

Citation for published version:

Di Lorenzo, M, Thomson, AR, Schneider, K, Cameron, PJ & Ieropoulos, I 2014, 'A small-scale air-cathode microbial fuel cell for on-line monitoring of water quality', *Biosensors and Bioelectronics*, vol. 62, pp. 182-188. <https://doi.org/10.1016/j.bios.2014.06.050>

DOI:

[10.1016/j.bios.2014.06.050](https://doi.org/10.1016/j.bios.2014.06.050)

Publication date:

2014

Document Version

Peer reviewed version

[Link to publication](#)

Publisher Rights

CC BY-NC-ND

University of Bath

Alternative formats

If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

A Small-scale Air-cathode Microbial Fuel Cell for On-line Monitoring of Water Quality

Mirella Di Lorenzo^{a,*}, Alexander R. Thomson^a, Kenneth Schneider^b, Petra J. Cameron^b, Ioannis Ieropoulos^c.

^a University of Bath, Department of Chemical Engineering, Bath, BA2 7AY, UK

^b University of Bath, Department of Chemistry, Bath, BA2 7AY, UK

^c Bristol Robotics Laboratory, University of the West of England, Bristol, UK

*Corresponding author:

Email: M.Di.Lorenzo@bath.ac.uk

Telephone: +44(0)1225 385574

Abstract

The heavy use of chemicals for agricultural, industrial and domestic purposes has increased the risk of freshwater contamination worldwide. Consequently, the demand for efficient new analytical tools for on-line and on-site water quality monitoring has become particularly urgent.

In this study, a small-scale single chamber air-cathode microbial fuel cell (SCMFC), fabricated by rapid prototyping layer-by-layer 3D printing, was tested as a biosensor for continuous water quality monitoring.

When acetate was fed as the rate-limiting substrate, the SCMFC acted as a sensor for chemical oxygen demand (COD) in water. The linear detection range was 3 - 164 ppm, with a sensitivity of $0.05 \mu\text{A mM}^{-1}\text{cm}^{-2}$ with respect to the anode total surface area. The response time was as fast as 2.8 minutes.

At saturating acetate concentrations (COD>164 ppm), the miniature SCMFC could rapidly detect the presence of cadmium in water with high sensitivity ($0.2 \mu\text{g l}^{-1} \text{cm}^{-2}$) and a lower detection limit of only $1 \mu\text{g l}^{-1}$. The biosensor dynamic range was 1 - 25 $\mu\text{g l}^{-1}$. Within this range of concentrations, cadmium affected only temporarily the electroactive biofilm at the anode. When the SCMFCs were again fed with fresh wastewater and no pollutant, the initial steady-state current was recovered within 12 minutes.

Keywords

Microbial fuel cell, Biosensor, Water quality, BOD, Cadmium

Introduction

Worldwide freshwater systems are contaminated with a plethora of trace compounds, originating from both synthetic and natural trace sources. Although the concentration of individual compounds in water may be low (micropollutant: picograms per litre to nanograms per litre), the effect of the mixing and inter-reaction of many compounds together (co-contamination) is a significant concern, primarily for long-term effects on aquatic life and human health (Schwarzenbach et al. 2006). The need for screening tools that help to understand the impact of these micropollutants and of their combination on the aquatic biota and potentially on human health is particularly urgent.

Microbial fuel cells (MFCs) have enormous potential as biosensors for water quality (Wang et al. 2013). MFCs are bioelectrochemical devices that produce electrical energy through the action of specific microbes (known as anodophiles), capable of transferring the electrons generated from the oxidation of organic compounds (the fuel), to an anode electrode. As a result, the current generated by MFCs directly reflects the metabolic activity of the anodophiles at the anode.

Under non-saturated fuel conditions, any variations in the concentration of the organic matter fed into the system, is directly proportional to the amount of electrons transferred onto the anode and therefore the output current. This is the basic principle behind the use of MFCs as amperometric sensors for the biochemical oxygen demand (BOD) in wastewater. MFC-type BOD sensors have proven to be a valid alternative to the traditional BOD₅ analytical method, and a very powerful

screening tool for *in-situ*, on-line, real time measurements with good operational stability (Di Lorenzo et al. 2009 ; Di Lorenzo et al. 2009; Kim et al. 2003b).

If, on the other hand, the MFC works at saturated fuel concentrations, with other parameters such as pH, salinity, temperature and anode potential remaining constant, then unexpected variations in the current output can be associated with the presence of toxicants in the feeding stream (Stein et al. 2010). The presence of a toxicant in the feeding solution can in fact affect the microbial metabolism and growth with consequent changes in the current generated (Cockerham and Shane 1994; Wang et al. 2013). The MFC can therefore act as an indicator for biologically active compounds in water (Kim et al. 2007). Currently, the methods to assay the bioavailability of a target toxicant involve the use of complex organisms such as fishes, daphnia, algae or bioluminescent microorganisms (Leal et al. 2012; Matsunaga et al. 1999; Qu et al. 2013; Zhang et al. 2012). These methods require long incubation time (up to several weeks), and have low reproducibility and stability. They also rely on the use of an external transducer to detect the signal from the organism, which complicates the measurement process and is often the cause of low accuracy.

