -0.1 \text{ V vs. Ag/AgCl}$), disregarding the nature of the inoculum or the growth medium used (Milner et al., 2017; Rothballer et al., 2015; Ter Heijne et al., 2010).

To the extent of our knowledge, this is the first time that electroactive biofilms (cathodic or anodic) are reported to grow and produce substantial biocatalytic currents in unamended tap water. The maximum biocatalytic currents delivered by our EABs on graphite rods or glassy carbon RDEs showed important variations, ranging from 1 to $22 \mu\text{A cm}^{-2}$ ($n = 10$). These current densities were more variable between EABs and 5–100 times lower than when the EABs were grown in saline M9 medium containing vitamins and trace elements ($\sim 80 \mu\text{A cm}^{-2}$, Fig. S4), demonstrating that (Ghent) tap water is not an optimal medium for growing these EABs. Stability of the bioelectrocatalysis in tap water will be discussed in Section 3.4.

3.2. Microbial communities

The microbial communities for five EABs grown on graphite rods were comparable to one another (Fig. 3). The most represented operational taxonomic units (OTUs) were two unclassified Gammaproteobacteria ($25 \pm 15\%$, $n = 5$ electrodes, red fractions on the bars of Fig. 3), followed by two unclassified Chlamydiales ($13 \pm 5\%$), two *Rhodococcus* spp. ($12 \pm 6\%$) and two Microbacteriaceae ($6 \pm 3\%$). One of the two Gammaproteobacteria was found dominating ($50 \pm 17\%$, $n = 5$) O_2 -reducing EABs grown in saline medium producing substantially higher current densities ($80 \mu\text{A cm}^{-2}$, unpublished data). While the present data are not sufficient to conclude which OTUs are involved in the (probable) electroautotrophic activity of these biofilms, populations of unclassified Gammaproteobacteria are commonly dominating EABs electroreducing O_2 at high potentials (Desmond-Le Quémener et al., 2016; Rothballer et al., 2015; Strycharz-Glaven et al., 2013). Meta-omics approaches have recently revealed that an uncultivated gammaproteobacteria ("*Candidatus Tenderia electrophaga*") was likely the key electroautotroph of the most studied of these communities (Eddie et al., 2017).

3.3. Toxic shock monitoring

Toxic shock monitoring was performed for several heavy metals and organic toxicants (Fig. 4). For instance, the addition of 0.5 mg L^{-1} of Hg (II) induced a sharp decrease of the cathodic current in less than 1 min before leveling off after 20 min ($-19 \pm 8\%$, $n = 2$ electrodes, duplicate displayed in Fig. S5A). Increasing the concentration of Hg(II) up to 2.5 mg L^{-1} led to an additional fast decline in current. Replacing the contaminated tap water by uncontaminated tap water immediately stopped the sharp decrease and stabilized the current with a slight negative drift persisting for $\sim 24 \text{ h}$. Eventually, the reduction current increased again (Fig. S6), illustrating the self-regenerative properties of the microbial biosensor.

The limits of detection for all tested toxicants were typically in the range of $1\text{--}10 \text{ mg L}^{-1}$. For three of the toxicants, the first measurable impact was an immediate decrease of the biocatalytic current (Hg(II), Cr(VI) and benzalkonium chloride). Comparing CVs recorded before

