Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-020-02620-w

RESEARCH PAPER

Online self-powered Cr(VI) monitoring with autochthonous *Pseudomonas* and a bio-inspired redox polymer

Irene C. Lazzarini Behrmann[1,2] · Matteo Grattieri[2] · Shelley D. Minteer[2] · Silvana A. Ramirez[1] · Diana L. Vullo[1,3]

Received: 12 January 2020 / Revised: 24 March 2020 / Accepted: 26 March 2020

Abstract

The assessment of water quality is critical to implement preventive and emergency interventions aimed to limit/avoid environmental contamination and human exposure to toxic compounds. While established high-resolution techniques allow quantitative and qualitative determination of contaminants, their widespread application is not feasible due to cost, time, and need for trained personnel. In this context, the development of easy-to-implement approaches for preliminary detection of contaminants is of the utmost importance. Herein, a portable self-powered microbial electrochemical sensor enabling online monitoring of Cr(VI) is reported. The biosensor employs a bio-inspired redox mediating system to allow extracellular electron transfer between a bacterial isolate from chromium-contaminated environments and the electrode, providing a clear response to Cr(VI) presence. The biosensor shows good linearity ($R^2 = 0.983$) and a limit of detection of 2.4 mg L^{-1} Cr(VI), with a sensitivity of 0.31 ± 0.02 μA cm^{-2} $mgCr(VI)^{-1}$ L. The presented microbial bioanode architecture enhanced biosensor performance thanks to the improved "electrical wiring" between biological entities and the abiotic electrode surface. This approach could be easily implemented in engineered electrode surfaces, such as paper-based multi-anodes that maximize bacterial colonization, further improving biosensor response.

Keywords Self-powered biosensor · Cr detection · Online sensor · Microbial fuel cell · Redox polymer · *Pseudomonas veronii*

Abbreviations

MFC	Microbial fuel cell
MOPS	3-(*N*-morpholino)propanesulfonic acid
NQ-LPEI	Naphthoquinone-modified linear polyethyleneimine polymer
SCE	Saturated calomel electrode

Introduction

Access to safe water is regarded as critical to fulfilling the broad set of goals of the 2030 Agenda for Sustainable Development set by the United Nations [1]. This includes access to safe water for agricultural purposes, which significantly contributes to economic development and food supply. Accordingly, monitoring the presence of contaminants in water is of the utmost importance to limit environmental harm and avoid human exposure. Classical and established environmental monitoring approaches through advanced analytical techniques (i.e., high-performance liquid chromatography, mass and atomic absorption spectrometry, etc.) allow for the detection of metals, pesticides, and other toxic compounds with high accuracy and at very low concentrations. However, a drawback of such approaches is the requirement of sample collection and transport to centralized laboratories,

Published in the topical collection featuring *Female Role Models in Analytical Chemistry.*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-020-02620-w) contains supplementary material, which is available to authorized users.

✉ Shelley D. Minteer
minteer@chem.utah.edu

✉ Diana L. Vullo
dvullo@campus.ungs.edu.ar

[1] Instituto de Ciencias, Universidad Nacional de General Sarmiento, J.M. Gutierrez 1150, B1613GSX, Los Polvorines, Buenos Aires, Argentina

[2] Department of Chemistry, University of Utah, 315 S 1400 E Room 2020, Salt Lake City, UT 84112, USA

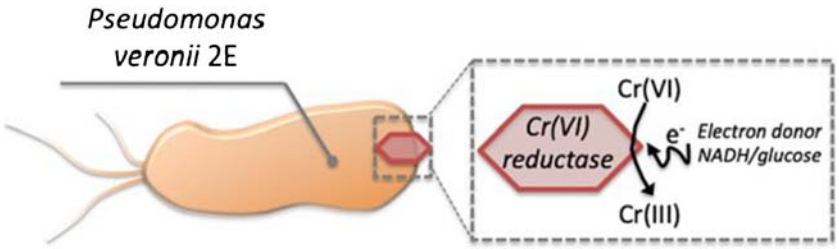
[3] CONICET, Godoy Cruz 2290, C1425FQB, Buenos Aires, Argentina

Published online: 08 April 2020

as well as trained personnel to perform the analysis. Furthermore, these approaches are time-intensive and expensive, making them not applicable for a preliminary, rapid, and easy-to-perform in-situ monitoring of water in remote or widespread areas [2, 3].