The unique advantage of MFC-based biosensors is that there is no need for an external transducer, since the presence of a pollutant in the feeding stream is immediately recorded as a change in electrical output response from the system (Stein et al. 2012b). The MFC technology can therefore lead to a cost-effective, simple, and sustainable screening tool that provides real-time, *in-situ* and continuous screening of biologically active pollutants in different water environments (Kim et al. 2007). Real-time screening systems are critical in the case of accidental large spills of toxicants, to allow immediate action and to minimise the negative environmental

impact. Moreover, real-time screening is a vital step in assessing and monitoring the efficacy of the wastewater treatment in wastewater re-use programmes, for which there is increasing need as pressure on water resources grow and water directives become more stringent.

To date, the research on MFC-based sensors for toxicants in water considered only the two-chamber configuration. Single-chamber air-cathode MFCs (SCMFCs), on the other hand, are easier to miniaturise for cost-effective sensor development (Yang et al. 2013). The advantage of miniature MFC sensing devices relies mainly on the improvement of the mass transport element, which minimises any differences in the analyte concentration at the input phase and the biofilm on the electrode, thus leading to a more reliable sensor (Stein et al. 2012b). Furthermore, the better oxygen supply at the air-cathode of the SCMFC can improve the system operation and therefore enhance the detection range (Di Lorenzo et al. 2009). Due to the shorter distances within, the sensor response time is also faster for miniature MFCs (Dávila et al. 2011).

This work investigates the use of a small-scale air-cathode single-chambered microbial fuel cell (SCMFC) as a sensor for water quality. The SCMFC is a very compact and handy device that was fabricated using layer-by-layer 3D printing. The miniature SCMFC was first tested as sensor for the labile organic carbon in water, by assessing its amperometric response to increasing concentrations of acetate in water. Subsequently, the SCMFC was tested as a tool to rapidly detecting the presence of toxicants in water. In particular, cadmium was chosen as model toxic compound. This chemical is considered carcinogenic and in 1976 it was classified in

Group 2A by the International Agency for Research on Cancer (IARC). The concentrations range of cadmium in rainwater, fresh waters, and surface waters in urban and industrialised areas, typically varies within $0.01 \mu\text{g l}^{-1}$ to $4 \mu\text{g l}^{-1}$, depending on specific location (Elinder 1985). The direct discharge from industrial operations, and the heavy use of fertilisers in agriculture can however cause accidental increase in these values, which might exceed the Maximum Contaminant Level (MLC) of cadmium in water systems ($5 \mu\text{g l}^{-1}$).

The aim of this study was to provide proof-of-concept evidence that the small-scale SCMFC can be used as valid alternative to traditional expensive and time-consuming analytical methods, such as atomic absorption spectroscopy, for the real-time detection of cadmium in water. With this purpose, the amperometric response of the SCMFC to accidental spills of cadmium in water was tested in continuous mode. The performance of the biosensor was evaluated in terms of detection range, sensitivity, response time and reproducibility.

2 Material and methods

2.1 Materials

All the reagents used were of analytical grade with no further purification prior to use, and were purchased from Sigma-Aldrich. All aqueous solutions were prepared with reverse osmosis purified water.

2.2 Microbial fuel cells

The air-cathode single-chamber microbial fuel cells were realised in polycarbonate acrylonitrile butadiene styrene (PC/ABS, Makerbot) by means of a rapid prototype 3D

printer (Replicator® 2, Makerbot). The SCMFC components were layer-by-layer printed along with the incorporation while printing of the anode, proton exchange membrane (PEM), and cathode (Figure 1). No fixtures, fittings or gaskets for sealing were necessary for the devices, thus leading to very compact, easy to use systems that could rapidly be produced.

The SCMFCs consisted of an anodic chamber (2 cm^3 total volume), and a fixed electrode spacing of 0.5 cm. The anode and the cathode were made of carbon cloth (untreated carbon cloth type B, E-Tek USA) with a total exposed surface area of $2\text{ cm} \times 2\text{ cm}$. Nafion 117 (Sigma-Aldrich) was used as the proton exchange membrane (PEM), which was hot pressed to the cathode by applying a pressure of 3 bars for 30 s at a temperature of $100\text{ }^{\circ}\text{C}$. Titanium wire (Goodfellow Cambridge Ltd) was threaded along the carbon cloth electrodes and used for the electrical contacts. Opposing inlet and outlet ports were introduced by drilling a 1.1 mm hole on in the anodic chamber, and tubing (ID 1mm, 1 cm long) for continuous flow was glued inside the holes.