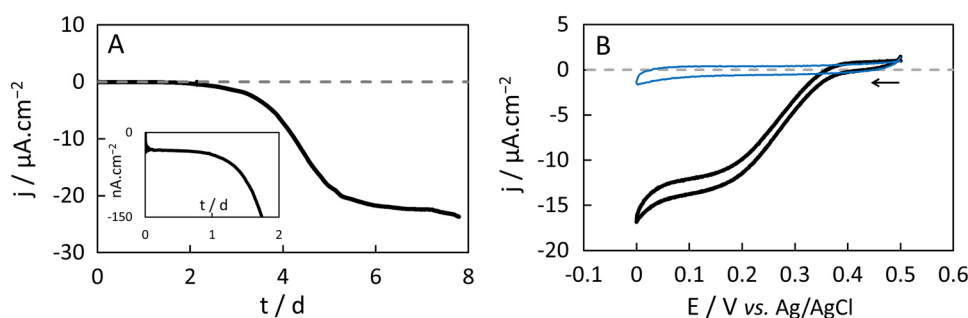


Fig. 2. (A) Increase of cathodic current associated with EAB growth in unamended tap water, glassy carbon RDE polarized at $+0.2 \text{ V vs. Ag/AgCl}$, 160 rpm, under air, inoculation at $t = 0$. The inset displays a zoom on the two first days. (B) Representative cyclic voltammograms of a mature EAB in tap water under air (thick black line) or anoxic conditions (thin blue line), at 0.2 mV s^{-1} .

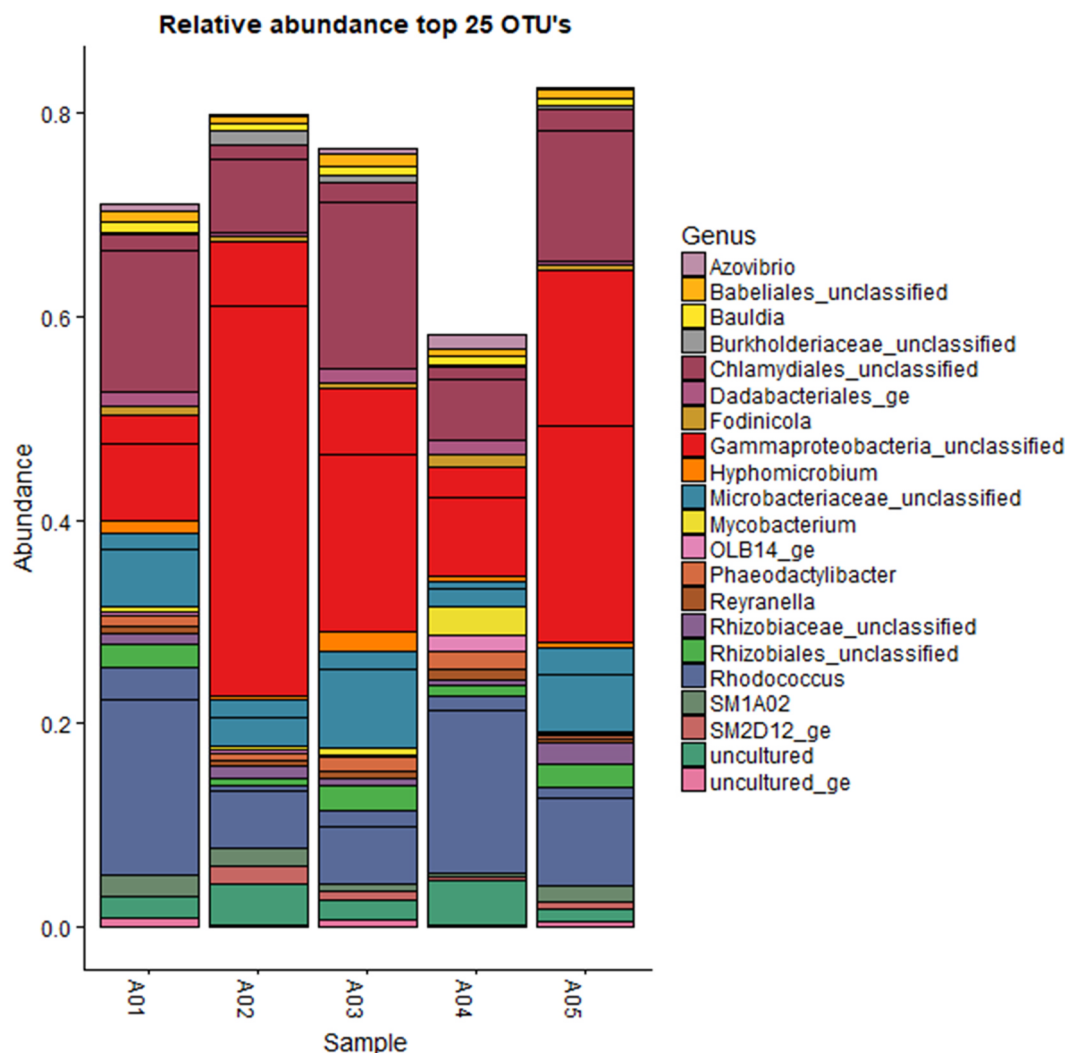


Fig. 3. Relative abundance of the microbial communities harvested on five O_2 -reducing EABs. Only the top 25 OTUs are represented.