Hexavalent chromium (Cr(VI)) is highly toxic and carcinogenic, as well as highly soluble in water. It usually exists as $HCrO_4^-$, CrO_4^{2-}, or $Cr_2O_7^{2-}$ and contaminates both surface and groundwater due to its extensive use in several industrial and human activities [4]. The industrial use of Cr(VI) is widely spread as it is applied in tannery and electroplating processes. As a consequence, Cr(VI) could be discharged in water bodies with dramatic effects on biota if no treatment is applied. The United States Environmental Protection Agency recommended limit of total chromium in dischargeable industrial effluents is 0.1 mg L^{-1} (0.0019 mM) with a monthly average Cr(VI) maximum value not higher than 0.077 mg mL^{-1} [5]. Similar limits are fixed also for other countries, such as the case of Argentina, setting 2 mg L^{-1} as the limit for total chromium and 0.2 mg L^{-1} (0.0038 mM) for maximum Cr(VI) levels in industrial wastewaters to be discharged into either sewage or surface water systems [6]. In electroplating rinse baths, Cr(VI) levels as high as 10 g L^{-1} are used, with the risk of the unwanted release of Cr(VI) in concentration orders of magnitude higher than regular conditions, defined as a Cr(VI) shock [2]. To prevent environment and human exposure to high Cr(VI) levels, it is important to develop easy-to-implement monitoring approaches applicable over widespread areas, which should then be coupled to established and/or novel removal techniques (i.e., chemical precipitation, sorption and biosorption, membrane separation, etc.), as well as biotransformations [7–9]. Colorimetric detection of Cr(VI) can be used in a wide range of concentrations as low as 10^{-7} M (5.2 μg L^{-1})/10^{-8} M (0.52 μg L^{-1}) range [10–12]. However, these colorimetric approaches have some limitations, such as their difficult application for continuous online monitoring. In this context, the application of microbial electrochemical sensors constitutes an interesting alternative to colorimetric sensors, as they provide an easy-to-measure electric signal. The sensing unit is constituted by intact bacterial cells exchanging electrons obtained from their metabolism of organic substrates with an external electron acceptor (the electrode surface), a process defined as extracellular electron transfer [13–15]. The presence of contaminants altering the catalytic activity of the microorganisms leads to variations in the current response, enabling the development of a cost-effective and sustainable monitoring approach [16]. The use of microbial electrochemical sensors has been successfully reported for the monitoring of formaldehyde [17–19], arsenic [20], organic contaminants [21–25], atrazine [26, 27], and chromium [28–31], among others. Di Lorenzo and co-workers reported different approaches to develop miniaturized and cost-effective devices that could be employed for the detection of various compounds [19, 32, 33]. Self-powered operation of a biosensor refers to a condition where no external power supply is required, and the generated current/power response is proportional to the analyte of interest, constituting itself the biosensor signal [34]. Such a configuration was extensively investigated for Cr(VI) monitoring by Li and co-workers, which employed different engineering approaches for the sensing bioanode coupled to Pt-based cathodes (0.5 mg cm^{-2} Pt load) in microbial fuel cells (MFCs). Specifically, a batch-mode cube microbial fuel cell allowed Cr(VI) shock (1 and 8 mg L^{-1}) monitoring based on the potential difference of the MFC [28]. Later, they used flat filter membranes to develop the bioanode sensing unit and studied Cr(VI) shocks in a range of 5–20 mg L^{-1} [29]. A multi-anode geometry allowed enhancing power output compared to MFCs with a single anode, resulting in a better signal-to-noise response [30]. While the approaches reported by Li and co-workers dealt with the engineering of the bioanode, the enhancement of the extracellular electron transfer process through an improved "electrical wiring" of bacterial cells for Cr(VI) monitoring was not explored. Accordingly, the aim of this work was to develop a portable self-powered microbial electrochemical biosensor for the online monitoring of Cr(VI) shocks. *Pseudomonas veronii* 2E (*P. veronii* 2E), an autochthonous bacterial isolate belonging to a Cr-contaminated environment in Buenos Aires (Argentina), is utilized as the microbial sensing unit of the biosensor in a Cr(VI) range of 4–50 mg L^{-1}. The selection of this bacterial strain was motivated by evidence of Cr(VI) reduction to Cr(III) performed by *P. veronii* 2E in both growing and non-growing states, as reported in previous studies [7, 9]. Specifically, in non-growing states, a buffered viable cell suspension without any nutrient supply showed Cr(VI) biotransformation. Such a process was increased by the presence of an external electron donor, such as NADH or glucose. As no changes in total Cr concentration were registered in the supernatant during the biotransformation kinetics experiments, an extracellular Cr(VI) reduction to Cr(III) mediated by a biological catalysis associated to cell envelopes is suggested. Scheme 1 shows the Cr(VI) biotransformation process for *P. veronii* 2E. Based on this metabolism, it was assumed that the extracellular electron transfer between intact *P. veronii* 2E cells and an electrode surface would be affected by the presence of Cr(VI), as electrons would be diverted to accomplish the reduction to Cr(III) catalyzed by Cr(VI) reductase (Scheme 1).

