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Abstract: The traditional five-day test of the biochemical oxygen demand (BOD5 test) has many disadvantages, and principally it is unsuitable for process control and real-time monitoring. As an alternative, a single chamber microbial fuel cell (SCMFC) with an air cathode was tested as a biosensor and the performance analysed in terms of its measurement range, its response time, its reproducibility and its operational stability. When artificial wastewater was used as fuel, the biosensor output had a linear relationship with the BOD concentration up to 350 mg BOD cm-3; very high reproducibility; and stability over 7 months of operation.
The system was further improved by reducing by 75 % the total anolyte volume. In this way a response time close to the hydraulic retention time (HRT) of the biosensor (i.e. 40 min) was reached. When the small volume SCMFC biosensor was fed with real wastewater a good correlation between COD concentration and current output was obtained, demonstrating the applicability of this system to real effluents. The

measurements obtained with the biosensor were also in accordance with values obtained with standard measurement methods.

30th September 2008

Dear Editor

We are pleased to submit this manuscript for consideration by Water Research:

A single chamber microbial fuel cell as a biosensor for wastewaters

This manuscript has been written by following the comments received on the two manuscripts which we have previously submitted to Water Research and which regarded the development of a biosensor for the wastewater organic content based on a single chamber microbial fuel cell (SCMFC) with an air cathode, and its further improvement.

In particular, the two articles are here merged in a single one, in order to generate a paper strong enough to be published in your Journal.

We strongly believe in the potential applications of this novel biosensor, and in its advantages in the field of water industry, environmental treatment processes, and environmental regulation and we hope that you find this new version of the manuscript interesting and of good-quality.

Yours faithfully,

Dr Mirella Di Lorenzo

Prof Tom P. Curtis

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A single chamber microbial fuel cell as a biosensor for wastewaters

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Running title: **A single chamber microbial fuel cell as a biosensor for wastewaters**

Abstract

The traditional five-day test of the biochemical oxygen demand (BOD_5 test) has many disadvantages, and principally it is unsuitable for process control and real-time monitoring. As an alternative, a single chamber microbial fuel cell (SCMFC) with an air cathode was tested as a biosensor and the performance analysed in terms of its measurement range, its response time, its reproducibility and its operational stability. When artificial wastewater was used as fuel, the biosensor output had a linear relationship with the BOD concentration up to 350 mg BOD cm^{-3}; very high reproducibility; and stability over 7 months of operation.

The system was further improved by reducing by 75 % the total anolyte volume. In this way a response time close to the hydraulic retention time (HRT) of the biosensor (i.e. 40 min) was reached. When the small volume SCMFC biosensor was fed with real wastewater a good correlation between COD concentration and current output was obtained, demonstrating the applicability of this system to real effluents. The measurements obtained with the biosensor were also in accordance with values obtained with standard measurement methods.

Key words: microbial fuel cell, BOD, sensor, wastewater

1 Introduction

Since it was selected by the Royal Commission on Sewage Disposal in 1908 as a definitive test, the 5-day biochemical oxygen demand (BOD_5) has become the most widely used parameter to measure organic content of wastewaters. This determination involves the measurement of the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter. Despite its widespread use, the BOD test has a number of limitations, such as questionable accuracy and irreproducibility, and is labour intensive and time consuming. It is therefore, not suitable for process control and real-time monitoring where rapid feedback is essential.

In recent years biosensors have demonstrated great potential as an alternative to the conventional analytical method for BOD measurement. The main advantages offered by biosensors over conventional analytical techniques are the possibility of portability, miniaturization and working on-site, furthermore they do not require additional processing steps such as reagent additions (Rodriguez-Mozez et al., 2006). Various BOD biosensors have been reported which are based on dissolved oxygen monitoring and use either specific microorganisms such as *Bacillus subtillis* (Riedel et al., 1988), *Arxula ardeninivorans* (Riedel et al., 1998), and *Serratia marcescens* (Kim and Kwon, 1999), mixed cultures (Tan and Wu, 1999) or activated sludge (Liu and Mattiasson, 2002). A biosensor based on a luminous bacterium has also been used, and the intensity of luminescence, proportional to the amount of organic compounds, was measured with a photodiode (Hyun et al., 1993). Other BOD sensors recently reported are based on the photocatalysis of the sample (Chee et al., 2005) and on a novel microbial membrane (Jang et al., 2004).