and after the addition of a compound allowed to confirm the toxicity of the latter if a doubt subsisted on its putative abiotic electrochemical response (example for Cr(VI) in Fig. S7). Conversely, the microbial cathodes showed rather unusual responses to the additions of three other pollutants (Pb(II), formaldehyde and 2, 4 dichlorophenol). The cathodic current actually increased stepwise with formaldehyde concentration up to 25 mg L^{-1} , before quickly declining once 100 mg L^{-1} was reached (Fig. 2D). When Pb(II) or 2,4-dichlorophenol were added, the cathodic current first transiently increased before quickly decreasing (Fig. 2C and F).

None of these compounds are expected to be abiotically reduced on carbon at $+0.1 \text{ V}$ vs. Ag/AgCl (e.g. $E_{\text{Pb}^{2+}/\text{Pb}}^0 = -0.34 \text{ V}$ vs. Ag/AgCl), which was confirmed by abiotic controls (Fig. S8). Furthermore, the very small increase in conductivity and/or osmolarity induced by the compound additions does not increase the current (Fig. S9A). These controls confirm that the additions of pollutants caused variations of the microbially-induced current.

The possible reasons are yet unclear for the initial current increase induced by the addition of certain toxic compounds. It is not excluded that these compounds may trigger a quick and transient stimulation of a step limiting the electroactivity of the key electroautotroph(s), before inhibiting another biochemical step involved in current production. An initial increase in respiration rate could also be an indication that the bacteria are trying to cope with the pollutant by investing in detoxifying metabolisms (e.g. by pumping the pollutant out of their cells). It

could also be possible that these toxicants first inhibit the metabolism of microorganisms not producing current but competing for O_2 , such as heterotrophs (Ghent tap water contains $2.5 \pm 1.8 \text{ mg L}^{-1}$ of total organic carbon), before impacting the current-generating bacteria. This hypothesis would however involve the existence of an important O_2 mass transfer limitation for current production, but the later was not substantial (Fig. S9B). Although entirely speculative, a so-called direct interspecies electron transfer (DIET) from heterotroph species to the electroautotroph would provide the later with another electron source than the electrode, therefore competing for current production. In this case, any stimulus negatively impacting the heterotroph activity and/or the DIET itself would generate an increase of the cathodic current. From an applied perspective, it cannot be excluded that the simultaneous occurrence of several pollutants of antagonistic effects (positive and negative) induces a combined impact of lower amplitude on the current.

Duplicate experiments with different EABs showed similar limits of detection and response times for the respective toxicants (Fig. S5). Conversely, the relative sensitivities (relative current losses for a specific concentration shift in toxicant) showed variability between duplicates. This, in addition to the non-specific response towards toxicants, further highlights the possibility to use these microbial cathodes only as non-selective, non-quantitative biosensors for early warning systems. The fast response for all tested toxicants (typically $\sim 1 \text{ min}$) would be beneficial to effectively monitor a transient chemical