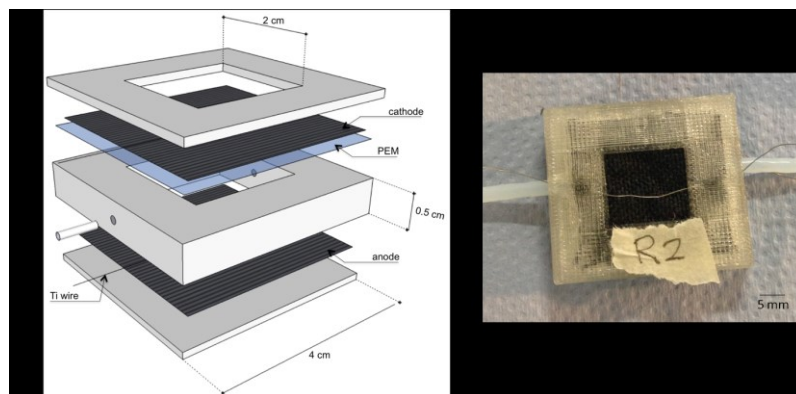


Figure 1. Layer-by-layer 3D-printed miniature microbial fuel cell. The device consisted of: a bottom layer (B); an intermediate layer (I); and a top layer (T). The anode and the PEM/cathode were inserted manually before the printing respectively of the intermediate and the top layer. Volume of the anodic chamber: 2 cm^3 . Total area of both electrodes: 4 cm^2 .

2.3 Operation of the microbial fuel cells

Artificial wastewater (AW) was prepared as previously described (Di Lorenzo et al. 2009) and autoclaved prior to use. Acetate was added to the AW as the carbon-energy source at a concentration varying in the range 0.2 - 10 mM. The resulting fuel was purged with nitrogen before being fed to the SCMFC.

The anode and cathode of the SCMFCs were connected through a voltmeter (ADC-24 Pico data logger), and a fixed external resistance (R_{ext}) of 1 k Ω was applied to polarise the cells and monitor the cell potential (V) under closed circuit conditions.

The resultant current (I) was calculated with the Ohm's law ($I = \frac{V}{R_{ext}}$)

The cells were fed with AW at a flow rate of 0.1 cm³ min⁻¹ by using a programmable multichannel peristaltic pump (Masterflex®, Cole Parmer) equipped with 2-stopped pump tubing (Masterflex®, ID 1 mm). The resulting hydraulic retention time (HRT) inside the cells was 2 min.

The maturing of the electrochemically active bacteria at the anode (enrichment), was performed by inoculating the SCMFCs with a 5%(v/v) mixed culture of bacteria. These were extracted from the anodes of operating in continuous flow MFCs at the Bristol Robotics Laboratory (Ieropoulos et al. 2012). The cultures were allowed to grow for 18 - 24 hours at room temperature in a growth medium flask containing: 1% tryptone; 0.5% yeast extract; 2 mM potassium acetate; 1% sodium sulphate.

During the enrichment, the feeding solution was freshly prepared on a daily basis and was continuously recirculated in the SCMFCs. Once a stable current was observed, the cells were fed with only AW with a specific COD value.

2.4 Testing the miniature SCMFCs as biosensors

The MFCs with enriched electroactive bacteria at the anode were initially tested as sensors for the labile organic carbon content in water.

The tests involved the continuous monitoring of the output current generated by the SCMFCs fuelled by AW with chemical oxygen demand (COD) values in the range 0.8-800 ppm obtained by varying the concentration of acetate in AW.

Subsequently, the SCMFCs were tested as a tool for detecting the presence of cadmium (as CdSO_4 , anhydrous, 99.99%, Sigma-Aldrich) in water. The tests involved feeding the SCMFCs with AW containing 3 mM of acetate and cadmium as specified (concentration within the range 0.1 - 100 $\mu\text{g l}^{-1}$) for a total period of 6 min. Afterwards the SCMFCs were fed with fresh AW containing 3 mM acetate and no toxicant. To avoid irreversible damage to the electroactive bacteria at the anode, only one test was performed per SCMFC (three in total) per day.

All the tests were carried out at room temperature, which was 20 ± 3 °C. Each experiment was performed in triplicate

2.5 Analyses

This study refers to the COD, as it was more convenient to measure than the five-days test for the biological oxygen demand (BOD_5). For the specific AW used in this study with acetate as the only organic compound, the COD was equal to the BOD_5 . The COD was determined by the standard method using chromate as the oxidant.

The internal ohmic resistance was measured by electrochemical impedance spectroscopy using a potentiostat (Autolab PGSTAT128N) with the cathode as working electrode and the anode as counter electrode and reference electrode.

Impedance measurements were carried out at open circuit voltage as previously specified (Di Lorenzo et al. 2009).

The coulombic efficiency, ε_c , was calculated according to (Logan et al. 2006):

$$\varepsilon_c = \frac{MI_{ss}}{FzQ\Delta COD} \quad (1)$$

Where: F is the Faraday's constant; M is the molecular weight of oxygen; I_{ss} (A) is the current at the steady state; $z=4$ is the number of electrons exchanged per mole of oxygen; Q ($\text{dm}^3 \text{s}^{-1}$) is the flow rate through the MFC; ΔCOD (g dm^{-3}) is the difference in the inlet and outlet COD.

The sensitivity of the SCMFC biosensor was referred against the anode total area (A, cm^2) and was calculated with the following formula (2):

$$\text{sensitivity} = \frac{\Delta I}{\Delta c} \frac{1}{A} \quad (2)$$

Where ΔI (μA) is the unit change in the current output; Δc (mM) is the change in the COD value or in the concentration of cadmium, depending on whether the SCMFCs were tested as a COD or as a cadmium biosensor, per unit change of the current output. The ratio $\frac{\Delta I}{\Delta c}$ was obtained from the slope of the current output versus concentration curve.