Self-powered operation for the microbial sensor is achieved by coupling the anodic sensing unit, where a bio-inspired naphthoquinone redox polymer enhances electron harvesting from microbial metabolism, to a Pt-free activated carbon–based cathode in a single-chamber MFC. The current generated by applying a 1000-Ω resistor between the anode and cathode electrode allows Cr(VI)



Scheme 1 Cr(VI) biotransformation process in *P. veronii* 2E based on Cr(VI) reductase and an external electron donor

shock monitoring with a linear response up to 18.5 mg L^{-1}. It should be noted that in general other toxic and inhibiting compounds could affect the current response of the biosensor. While this is an important aspect that will require future studies, the non-specificity of the microbial sensor is not considered a drawback. In fact, application of the biosensor in the environment could provide early warning for the presence of various toxic compounds, while the application in specific environment as water streams resulting from the electroplating process could allow for a preliminary quantification of chromium. It remains clear that the current method is not supposed to substitute advanced analytical techniques, but to provide an easy-to-use and cost-effective early monitoring approach.

Material and methods

Chemicals

All of the chemicals used in this work, except magnesium chloride hexahydrate (Fluka), dextrose anhydrous (Fisher Scientific), calcium chloride (Fisher), and carbon black (Alfa Aesar), were purchased from Sigma. They were utilized without further purification.

Microorganism

P. veronii 2E is a native strain isolated from polluted sediments associated with the Reconquista River basin (Buenos Aires Metropolitan Area) and identified by 500-bp 16S rRNA gene sequencing. Previous studies proved its ability to remove Cd(II), Zn(II), and Cu(II) by biosorption processes, as well as to biotransform Cr(VI) to Cr(III) in both laboratory samples and industrial wastewaters [7, 9, 35]. *P. veronii* 2E cultures were developed in nutrient broth (beef extract 3.0 g and bactopeptone 5.0 g per 1 L ultrapure water 18.2 MΩ cm^{-1}) at 30 °C for 24 h at 120 rpm. Cells were harvested by centrifugation at 4500*g* for 20 min and suspended in 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer with 10 mM $MgCl_2$ (pH 7) for a final concentration of 1 g mL^{-1}.

Bioanode preparation

Carbon felt (Sigracell® GFA 6EA, SGL group) was utilized as electrode support, with a geometric area of 1 cm^2. The electrode surface was first modified with the naphthoquinone-modified linear polyethyleneimine polymer (NQ-LPEI) synthetized as previously reported [36]. Initial electrode modification was performed as follows: 37.5 μL of 10 mg mL^{-1} ethylene glycol diglycidol ether was added to 1 mL of a 7.0 mg mL^{-1} NQ-LPEI polymer solution, and 120 μL of the obtained solution was deposited on one side of the 1 cm^2 electrode. The modified electrode was dried in darkness for 15 h. Following, the modified electrodes were soaked for 60 s in a *P. veronii*-sodium alginate suspension, prepared as follows: a bacterial cell suspension (10 g dry weight L^{-1}) mixed with an equal volume of 15 g L^{-1} sodium alginate solution, and immediately immersed in 0.2 M $CaCl_2$ for 20 s. Finally, the obtained bioanodes were washed in ultrapure water prior to their use for electrochemical characterization. For negative control experiments, autoclaved cells (125 °C for 25 min) and a cell-free sodium alginate solution replaced living bacteria. For application in the MFC setup (self-powered biosensor), the bioanode was prepared with a geometrical area of 4 cm^2, modified with 240 μL of polymer solution deposited on each side of the electrode, and following the same procedure to soak the electrodes in the *P. veronii*-sodium alginate suspension.

Additional control experiments were performed with bacterial cells directly deposited on an electrode surface (direct electron transfer), or utilizing a diffusible redox mediator (*p*-benzoquinone), rather than the NQ-LPEI redox polymer, to confirm its role in the extracellular electron transfer process. Details on the preparation of the control bioanodes are reported in the Electronic Supplementary Material (ESM).

Electrochemical setup

A three-electrode cell was set up for initial characterization of the bioanode utilizing a saturated calomel electrode (SCE, CHI 150, CH Instruments, Inc.) and a Pt mesh as a counter electrode. Cyclic voltammetry (CV) and amperometric i-t experiments were performed with CH Instruments potentiostats (CH Instruments, Inc.). A BioLogic potentiostat was used to test the biosensor in self-powered configuration (the MFC

setup). The supporting electrolyte was 20 mM MOPS-10 mM $MgCl_2$ (pH adjusted to 7). Glucose solution was added as the substrate for the bacterial cells to a 20-mM final concentration. 19.5 mM K_2CrO_7 was used as Cr(VI) stock solution. Cr(VI) shocks were explored in a concentration range from 4 to 50 mg L^{-1}.