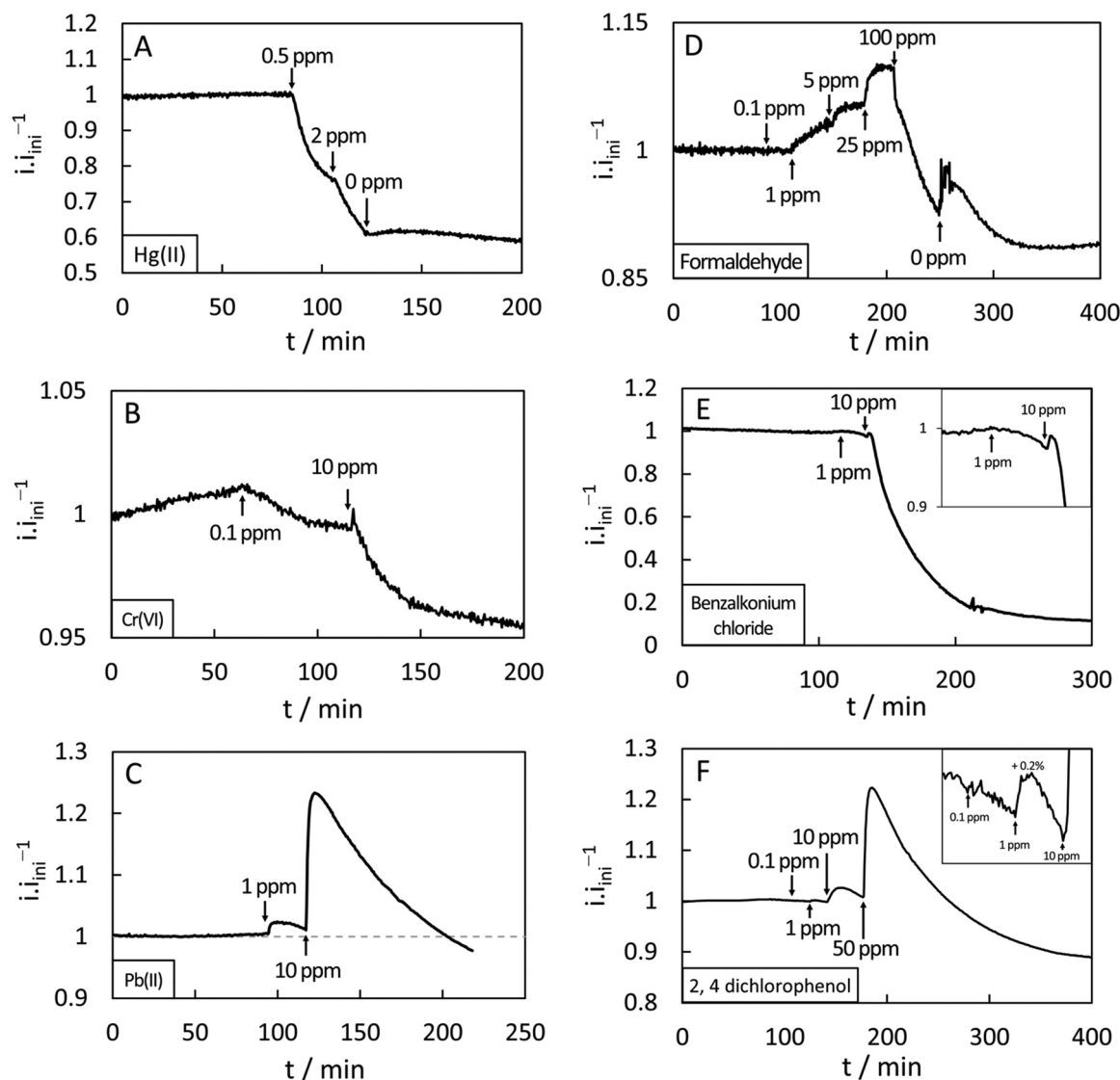


Fig. 4. Toxic shocks monitored for several heavy metals: (A) Hg(II), (B) Cr(VI), (C) Pb(II); and organic toxicants: (D) formaldehyde, (E) benzalkonium chloride, (F) 2, 4 dichlorophenol. Currents are normalized with respect to the initial baseline current in absence of toxicant. The additions of toxic compounds are represented by arrows with the corresponding new concentrations of the toxic element; '0 ppm' correspond to a replacement of the contaminated water by fresh tap water devoid of contaminant. Zooms in inset for smaller impacts. Baseline currents are provided in Table S3. Note differences in scales.

contamination occurring along the flow of a watercourse or a continuous bioprocess, which could otherwise remain unnoticed.