3 Results and discussion

3.2 Amperometric response of the small-scale SCMFCs to variations of the labile organic carbon in water

The anodes of the SCMFCs were enriched with the electroactive bacteria by feeding the devices with AW containing acetate (2 mM) and a mixed culture of anaerobic bacteria. Acetate is a byproduct of microbial metabolism in many wastewater streams and its use as the carbon-energy source in MFCs has been extensively reported (Ieropoulos et al. 2010; Liu et al. 2005; Pant et al. 2010). The loading rate was 0.5 mg COD h⁻¹ per cm³ of anode compartment. The cells were initially operated at open circuit for two days, with a stable open circuit potential of 400 ± 30 mV. Following this, an external load of 1 kΩ was applied to the system. Figure 2 shows the variation of the current output with time during the enrichment over a total period of seven days. As shown, after the fifth day of enrichment, the MFC performance reached a steady state current of 32.8 ± 0.6 μA. This was equivalent to a power density of 0.51 ± 0.17 μW cm⁻³, with respect to the anode volume. The coulombic efficiency was of 15 ± 7% and the COD removal rate was 0.1 ± 0.02 mg COD h⁻¹. The internal ohmic resistance of the MFCs was 2 Ω, which is a value 10 times lower than the resistance previously observed with larger devices (Di Lorenzo et al. 2009). This is a consequence of scaling down the device. In a miniature biofuel cell the ohmic losses are minimised thanks to the very short distance between the two electrodes (Lee and Kjeanga 2010).

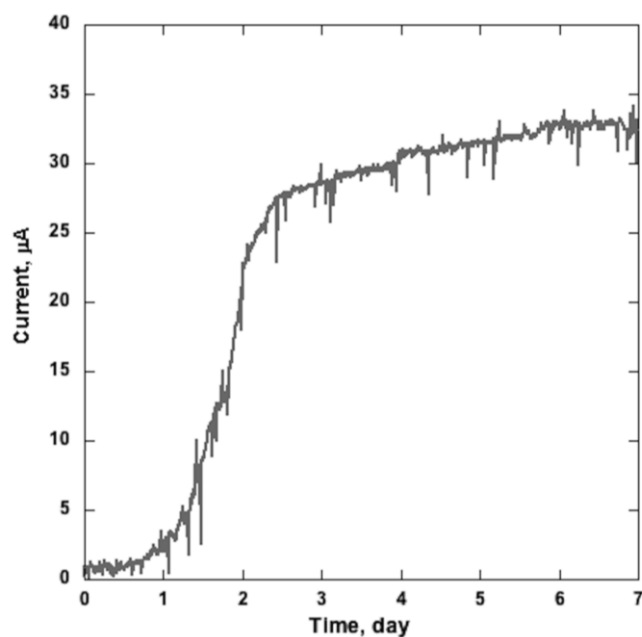


Figure 2. Electrochemically-active bacterial enrichment at the anode of the miniature SCMFCs. Artificial wastewater containing 1% by volume of anaerobic sludge as inoculum (total COD: 170 ppm) was continuously fed to the MFCs at a flow rate of $0.1 \text{ cm}^3 \text{ min}^{-1}$. Data is the average from three reactors with 5% accuracy.

After the seventh day of enrichment, it was assumed that a stable biofilm was formed at the anode of the SCMFCs, therefore no more bacteria were added to the feeding solution. This caused a 3.5% decrease in the inlet COD but no marked changes were recorded in the current output.

The enriched SCMFCs were fed with AW at several COD values obtained by varying the concentration of acetate in the range of 0.01 - 10 mM. Figure 3 shows the amperometric response of the MFCs to the changes in the AW COD values.

A linear response between COD values and current output was observed in the range of 0.04 - 2 mM (3 - 164 ppm), with a sensitivity of $0.05 \text{ } \mu\text{A mM}^{-1}\text{cm}^{-2}$ with respect to the anode total area. This linearity range defines the non-saturating fuel conditions for the SCMFCs. For acetate concentration higher than 2 mM (i.e. COD values higher than $\sim 164 \text{ ppm}$) the current output was no longer affected by the fuel

concentration. This is consistent with the substrate saturation behaviour of microbial community growth kinetics, since the biofilm population cannot metabolise at a rate of growth, $\mu_{\max}$, faster than the maximum (Ledezma et al. 2012). No detectable current was observed for acetate concentrations lower than 3 ppm.

Very good reproducibility within the three SCMFCs tested was observed, with a maximum variance of 1.5%.