All these biosensors demonstrated a very good relationship with the BOD concentration but presented the limitation of having a very short operational stability

and, in the case of biosensors based on a single organism, also very low substrate versatility. The measuring range is also very low, generally on the order of 0- 110 ppm (Kim et al., 2003a).

Microbial fuel cells (MFCs) have been tested as BOD sensor and several types of MFC-based BOD sensor have been developed, (Chang et al., 2004, Chang et al., 2005, Gil et al., 2003, Kang et al., 2003, Kim et al., 2003a, Kim et al., 2003b, Kumlanghan et al., 2007, Moon et al., 2004, Pasco et al., 2004) showing an operational stability of over 5 years with minimum maintenance (Chang et al., 2004, Chang et al., 2005, Kim et al., 2003a, Kim et al., 2003b).

All these studies used two chambered-MFC with an aqueous cathode.

In this study, a single chamber MFC (SCMFC) with an air cathode, was tested as BOD biosensor. The aim was to make a more compact and simple system with reduced cost of operation. An air-cathode MFC provides potential advantages over the two chamber system because neither aeration, recycle or chemical regeneration of catholyte is required (Fan et al., 2007). Moreover the range of BOD concentrations detected by the biosensor may be increased because of a better oxygen supply to the cathode.

In previous studies, a linear relationship between wastewater strength and current generated was observed only up to 150 ppm (Kim et al., 2003a). Chang et al., (2004) applied a model fitting to predict BOD concentrations higher than 100 ppm.

The performance of a SCMFC biosensor was evaluated in terms of its COD range, response time, reproducibility and operational stability. The effect of the reactor volume was also investigated. Initially artificial wastewater was used and afterwards the sensor was tested with real wastewater.

2 Materials and methods

2.1. Microbial fuel cell and its operation

The single chamber, microbial fuel cells (SCMFC) used were made from polyacrylate tubes, 12.5 cm inner diameter, 4 cm length. The cells had three flow ports (each 1.0 cm diameter); one at the bottom and two on the top, (see Figure 1A). The open volume of the anode chamber was of 50 cm^3.

In some experiments a smaller reactor was machined. The new SCMFC biosensor had a similar design to the original SCMFC biosensor. The anode and cathode cross sectional area were kept at 12.5 cm^2, while the void volume was approximately 75% smaller, obtained by reducing the reactor length from 4 cm to 1 cm, and resulting in a volume of 12.6 cm^3.

The cell had two sections at both ends of the tube: one to hold the anode and the other to hold the cathode. The anode was a circular piece of carbon cloth (BASF, no wet proofing), 12.5 cm^2 in area and 0.1 cm thickness. The cathode was exposed to air on one side and on the other it was separated from the anode by a cation exchange membrane (Nafion® 117, DuPont).

The cathode was a carbon supported platinum catalyst (0.3 mg Pt cm^{-2}; 60% on Vulcan, E-tek), deposited on wet proofed (20 wt % Teflon) Toray carbon paper from E-tek. The cross section area was 12.5 cm^2.

In this study the anode and cathode were connected through a voltmeter (Pico® data logger) and an external resistance was applied to polarise the cell and monitor the current variation under closed circuit conditions. The external resistance was controlled by utilizing a resistor substitution box (RS 500, 1% accuracy, Elenco electronics).

In operation of the cell, wastewater was fed through the injection port at flow rates in the range of 0.12-1.5 cm^3 min^{-1} using a peristaltic pump (Watson-Marlow, 520S) equipped with Marprene II tubing (0.14 cm internal diameter). Figure 1B shows the system layout. The MFCs were operated at room temperature which was approximately 21 ± 2 °C. Each experiment was performed in duplicate.