Self-powered biosensor setup

All MFC experiments were performed in a single-chamber electrochemical cell with 60 mL 20 mM MOPS-10 mM $MgCl_2$, pH 7, and glucose 20 mM. Carbon felt (4 cm^2 geometrical area) electrodes were used as a support to immobilize *P. veronii* 2E and used as the anode. Air-breathing activated carbon–based electrodes were used as the cathode, prepared as previously reported [37, 38]. Briefly, 3.5 g activated carbon, 0.5 g carbon black, 2.5 mL poly(tetrafluoroethylene) (PTFE), and 2.0 mL water were mixed and the paste was pressed onto a stainless steel mesh disk at 140 °C with 5000 psi for 5 min. The electrical circuit between anode and cathode electrodes of the MFC was completed by setting a specific ohmic resistance (1000 Ω) between the cathode (utilized as the working electrode), and the anode (utilized as counter and reference electrode), employing the BioLogic potentiostat. By utilizing this setup, the ohmic resistance between the electrodes was accurately controlled while continuously monitoring the current response (presented as current density, j, based on the anode geometric area) of the microbial fuel cell. Cr(VI) shocks were studied by performing subsequent additions of Cr(VI) from a 19.5 mM K_2CrO_7 stock solution.

Scheme 2 shows the setup that was employed.

Statistics and data analysis

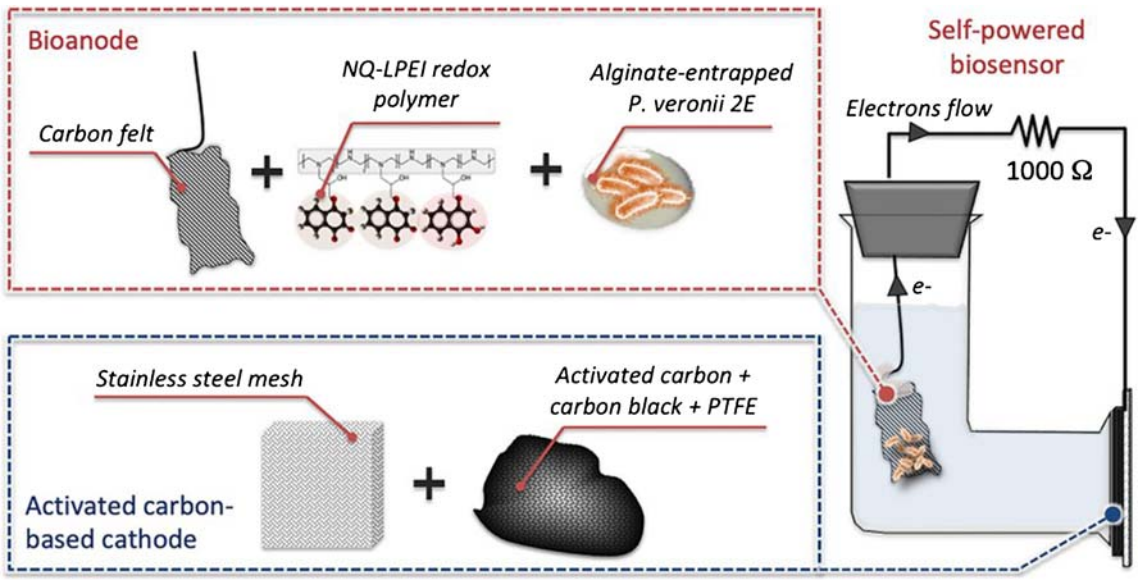
At least three independent replicate experiments were performed for all the measurements. Mean values are reported, with the errors and error bars indicating one standard deviation. Data were evaluated for outliers by the *Q*-test.

Results and discussion

Bioanode evaluation

Preliminary experiments were aimed to investigate the possibility to perform direct electron transfer between *P. veronii* 2E and the electrode surface, as well as the optimal glucose concentration for bioelectrocatalysis studies. The results obtained by cyclic voltammetries showed that direct electron transfer between bacterial cells and the electrode was possible, but only limited current values were achieved (ESM Fig. S1), motivating our interest in exploring different approaches of mediated electron transfer. Furthermore, a broad concentration range of glucose was tested, (0–180 mM, ESM Fig. S2), with the best results in terms of maximum current response obtained at a concentration of 20 mM glucose, which was selected for further experiments.

In order to facilitate the extracellular electron transfer, a bio-inspired NQ-LPEI redox mediating system developed in our group was employed. In this artificial mediating system, naphthoquinone redox moieties resembling redox carriers present in bacterial cells are confined on the polymer backbone. Such a bio-inspired redox system was recently shown to enhance extracellular electron transfer for *Shewanella*



Scheme 2 Bioanode and activated carbon–based air-breathing cathode components and their setup in the self-power biosensor. Electrons flow from the anode to the cathode through a 1000-Ω resistor, as indicated in the right side of the scheme