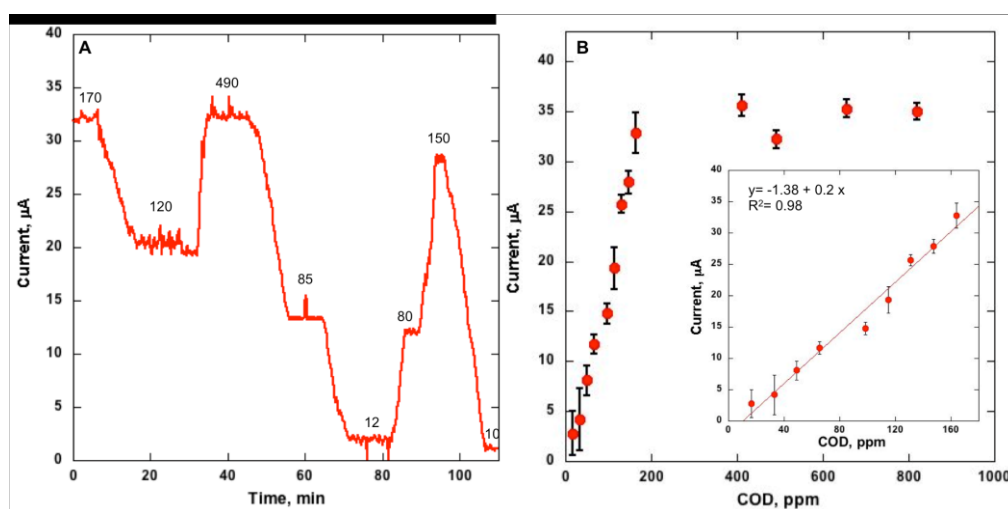


Figure 3. MFC response to AW with COD values ranging from 0.75 – 820 ppm (0.01 - 10 mM) obtained by varying the concentration of acetate. A: current output change with time at various COD values (indicated in ppm with the numbers in the figure) for one SCMFC. B: Average steady-state current output from three SCMFCs as a function of the COD concentration. Error bars refer to the standard deviation among three replicates.

The observed dependence of current output to the concentration of labile organic carbon in water confirms previous results that demonstrate the use of MFCs as BOD sensor (Chang et al. 2004; Di Lorenzo et al. 2009 ; Kim et al. 2003a).

The response time (t_r) of the small-scale SCMFC sensor was defined as the time required to reach 95% of the steady-state current (Di Lorenzo et al. 2009). This resulted to be much shorter than other MFC-type BOD sensors previously reported (Table 1). In particular, t_r of 2.8 ± 0.5 min was observed for when the fuel concentration was stepped-up, while in the case of a concentration step-down, t_r was 8.7 ± 1.4 min. The reduced response time is a consequence of the system

miniaturisation and the associated very low Reynolds numbers inside the anodic compartment, which leads to a response time close to the HRT (Moon et al. 2004). The longer response time in the case of a step-down in the COD value is in agreement with previous findings and is attributed to the dissipation of the residual reducing power from the previous feeding with a high level of electron donors at the anode (Di Lorenzo et al. 2009).

Table 1 compares the performance of the small-scale SCMFC developed in this study with other recently reported MFC-type BOD sensors. As observed, the dynamic range of the MFC-type biosensor is wider in single-chamber MFCs, due to a greater oxygen supply at the cathode (no oxygen mass transfer limitation in water). A single-chamber design is also preferable to two-chamber configurations, since the costs of operation are reduced with no catholyte pumping or air/oxygen purging required. Moreover, single-chambered MFCs are more compact and handy, and are simpler to further miniaturise.

Table 1 Comparison of MFC-type BOD biosensors according to the device configuration and the volume of the anodic chamber.

Configuration	Anodic chamber volume cm ³	Linearity range ^a ppm	t _r min	Reference
Single chamber	2	3 - 164	2.8	This study
Single chamber	12.6	50 - 350	40	(Di Lorenzo et al. 2009)
Single chamber	73	5 - 120	132	(Yang et al. 2013)
Two chambers	20	20 - 100	60	(Chang et al. 2004)
Two chambers	25	2.5 - 50	30	(Kim et al. 2003b)

^a With respect to the maximum current output versus BOD curve

Notably, the detection range obtained with the miniature SCMFC is better suited towards lower COD values compared to a system previously reported (Di Lorenzo et al. 2009). The main difference between the two systems is in the anodic chamber size, with configuration, electrodes material and fuel used, being the same. Therefore, the enhanced detection limit at lower COD values can be attributed to the increased electrode surface area to volume ratio in the miniature device, and the consequent improvement in the mass transfer rates (ElMekawy et al. 2013).

3.2 Use of the micro SCMFCs as sensors for toxicants in water

The miniature SCMFCs were subsequently tested as sensors for the rapid detection of toxicants in water. In particular cadmium (Cd^{2+}) was chosen as toxic chemical.

Cadmium is an extremely toxic metal that is widely found in the aquatic environment (Zhang et al. 2012). It enters the aquatic environment mainly from industries, being the key component in the production of batteries, electroplating and paint pigments. However, it can also come from rocks and soils that are directly exposed to water systems. Cadmium is classified as a severe environmental pollutant (Friberg et al. 1986) and as a human carcinogen (Koriem et al. 2013).

The toxic event was simulated by feeding the SCMFCs, for a total period of 6 min, with artificial wastewater containing a saturating concentration of acetate (i.e. 3 mM), and cadmium (as $\text{Cd}^{2+} \text{SO}_4^{2-}$) at a concentration ranging from 0.1 to 100 $\mu\text{g l}^{-1}$. Afterwards, the inlet was replaced with fresh AW without cadmium.