In some experiments the effect of temperature on system performance was investigated by locating the reactors in an incubator at a controlled temperature.

2.2. Fuel

Artificial wastewater (AW) was made with the following constituents:

NH_4Cl, 40 mg dm^{-3}; $MgCl_2$, 10 mg dm^{-3}; $CuSO_4$, 0.1 mg dm^{-3}; $CaCl_2$, 5 mg dm^{-3}; $MnSO_4$, 0.1 mg dm^{-3}; $ZnCl_2$, 0.1 mg dm^{-3}; phosphate buffer (1.0 mol dm^{-3} (M), pH7); and distilled water. The COD concentration of the AW was controlled by adding an appropriate amount of glucose.

The AW was autoclaved at 121 ºC for 10 min prior to use.

Treated wastewater (WW) was collected from the primary clarifier of the wastewater treatment system at Cramlington treatment plant (Northumbrian Water, U.K.). The wastewater was collected during a period of heavy rain and had a COD of 175 ± 50 mg dm^{-3} and other characteristics shown in Table 1. It was collected in one batch and stored at 4°C until use and its COD did not markedly change during storage. To prevent clogging of the microbial fuel cell, the WW was filtered with a woven cloth prior to use.

In some tests, WW was diluted with tap water. In this case phosphate buffer (5 M, pH 7) was added in order to keep the wastewater conductivity constant and equal to the non diluted wastewater.

2.3. Enrichment

The enrichment and adaptation of the electrochemically-active bacteria in the SCMFCs was performed in batch mode under a fixed external load of 500 Ohms, and was carried out for a period of approximately four weeks.

When artificial wastewater was used as fuel, a COD of 1000 ppm was selected for enrichment and anaerobic sludge (Northumbrian Water treatment plant in Cramlington, UK) was added to the fuel as a bacterial inoculum. Once a stable peak current was observed, the cells were fed AW with a COD of 200 ppm and no inoculum.

When real wastewater was used as fuel, during the enrichment the COD was increased to the final value of 1000 ppm by adding a suitable amount of glucose. The wastewater itself provided the inoculum, and no additional inoculum was added in this case.

2.4. Analyses

In this work we based analysis on the chemical oxygen demand (COD) since it was more conveniently measured than BOD_5. For the artificial wastewater the COD was equal to the BOD, while for the real wastewater the BOD was approximately half (Table 1). The COD was determined by the standard method using chromate as the oxidant as previously described (Eaton et al., 2005). All samples were filtered through a 0.22 μm pore diameter membrane filter (VWR International) prior to COD measurements.

The COD consumption rate was calculated when the steady state was reached with the formula:

$$Q \times (COD_{IN} - COD_{OUT}) \quad (1)$$

Where Q ($dm^3\ h^{-1}$) was the fuel flow rate, and COD_{IN} and COD_{OUT} (mg dm^{-3}) were the COD of the influent and the effluent, at steady-state, respectively.

Sulphate and phosphate concentration in the real wastewater were determined using a Dionex ICS-1000 ion chromatograph with an AS40 automated sampler and with an IonPac AS14A, 4 x 125 mm analytical column; a 8 mM Na_2CO_3 solution was used as eluent at a flow rate of 1 $cm^3\ min^{-1}$. A sample loop of 25 µL was used; the detector was an electrochemical conductivity detector.

Conductivity measurements were performed with a conductivity meter provided by Hanna instruments.

The internal resistance was measured by electrochemical impedance spectroscopy using a potentiostat (Gillac, ACM Instruments) with the cathode as the working electrode and the anode as counter electrode and reference electrode. Impedance measurements were conducted at open circuit voltage (OCV) over a frequency range of 10^4 down to 10^2 Hz with a sinusoidal perturbation of 15 mV amplitude.

Polarization curves were recorded by means of a potentiostat (Gillac, ACM Instruments) at a scan rate of 1 mV s^{-1} and a prior open circuit potential of over 4 hours.