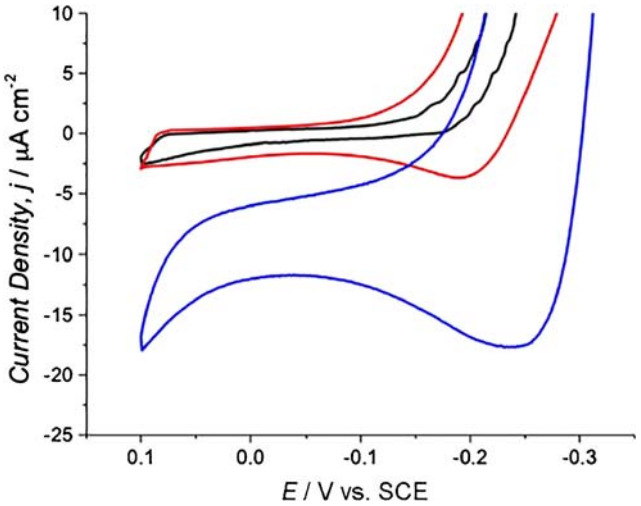


Fig. 1 Cyclic voltammograms for *Pseudomonas veronii* 2E immobilized on carbon felt electrodes modified with NQ polymer (blue line). Control experiments with autoclaved bacteria (red line) or cell-free electrodes (black line) in 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$. Substrate, 20 mM glucose; scan rate, 1 mV s^{-1}; reference electrode, saturated calomel electrode; counter electrode, Pt

oneidensis MR-1 [39], for the photosynthetic purple bacterium *Rhodobacter capsulatus* [40], as well as for a gram-positive bacterium [41]. As shown in Fig. 1, the polymer in the presence of *P. veronii* 2E cells allowed a clear bioelectrocatalytic response (blue line) compared to cell-free electrodes (black line) and electrodes with inactive cells (autoclaved, red line). Furthermore, it is important to note that extracellular electron transfer was significantly enhanced compared to direct electron transfer conditions, reaching a current density of -16 ± 4 μA cm^{-2}. To further explore the effectiveness of the NQ-LPEI redox system in enhancing the extracellular electron transfer, bioanodes employing a quinone-based diffusible redox mediator were also prepared. Accordingly, *P. veronii* 2E cells were immobilized in a calcium alginate matrix and 0.3 mg mL^{-1} of activated carbon was incorporated to the matrix to improve conductivity, following the approaches recently reported by our group [42, 43]. *p*-Benzoquinone was added to the cell-alginate suspension as an exogenous electron transfer mediator, since its capability to diffuse through the cellular membrane of bacterial cells and act as a redox shuttle has been reported for several bacterial species [44, 45]. As shown in ESM Fig. S3A, the maximum current density for such a mediating system was -9 ± 5 μA cm^{-2}, confirming the outstanding role of the redox polymer in harvesting the electrons from microbial metabolism.

Chromium(VI) effect and online monitoring

The effect of Cr(VI) on the biocatalytic response of *P. veronii* 2E was first explored by cyclic voltammetry utilizing a Cr(VI) shock of 50 mg L^{-1} (Fig. 2a). The current signal obtained from the bioanode (blue continuous line) was considerately suppressed in the presence of the contaminant (blue dashed line), opening for the possibility to utilize the developed system for Cr(VI) monitoring. Conversely, when inactive cells were employed (red), no significant difference was obtained in the presence or absence of Cr(VI) shock. Following this preliminary test, the quantitative application of Cr(VI) inhibitory effect was investigated for Cr(VI) shocks in a concentration range from 4 to 50 mg L^{-1} (Fig. 2b). As shown, the biosensor presented a linear current response to Cr(VI) concentration in the range between 4 and 18.5 mg L^{-1} of Cr(VI). The performance of the biosensor with the NQ-LPEI redox polymer was again compared to the biosensor prepared employing *p*-benzoquinone as a diffusible redox mediator (ESM Fig. S3B), revealing that the use of the redox polymer outperformed the diffusible redox mediator (0.31 ± 0.02 μA cm^{-2} $mgCr(VI)^{-1}$ L, $R^2 = 0.98$; 0.22 ± 0.04 μA cm^{-2} $mgCr(VI)^{-1}$ L, $R^2 = 0.94$, respectively). It should also be noted that a satisfactory calibration curve could not be obtained for the bioanode employing the diffusible redox mediator, and a lower concentration range was considered for the comparison of the two bioanode architectures. These results further underline the importance of utilizing the NQ-LPEI redox polymer for biosensor development. It is important to note that the low redox potential of the NQ-LPEI redox polymer allows harvesting the electrons obtained from bacterial metabolism with a lower overpotential compared to the diffusible mediator.

Analyzing the performance of the biosensor in view of current legislation, it is important to note that even if the biosensor employing NQ-LPEI ensures a lower limit of detection (2.4 mg L^{-1}) and limit of quantification (8.1 mg L^{-1}) compared to the configuration employing p-benzoquinone (0.7 mM) + activated carbon (lower limit of detection 6.4 mg L^{-1}, limit of quantification 21.2 mg L^{-1}), such performance does not allow reaching the required limits settled by the Argentinian legislation for chromium release in industrial wastewaters. However, the current system is of interest for the early detection of Cr(VI) shocks, where concentrations can largely exceed the limits settled by law resulting from the malfunctioning of chromium removal processes, and to allow the implementation of preventive/emergency interventions. *P. veronii* 2E electroactivity and the discussed chromium-inhibiting effect constitute the bases for the development of an online Cr(VI) monitoring system. Based on these results, the possibility to use the bioanode employing NQ-LPEI as a redox mediating system in a MFC was explored as a self-powered Cr(VI) biosensor.