Figure 4 show the schematic of the experiments performed that required the

operation of several SCMFCs in parallel. SCMFCs 1, 2 and 3 were fed with the AW to be analysed to give a response averaged on three replicates. The other three MFCs (4,5,6) were fed with fresh AW to provide the reference current baseline. The latter were also used as reserve SCMFCs to replace any of the sensing SCMFCs in case their anode biofilms were irreversibly damaged due to the toxic event.

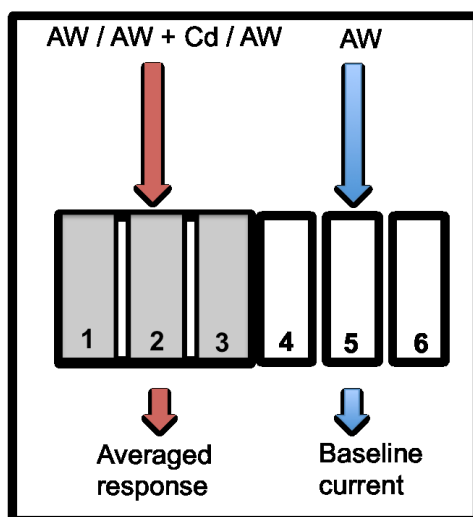


Figure 4. Schematic of the experiments performed. The toxic event was simulated by instantaneously feeding AW + cadmium to the SCMFCs 1, 2 and 3 for a total of 6 minutes. Afterwards, the devices were fed with fresh AW to re-establish the baseline current generated by the SCMFCs 4, 5, and 6. The AW contained 3 mM acetate as source of organic carbon.

Figure 5 shows the effect that the injection of cadmium had on the current generated by the SCMFCs 1, 2 and 3. No detectable effects were observed for cadmium concentrations lower than $1 \mu\text{g l}^{-1}$ and therefore the relative data are not reported. On the other hand, the addition of cadmium in the inlet at concentrations higher than $1 \mu\text{g l}^{-1}$ caused a rapid drop in the current output that was depended on the concentration added (Figure 5A). For cadmium concentrations below $50 \mu\text{g l}^{-1}$, once the normal operational conditions were re-established (i.e. no cadmium in the feeding solution), the current generated by the SCMFCs returned to the baseline value of $32.2 \pm 1.2 \mu\text{A}$. In this case it was therefore assumed that the presence of cadmium caused only temporary changes in the metabolism of the electroactive bacteria. After

the addition of $50 \mu\text{g l}^{-1}$ of cadmium, the re-established steady-state current output was 4.3% lower, and concentrations of cadmium higher than $50 \mu\text{g l}^{-1}$ were excessively toxic for the system, causing permanent damage to the electroactive biofilm. Figure 5A shows that the current output instantaneously dropped to zero upon the addition of $100 \mu\text{g l}^{-1}$ of cadmium, and did not recover again after the toxic event.

The time required for the biofilm to recover was related to the cadmium concentration injected, being as fast as 4 min for a concentration of $1 \mu\text{g l}^{-1}$. Generally, after approximately 12 minutes, a complete recovery was observed for all the cases of reversible anodic biofilm damage.

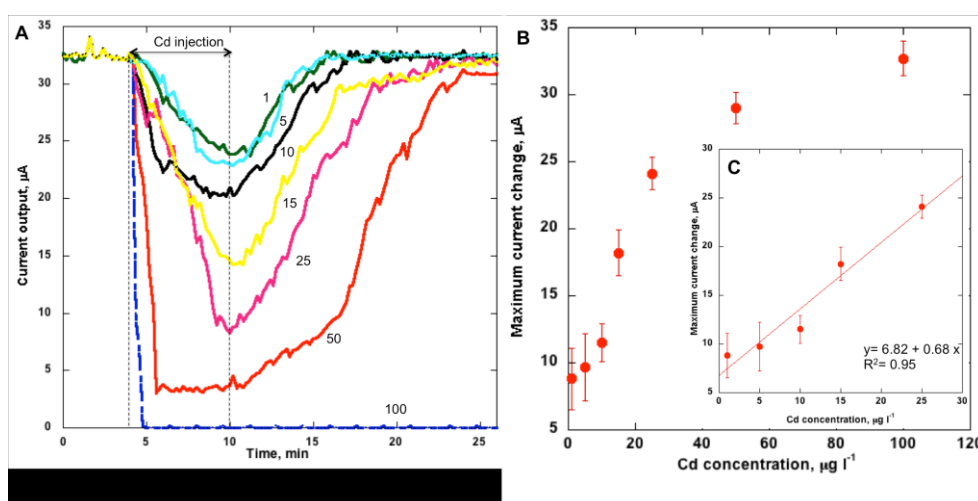


Figure 5. MFC-type biosensor response to cadmium. A: current output versus time. AW containing cadmium in the range $1\text{--}100 \mu\text{g l}^{-1}$ was injected in the SCMFCs for a total of 6 min. Subsequently the cells were fed with AW and no cadmium. The numbers indicate the concentration of Cd^{2+} ($\mu\text{g l}^{-1}$) added. Data are the average of three replicates with 5% accuracy. B: current output after 6 min from the toxicant injection versus cadmium (Cd) concentration. Error bars refer to the standard deviation among three replicates. C: linear regression of the data obtained for cadmium concentrations in the range $1\text{--}25 \mu\text{g l}^{-1}$.