The Coulombic efficiency (fractional), at the steady-state was calculated with the formula (2):

$$\varepsilon_c = \frac{M\ I}{Fz\ Q\ \Delta COD} \times 100\ \% \qquad (2)$$

Where F is Faraday's constant (96,485 C mol^{-1}); M = 32 the molecular weight of oxygen, I (A) the current at the steady-state; z = 4 the number of electron exchanged per mole of oxygen, Q ($dm^3\ s^{-1}$) the flow thought the system, ΔCOD (g dm^3) the difference in the influent and effluent COD.

3. Results and discussion

3.1. Effect of feeding rate

Figure 2 show the variation in current output of the MFC with time when fed with successive batches of AW. The system start up was slow and the current output rose after each batch feed, reaching a peak before declining due to consumption of the fuel (Figure 2). The peak current increased from a value of $0.02 \pm 6 \times 10^{-4}$ mA with the first batch of fuel, until a value of $0.12 \pm 4 \times 10^{-3}$ mA was reached in the fourth batch. After 400 hours (4 batches) no further increase in the output current was observed: it was assumed that the anode biofilm was enriched with electrochemically active bacteria, and it was stable. When the COD concentration of the AW was decreased to 200 ppm and no inoculum used, a stable current of approximately 0.04 mA was generated. The total enrichment period was approximately 550 hours.

Once the enrichment of the cells was complete, the system performance was investigated in a continuous mode. Wastewater (AW, 100 ppm as COD) was fed into the MFCs at different flow rates and the current monitored. The MFCs were fed at a specific flow rate until a stable current was generated and then the feed rate was increased.

Table 2 reports the change in current response on increasing the AW flow rate in steps of 0.12 to 0.56 cm^3 min^{-1}. The steady-state current increased with each step in flow rate, varying from 0.063 ± 0.002 mA for a flow rate of 0.12 cm^3 min^{-1} until a value of 0.27 ± 0.003 mA for a flow rate of 0.56 cm^3 min^{-1}. Indeed the increase was observed until a flow rate of 0.46 cm^3 min^{-1}; when the cells were fed at higher flow rates (i.e. 0.56 cm^3 min^{-1}) only a small increase of 4 % in the current was observed. No further increase in current was observed at flow rates above 0.56 cm^3 min^{-1} (data not shown). This trend also occurred on reducing the flow rate and at a specific flow rate the

current obtained had a precision of 0.38 %. The increase in current with flow rate would indicate an enhancement of the electrochemical activity of the bacteria by an increase in the rate of mass transport. The flow in the MFC was laminar with a Reynolds number of approximately 1.0 at the lowest flow rate used. Under such conditions mass transport coefficients would be very low (*ca.* 10^{-7} m s^{-1}) which would only be sufficient to generate small currents (mA). The geometry of the cell used did not enable any precise calculations to be made on transport rates or limiting currents. However the fact that a "peak" current was obtained at the higher flow rates used might well be a combination of "kinetic" limitations of bacterial processes as well as non-uniform fluid flow distribution in the cell.

Table 2 also describes the response of the MFC-based sensor to different flow rates in terms of the change in COD and COD consumption rates, and the Coulombic efficiencies observed for each feeding rate. By increasing the flow rate, and thereby reducing the hydraulic retention time (HRT), the COD removal rate and the COD of the effluent increased. The effect of increasing the effluent COD in steps produced successively lower increases in the COD removal rate, which for the first step increase was approximately 97 %, whilst for the last (0.46 cm^3 min^{-1} to 0.56 cm^3 min^{-1}) it was only of 17 %.

A high flow rate should ideally increase the dynamic response of the system by increasing the mass transport. Therefore it was decided to perform all the following experiments at a flow rate 0.46 cm^3 min^{-1} since it was the highest flow rate considered which still gave a substantial increase in the steady-state current.

For all flow rates, low values of Columbic efficiency (4-6%) were obtained; therefore a high percentage of COD removal was not related to the current generated.