Self-powered biosensor

The possibility to employ the developed bioanode with an air-breathing Pt-free activated carbon–based cathode in a MFC

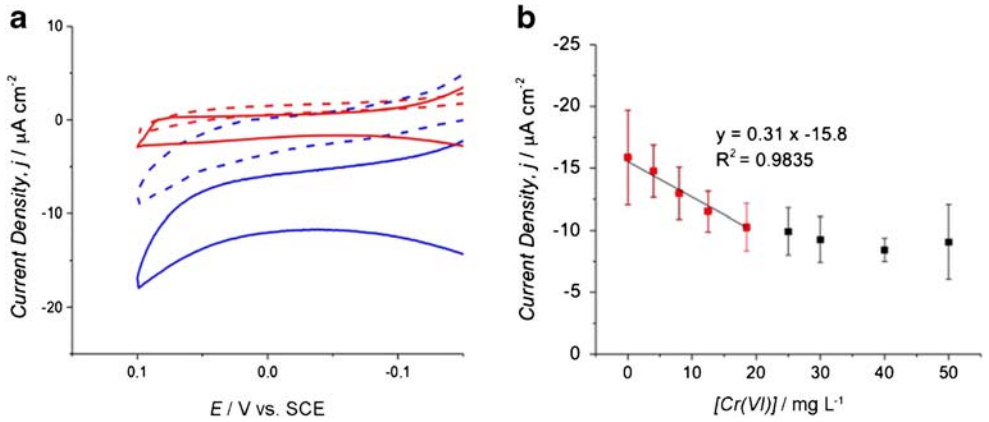


Fig. 2 **a** Cyclic voltammograms for *Pseudomonas veronii* 2E with Cr(VI) addition in 20 mM MOPS buffer (pH 7) + 10mM $MgCl_2$, 0 mg L^{-1} Cr(VI) (continuous line), 50 mg L^{-1} Cr(VI) (dashed line). Control experiments with autoclaved bacteria (red). Substrate, 20 mM glucose; scan rate, 1 mV s^{-1}; counter electrode, Pt; reference electrode, SCE. **b** Current density vs. [Cr(VI)] mg L^{-1} determined at the applied potential of + 0.1 V vs. SCE

was first explored by performing power curves, as shown in Fig. 3a. Quasi-stationary polarization curves at 0.1 mV s^{-1} with the cathode as the working electrode and the anode as the counter and reference electrode were performed to determine the power output. The power was calculated based on the formula $P = E \times I$, where P is the power, E is the potential, and I is the current density. *P. veronii* 2E cells immobilized in the presence of the NQ-LPEI redox polymer achieved a significantly higher power generation (57 ± 7 mW m^{-2}) that in the absence of the polymer (1.7 ± 0.7 mW m^{-2}). Interestingly, control experiments performed with autoclaved cells and the NQ-LPEI polymer, or only the NQ-LPEI polymer, also showed a limited power output, which could be explained with the limited capability of the polymer to catalyze the oxidation of the carbon substrate.

Based on the power outputs, the successful implementation of the developed bioanode in a MFC was demonstrated, allowing for its operation without the need for an external power supply (self-power mode). The current production of the self-powered biosensor is shown in Fig. 3b. Upon setting the 1000-Ω resistance between the anode and cathode, current density stabilized at 124 ± 4 mA m^{-2}. The addition of Cr(VI) resulted in an immediate decrease of the current response, which reached 50 ± 10 and 24 ± 7 mA m^{-2} for final Cr(VI) concentrations of 12.5 and 18.5 mg L^{-1}, respectively, suggesting a linear behavior. Comparison with the control experi-