In Figure 5B the current output after 6 min of cadmium injection is plotted against the relative concentration. As shown, a linear trend was observed in the range of $1\text{--}25$

$\mu\text{g l}^{-1}$ with a R^2 value of 0.90 (Figure 5C). In these conditions, the SCMFC-biosensor sensitivity towards cadmium was of $0.2 \mu\text{g l}^{-1} \text{ cm}^{-2}$.

If operated as an on-site continuous monitoring system for a water environment, the miniature SCMFC sensor could therefore act as a real time early-warning tool in the case of spills of traces of cadmium. The very low concentration detection range is a key feature of the SCMFC sensor, since the maximum contaminant level above which exposure to cadmium affects human health has been set to $5 \mu\text{g l}^{-1}$ (Ivari et al. 2013). Traditional bioassays have detection limits in the order of mg l^{-1} (Zhu et al. 2006), and other sophisticated analytical methods are too expensive, time-consuming and not suitable for on-line monitoring (Lu et al. 2002; Yaman 2005).

The sensitivity of a MFC biosensor depends on the specific characteristics of the target toxicant, the mechanism of interaction with the bacterial cells' and their affinity for the pollutant (Stein et al. 2012a). The MFC design also plays an important role, and microengineering can open new perspectives in the development of MFC-based sensors. In micro-MFCs the mass transport at the anode can in fact be markedly improved, thus minimising any difference in the toxicant concentration between the feeding stream and the biofilm (Stein et al. 2012a; Stein et al. 2011). Miniaturisation can thus be a tool for enhancing the sensitivity of MFC-based sensors.

Currently, there is an increasing interest in miniaturising the biofuel cell technology, due to the very high power densities associated to small-scale devices (You et al. 2014). Still, the process of MFC miniaturisation is only at the beginning (Lee and Kjeanga 2010). To the authors' knowledge, so far the only micro-scale MFC sensor produced by lithography was reported by Davila et al. This micro MFC-sensor consisted of two chambers, each of a volume of $144 \mu\text{l}$, which employed potassium ferricyanide at the cathode (Dávila et al. 2011).

The effective monitoring of a water environment in practical applications would require the operation of several SCMFCs in parallel, as suggested in the scheme reported in the supplementary data (Figure S1). An advantage of miniaturisation is that multiple miniature MFC units could be easily arranged in stacks, while keeping the overall device footprint still relatively compact and small. The layer-by-layer 3D printing technique adopted in this study can easily allow for a further miniaturisation of the SCMFCs, as well as for the realisation of a stack of multiple units. This will be the scope of future research.

4 Conclusions

This study shows the applicability of a small-scale single chambered air-cathode microbial fuel cell (SCMFC) as a screening tool for the real time detection of traces of cadmium in water. For the purposes of proving the principle, the MFC presented herein was tested with cadmium in pure water only. A natural water environment will contain other contaminants, which will affect the behaviour – and thus the electrical output – of the biofilm community. This will form part of our future work. The miniature SCMFC was fabricated with a rapid layer-by-layer 3D printing technique that easily allows further miniaturisation of the device as well as arrangements of multiple SCMFCs in stacks.

Under non-saturating fuel conditions (i.e. COD range: 3 - 164 ppm) the sensor is capable of monitoring changes in the labile organic carbon content in continuous mode with response times as fast as 2.8 minutes.

Under saturating fuel conditions (i.e. acetate concentration of 3 mM) and fixed operational conditions (e.g. pH, temperature), the instantaneous addition of cadmium in the feeding water caused changes in the output current. In particular, the decrease

in the current output generated by the SCMFCs was correlated to the concentration of cadmium injected, with a lower detection limit of $1 \mu\text{g l}^{-1}$. For cadmium concentrations up to $50 \mu\text{g l}^{-1}$ the baseline current output was recovered within 12 minutes after the toxic event.

Although the sensitivity of the proposed MFC biosensor towards cadmium is very good, its specificity is still a challenge compared to highly selective sensors recently developed (Ivari et al. 2013). Future work will investigate the response of the miniature SCMFC biosensor to other toxicants. The selectivity of the MFC biosensor towards several pollutants can be tuned by acting on the system overpotential (i.e. the potential difference between the substrate redox potential and the anode potential) at which the MFC is operated as previously suggested (Stein et al. 2012a). The simultaneous detection of a range of pollutants in water could then be performed with an array of miniature SCMFCs operated at various anode potentials.

5 Acknowledgments

The authors wish to thank the Royal Society Research Grant (RG110344) for funding; Dr Davide Mattia and Dr Laura Torrente, University of Bath, for the use of the Makerbot Replicator® 2 printer. Ioannis Ieropoulos is an EPSRC Career Acceleration Fellow (grant numbers EP/I004653/1 and EP/L002132/1).

6 References

- Chang, I.S., Jang, J.K., Gil, G.C., Kim, M., Kim, H.J., Cho, B.W., Kin, B.H., 2004. *Biosens Bioelectron.* 19, 607-613.
- Cockerham, L.G., Shane, B.S., 1994. *Basic environmental toxicology*, pp. 353-384.
- Dávila, D., Esquivel, J.P., Sabaté, N., Mas, J., 2011. *Biosens Bioelectron.* 26(5), 2426-2430.