All previous studies on toxic shock monitoring using microbial cathode reported limit of detection (LOD) for formaldehyde ranging from 5 to 15 mg L⁻¹ (Jiang et al., 2018, 2017; Liao et al., 2018b). These values are lower than the lowest LOD reported for anodic EABs (25 mg L⁻¹, (Jiang et al., 2017)). To the extent of our knowledge, all LODs observed in the present study were lower than (or similar to) the LODs reported for heterotrophic anodic EABs, with an exception for Pb (II) (Table 1). Considering the multiple studies on toxic shocks monitored by microbial anodes (including those integrated in MFCs (Zhou et al., 2017)), this further suggests that O₂-reducing microbial cathodes are generally more sensitive to toxic shocks than anodic, heterotrophic EABs, as previously advocated (Jiang et al., 2017). This could be induced by the different intrinsic sensitivities of the distinct electroactive microorganisms. It can also be hypothesized that the thick (tens of μm) and dense anodic EABs (Zhang et al., 2018) provides better shielding against toxic shocks than the patchy cell clusters (5–25 μm diameter) typically forming the electroautotrophic EABs (Yates et al., 2016). Regardless, heterotrophic anodic EABs and autotrophic cathodic EABs are

Table 1

Comparison of the limits of detection of the present cathodic autotrophic biofilms with the smallest LODs reported for anodic heterotrophic biofilms. The LODs of the present study are also provided at molar concentration between parentheses.

Toxic compound	Limit of detection of toxic shocks		
	Cathodic EAB (this study) mg L ⁻¹ (μM)	Anodic EAB / MFC	
		mg L ⁻¹	Reference
Mercury(II)	0.5 (2.5)	1	(Yu et al., 2017)
Chromium(VI)	1 (19)	1	(Liu et al., 2014)
Lead(II)	< 10 (< 48) ^a	1	(Kim et al., 2007)
Formaldehyde	1 (33)	25	(Jiang et al., 2017)
Benzalkonium chloride	1 (N/A) ^b	N/A	N/A
2,4 dichlorophenol	10 (61)	N/A	N/A

^a a fraction of Pb(II) precipitated in tap water after an equivalent addition of 10 mg L⁻¹.

^b unavailable molar concentration because variable molecular weight.

not competing considering that their putative use as biosensors would be dedicated to quasi-anoxic solutions with high BOD levels for the former, and oxic solutions with low BOD levels for the latter.

A biosensor for non-specific, non-quantitative toxic shock monitoring does not need high measurement accuracy, but must avoid false positives and requires sufficiently small LOD for a dedicated use. The occurrence of false positives could be limited by implementing biosensors in series within a watercourse or a continuous bioprocess, considering their low cost.

Limits of detections of “natural” EABs – i.e. excluding uphill genetic engineering – will probably select for the possible applications. For example, Belgian regulations fix limits for Hg ($1 \mu\text{g L}^{-1}$), Cr ($50 \mu\text{g L}^{-1}$) and Pb ($10 \mu\text{g L}^{-1}$) in tap water which are between 20 and 1000 times lower than the respective LOD reported in the present study (FARYS, 2018). Although optimization is certainly possible, this suggests that EAB-based biosensors could be more relevant in detecting acute pollution, or monitoring industrial effluents whose discharge limits share similar magnitude with the LODs of the biosensor for the discharged compounds. The sensor could also be used in some process water in industrial plants, where the detection of toxicant would indicate some error upstream, for instance corrosion. The occurrence of antibiotic pollution in water resources (Gothwal and Shashidhar, 2015) could also be monitored.