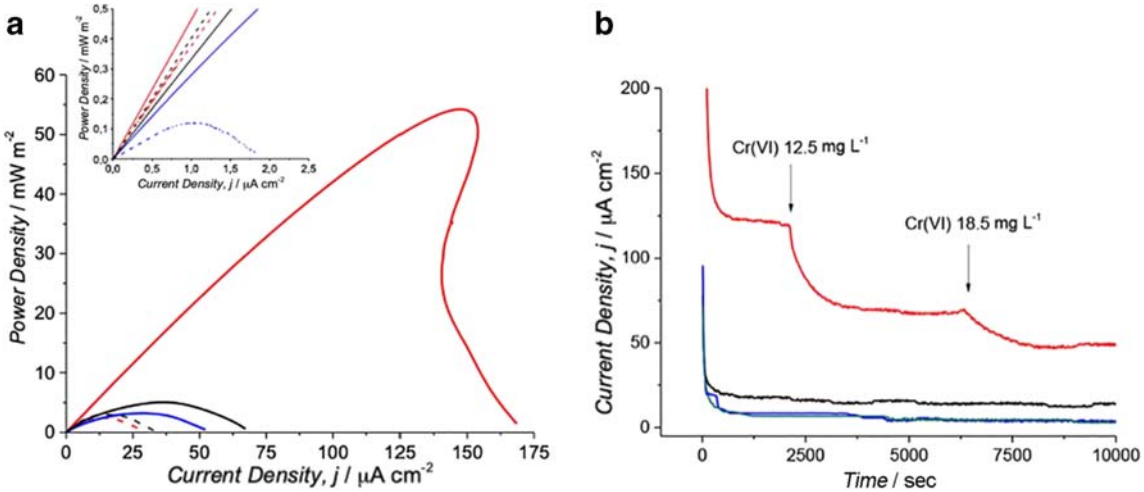


Fig. 3 **a** Power density (mW m^2) vs. current density for *P. veronii* 2E immobilized in calcium alginate with or without NQ-LPEI redox mediating system (continuous red and dashed red, respectively). Control experiments were performed with autoclaved bacteria with and without NQ-LPEI (continuous black and dashed black, respectively), and with cell-free electrode with and without NQ-LPEI (continuous blue and dashed blue, respectively). The inset shows the power curve for cell-free electrode without NQ-LPEI. **b** Self-powered operation of the biosensor showing current density vs. time for *P. veronii* 2E immobilized in calcium alginate (black), and with NQ-LPEI (red). Control experiments were performed using cell-free electrode with and without NQ-LPEI (light blue and blue, respectively). Arrows show Cr(VI) additions and final Cr(VI) concentration in solution

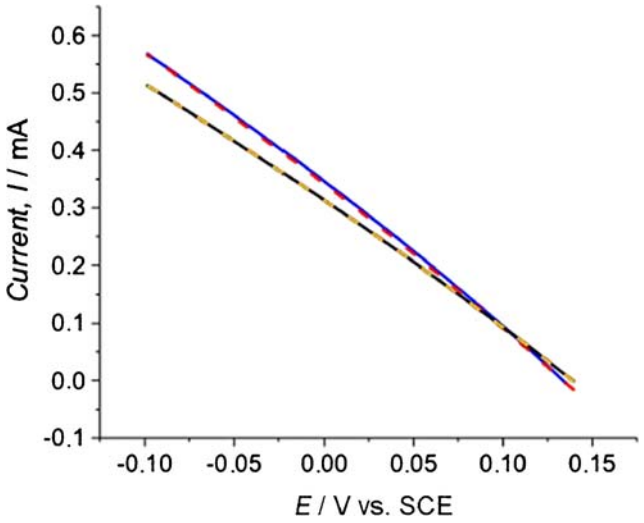


Fig. 4 Cathode polarization curves performed in the absence of Cr(VI) (blue and black continuous lines) or in the presence of 6.6 and 12.5 mg L^{-1} Cr(VI) (yellow and red dashed lines, respectively). Scan rate, 0.2 mV s^{-1}

ments (autoclaved cells and cell-free anodes) clearly shows that the current response is resulting from a biological activity related to bacterial cells. Furthermore, in order to confirm that Cr(VI) inhibition was affecting the bioanode and not the performance of the air-breathing activated carbon–based cathode, cathodic polarizations in the presence and absence of Cr(VI) were performed and are reported in Fig. 4. It can clearly be noted that no significant difference was obtained for the polarizations performed in the presence of different Cr(VI) concentrations (from 0 to 12.5 mg L^{-1}).

The result confirms that the oxygen reduction reaction taking place at the cathode electrode is not affected by the presence of Cr(VI), and the variation in current output obtained for the MFC after Cr(VI) addition is related with the inhibition of the *P. veronii* 2E cells at the bioanode. When comparing the performance of the portable self-powered biosensor developed in this work with self-powered microbial electrochemical sensors for Cr(VI) shocks reported in the literature, it can be noted that the range of Cr(VI) concentrations that could be monitored (2–50 mg L^{-1}) is comparable to other works [28–31]. Direct comparison of the sensitivities for the different biosensors reported is more complicated, since most of the work reported the effects of Cr(VI) in terms of variation of the difference of potential of the MFCs, or changes in power output, rather than current production. However, it is critical to note that while the approaches reported in literature focused on bioanode and MFC engineering, this work tackles for the first time the extracellular electron transfer process. By employing a bio-inspired redox polymer, an improved "electrical wiring" of the intact bacterial cells was achieved. Specifically, it was possible to enhance the extracellular electron transfer between the autochthonous *Pseudomonas* and the anode, enabling higher power and current densities in the developed self-powered biosensor. Accordingly, the presented system could be easily implemented also in other anode configurations reported, with the possibility to greatly improve the performance of self-powered biosensors.