Di Lorenzo, M., Curtis, T.P., Head, I.M., Scott, K., 2009. *Water Research* 43, 3145–3154.

Di Lorenzo, M., Curtis, T.P., Head, I.M., Velasquez-Orta, S.B., Scott, K., 2009.. *Water Science and Technology*, 2879-2887.

Elinder, C.-G., 1985. CRC Press, Inc., Boca Raton, Florida.

ElMekawy, A., Hegab, H.M., Dominguez-Benetton, X., Pant, D., 2013. *Bioresource Technology* 142, 672-682.

Friberg, L., Nordberg, G.F., Vouk, V.B., 1986. *Handbook of the Toxicology of Metals*, II. Amsterdam. pp. 130–184. Elsevier.

Ieropoulos, I., Greenman, J., Melhuish, C., 2010. *Bioelectrochemistry* 78(1), 44–50.

Ieropoulos, I., Greenman, J., Melhuish, C., 2012. *Phys. Chem. Chem.Phys* 14, 94-98.

Ivari, S.A.R., Darroudi, A., Arbab, Z.M.H., Zohuri, G., Ashraf, N., 2013. *Arabian Journal of Chemistry* In press.

Kim, B.H., Chang, I.S., Gil, G.C., Park, H.S., Kim, H.J., 2003a. *Biotechnology letters* 25, 541-545.

Kim, M., Hyun, M.S., Gadd, G.M., Kim, H.J., 2007. *J. Environ. Monit.* 9, 1323-1328.

Kim, M., Youn, S.M., Shin, S.H., Jang, J.G., Han, S.H., Hyun, M.S., Gadd, G.M., Kim, H.J., 2003b. *J. Environ. Monit.* 5, 640-643.

Koriem, K.M.M., Fathi, G.E., Salem, H.A., Akram, N.H., Gamil, S.A., 2013. *Toxicology Mechanisms Methods* 23(4), 263–272.

Leal, L.H., Soeter, A.M., Kools, S.A.E., Kraak, M.H.S., Parsons, J.R., Temmink, H., Zeeman, G., Buisman, C.J.N., 2012. *Water Research* 46(4), 1038–1044.

Ledezma, P., Greenman, J., Ieropoulos, I., 2012. *Bioresource Technology* 118, 615-618.

Lee, J.w., Kjeanga, E., 2010. *Biomicrofluidics* 4, 1-11.

Liu, H., Cheng, S.A., Logan, B.E., 2005. *Environ. Sci. Technol.* 39, 658–662.

Logan, B.E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. *Environmental Science Tehnology* 40(17), 5181–5192.

Lu, Y.K., Sun, H.W., Yuan, C.G., Yan, X.P., 2002. *Anal. Chem.* 74, 1525-1529.

Matsunaga, T., Takeyama, H., Nakao, T., Yamazaw, A., 1999. *Journal of Biotechnology* 70, 33-38.

Moon, H., Chang, I.S., Kang, K.H., Jang, J.K., Kim, B.H., 2004. *Biotechnology letters* 26, 1717-1721.

Pant, D., Van Bogaert, G., De Smet, M., Diels, L., Vanbroekhoven, K., 2010. *Electrochimica Acta* 55(26), 7710–7716.

Qu, R., Wang, X., Liu, Z., Yan, Z., Wang, Z., 2013. *Journal of Hazardous Materials* 262, 288–296.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U., Wehrli, B., 2006. *Science* 313, 1072-1077.

Stein, N.E., Hamelers, H.M.V., Buisman, C.N.J., 2010. *Bioelectrochemistry* 78, 87–91.

Stein, N.E., Hamelers, H.M.V., Buisman, C.N.J., 2012a. *Sensors and actuators B: Chemical* 163, 1-7.

Stein, N.E., Hamelers, H.M.V., van Straten, G., Keesman, K.J., 2012b. *Journal of Process Control* 22, 1755–1761.

Stein, N.E., Keesman, K.J., Hamelers, Hubertus V.M., van Straten, G., 2011. *Biosensors and Bioelectronics* 26 3115–3120.

Wang, X., Gao, N., Zhou, Q., 2013. *Biosens Bioelectron.* 43 264–267.

Yaman, M., 2005. *Anal. Biochem.* 339, 1-8.

Yang, G.-X., Sun, Y.-M., Kong, X.-Y., Zhen, F., Li, Y., Li, L.-H., Lei, T.-Z., Yuan, Z.-H., Chen, G.-Y., 2013. *Water Science & Technology* 1914-1919.

You, J., Greenman, J., Melhuish, C., Ieropoulos, I., 2014. *Sustainable Energy Technologies and Assessments* 6, 60-63.

Zhang, H., Cao, H., Meng, Y., Jin, G., Zhu, M., 2012. *Ecotoxicology and Environmental Safety* 80, 258–265.

Zhu, J.-Y., Huang, H.-Q., Bao, X.-D., Lin, Q.-M., Cai, Z., 2006. *Aquatic Toxicology* 78, 127-135.