3.4. Long term stability and drift of baseline current

Two EABs were maintained to assess the long-term stability of their electroactivity in tap water. They delivered a continuous biocatalytic current at +0.1 V for more than 8 months only by periodically replenishing the recirculating tap water (without adding other chemicals). The polarization curves recorded after 3, 5 and 8 months are displayed Fig. 5. They exhibit the sigmoidal shape at the high potential reduction induced by the microbial electrocatalysis. A representative two-month-long chronoamperometry of a cathodic EAB in tap water is displayed in Fig. S10A. This baseline current shows variation over time, which is inherent to any microbial electrode. The maximum relative drifts in current were below 2% per hour (see corresponding zoom on Fig. S10B). The magnitude of this current variation is much lower than the current changes typically recorded during the toxic tests presented in Fig. 4 (generally closer to the percent per minute). Yet the impact of variable environmental parameters on the baseline current should also be assessed. For example, seasonal and daily temperature cycles would impact the current during an environmental monitoring. It should however be rather straightforward to discriminate between a rather sharp current change induced by a toxic shock and a slow, periodic current variation due to a temperature cycle, for example by using detrending algorithms and autoregressive models.

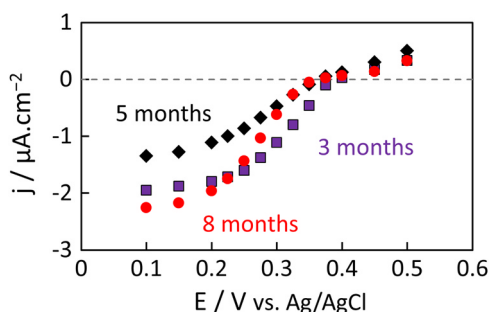


Fig. 5. Polarization curves of a cathodic EAB on graphite rod after 3 months (purple squares), 5 months (black lozenges) and 8 months (red circles) of continuous polarization at +0.1 V vs. Ag/AgCl in tap water refreshed every few days in a recirculating setup as schematized in Fig. 1. The polarization curves were obtained by successive chronoamperometries recorded at decreasing potentials and the current reported are at quasi steady-state.

4. Conclusion and perspectives

Oxygen-reducing microbial cathodes were grown for the first time in tap water. Some EABs survived for at least 8 months in renewed tap water without addition of exogenous compounds. The current density quickly responded to the additions of the six toxic compounds tested, with LODs similar or below the lowest ones reported the commonly studied heterotrophic anodic EABs. These O_2 -reducing microbial cathodes offer perspectives for cheap, non-selective monitoring of toxic compounds in freshwater with a high level of dissolved O_2 . Further research is needed to assess the applicability of such biosensors for environmental or bioprocess monitoring. For example, the impact of BOD-level variation on cathodic current could be studied, considering that higher organic load would favor the development of heterotrophic bacteria which can compete with electroautotroph for the electrode surface and O_2 consumption. Environmental communities specific to different waterbodies could affect the EAB stability (e.g. if they include predatory protists that are bacterivorous, or bacteriophage viruses). Biofilms used to monitor recurrent toxic shocks would likely evolve a tolerance towards specific toxicants, which should be investigated. The development of electroautotrophic biofilms is slow, and strategies used to decrease the startup-time of anodic EAB could therefore be tested (Guo et al., 2014). Finally, higher current densities would likely enhance the sensitivity of the biosensor. Repeated transfer can enrich microbial consortia producing higher cathodic current (Liao et al., 2018a). Although the current of anodic EABs is highly dependent on the electrode material properties (Guo et al., 2017), very little studies have investigated the corresponding impact on the current generated by cathodic EABs performing attested direct electron transfer.

Credit authorship Taxonomy statement

Antonin Prévotau: Conceptualization; Data curation; Formal analysis; Investigation ; Methodology; Validation; Writing - original draft

Peter Clauwaert: Conceptualization; Data curation; Writing - review & editing.

Frederiek-Maarten Kerckhof: Formal analysis; Methodology; Writing - review & editing.

Korneel Rabaey: Project administration, Funding acquisition, Writing - review & editing.

CRediT authorship contribution statement

Antonin Prévotau: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft. **Peter Clauwaert:** Conceptualization, Data curation, Writing - review & editing. **Frederiek-Maarten Kerckhof:** Formal analysis, Methodology, Writing - review & editing. **Korneel Rabaey:** Project administration, Funding acquisition, Writing - review & editing.

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Competing financial interests

The authors declare no competing financial interests.

Declaration of interests

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2019.02.037.

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