Finally, we further investigated the possibility to reuse the self-powered biosensor after initial exposure to Cr(VI). Accordingly, the solution containing Cr(VI) was removed and replaced with fresh electrolyte. Power generation and current production in self-powered operation were then tested with new additions of different Cr(VI) concentrations (Fig. 5a and b, respectively). Replacing the electrolyte did not allow recovering the initial performance of the biosensor; however, it was still possible to monitor the presence of Cr(VI) due to the variation in power/current production. Future studies will be aimed to explore the possibility to recover the initial performance of the biosensor in order to monitor multiple Cr(VI) shocks with the same system.

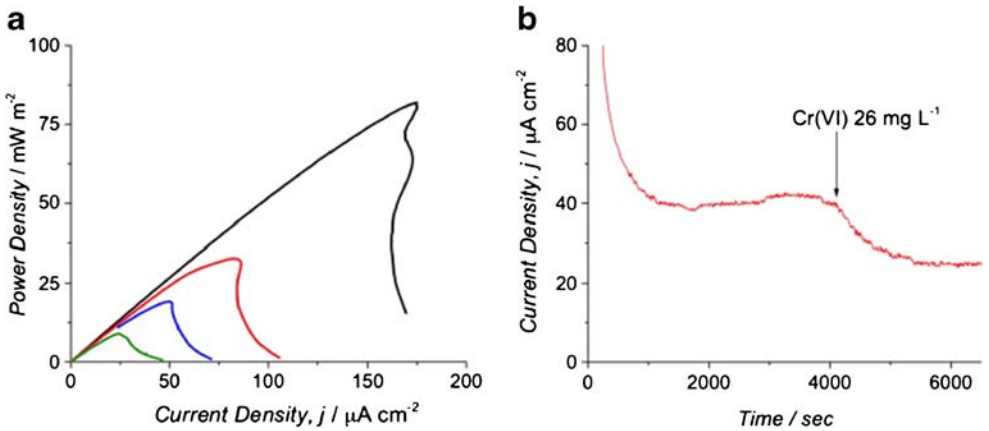


Fig. 5 **a** Power density (mW m^2) vs. current density for *P. veronii* 2E immobilized in calcium alginate with NQ-LPEI system performed in the absence or with chromium addition: in absence of Cr(VI) (black), after the first addition of Cr(VI) 12.5 mg L^{-1} (red). Replaced with fresh electrolyte (blue), and after a new addition of Cr(VI) 12.5 mg L^{-1} (green). **b** Self-powered operation of the biosensor showing current density vs. time for *P. veronii* 2E immobilized in calcium alginate NQ-LPEI after replacing the solution with fresh electrolyte (red). The arrow shows Cr(VI) addition and final Cr(VI) concentrations in solution

Recent literature on microbial electrochemical sensors highlighted the critical challenge of establishing electrochemical communication between intact bacterial cells capable to operate in contaminated solutions and an electrode surface [26, 46–48]. However, in view of the future implementation of the presented self-powered biosensor in the field, future studies should focus on the detection of Cr(VI) in real water samples. In this context, it should be noted that together with Cr(VI) levels as high as 10 g L^{-1}, electroplating rinse baths contain also Pb (ca. 0.1 ppm), Cu, and Zn (ca. 100 ppm each). In view of future studies focused on the application and optimization of the biosensors for the monitoring of Cr(VI) released from these baths, it is important that the utilized bacterial strain can cope with the presence of the indicated toxic compounds. Outstandingly, it was previously shown that, regardless of the presence of other metals, a 100% of Cr(VI) reduction was achieved by *P. veronii* 2E immobilized in calcium alginate while working with electroplating rinse baths with the described composition [9], opening a door for the application of the biosensors with real samples.

Conclusions

Cost-effective and easy-to-apply approaches for online water monitoring are critical to avoid the release of toxic compounds in the environment. This work represents a proof of concept for the possibility to use the current produced by *P. veronii* 2E employing NQ redox polymer for the monitoring of Cr(VI) in water with a linear range of 4 to 18.5 mg L^{-1} and a sensitivity of 0.31 ± 0.02 μA cm^{-2} $mgCr(VI)^{-1}$ L. The system could be utilized to obtain a portable self-powered biosensor, which could be employed in the field for early detection of Cr(VI) with no need of trained personnel. The use of this autochthonous bacterial strain able to biotransform Cr(VI) opens for the possibility to develop bioelectrochemical systems for its simultaneous monitoring and removal. Furthermore, the presented approach could be coupled with bioanode engineering and MFC miniaturization approaches reported in the literature, allowing for the development of easy-to-apply self-powered biosensors with improved performances.

Funding information This work was financially supported by the Universidad Nacional de General Sarmiento, Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT 2014-0964 and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). This research was financially supported by Research Grant Award No. IS-5060-18 from BARD, The United States - Israel Binational Agricultural Research and Development Fund.

Compliance with ethical standards

This research did not involve human and/or animal participants.

Conflict of interest The authors declare that they have no conflict of interest.

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