3.2. Effect of external resistance

It has been previously demonstrated, that the external resistance can affect the MFC dynamic performance and could therefore be a limitation in MFC sensor response (Kang et al., 2003, Kumlanghan et al., 2007). A series of tests was therefore, performed in which the external resistance (R_{ext}) was varied from 50 to 500 Ω at a constant inlet COD concentration of 100 ppm and a flow rate of 0.46 cm^3 min^{-1}. The aim was to determine the value of the external resistance which gave the shortest dynamic response of the system. The MFCs were fed until a steady current was generated. Subsequently cell starvation was performed by interrupting the feed until the output current reached approximately 0.015 ± 0.05 mA. During starvation, the anolyte solution in the reactor was re-circulated at the same flow rate, i.e. 0.46 cm^3 min^{-1}, to ensure mixing inside the MFC-based sensor. Table 3 reports the results obtained. The peak current increased and the response time to reach steady state decreased as the resistance was reduced. Decreasing the external resistance to 50 Ω, the response time decreased by approximately 67 %, with respect to a R_{ext} of 500 Ω. The steady-state current was in fact reached after 4.5 hours compared with 13.75 hours when the external resistance was 500 Ω. An external resistance of 50 Ω was the lowest that could be used that gave reliable cell voltages. Table 3 also shows that changes in the external resistance had no major effect on the COD removal rate. Essentially the majority of COD removal was associated with non-anodic processes (low Coulombic efficiency)

3.3. Sensor response to COD concentration and calibration under an external load of 50 Ω

A calibration of steady state current versus COD (50 to 1000 ppm) was conducted with an external load of 50 Ω corresponding to the fastest response of the system. It

was not possible to consider COD concentrations lower than 50 ppm due to the sensitivity of the data logger used. Figure 3A shows the dynamic response of the system to different inlet COD concentrations. The cells were continuously fed at a specific COD until a steady current was generated, followed by starvation until a current of approximately 0.015 ± 0.05 mA was obtained. Very good reproducibility of the system was observed: for a fixed inlet COD, either in the case of two consecutive batches or when batches with other concentrations were interposed: a stable current was obtained with a coefficient of variation of 1%. Figure 3B shows the variation in current with COD concentration. A linear response (see Figure 3C) was obtained up to COD values of 350 ppm ($r^2 = 0.96$). For a two chamber MFC, a linear response has been reported up to COD concentrations of 150 ppm (Chang et al., 2004, Kim et al., 2003b). The greater dynamic range of the SCMFC with an air cathode studied here, suggests that improving oxygen supply at the cathode improved the dynamic range of the sensor.

MFC biosensors have previously been calibrated also by considering the relationship between COD concentration and the total charge generated (Gil et al., 2003, Kim et al., 2003a). In this case a linear relationship between COD concentration was obtained up to 250 ppm COD (Gil et al., 2003, Kim et al., 2003a). Figure 4 shows a calibration of charge generated vs COD. In this case a linear response was obtained up to a COD concentration of 500 ppm, though the linearity was slightly lower than for calibration against current ($r^2 = 0.93$ compared to $r^2 = 0.96$).

The reproducibility of the SCMFC, under an external resistance of 50 Ω, was confirmed also in the case of feeding continuously, as shown in Figure 5. The Figure represents in fact, the dynamic response of the current under variable COD

concentration, ranging from 60 to 210 ppm, at a constant flow rate of 0.46 cm^3 min^{-1}. The MFC gave reasonably constant values of current response when the input COD was the same. For example, when the reactors were fed with a solution having 60 ppm COD the current output was 0.07 mA with a relative standard deviation of 0.22 %. With 160 mg COD L^{-1} the current output was 0.147 mA with a standard deviation of 0.53 %.

3.5. Effect of reactor volume

The effect of the reactor volume on the system response was investigated by considering a new reactor configuration with a 75% smaller volume. In this way, for a feeding rate of 0.46 cm^3 min^{-1}, the HRT was reduced to 27 min. Figure 6 shows current generation during the period of enrichment of the anode biofilm with electrochemically active bacteria in the smaller volume SCMFC biosensor.

The small volume SCMFC biosensor required 400 hours to reach a stable peak. The batches of fuel added during the first 250 hours of enrichment did not cause any current increase and therefore are not shown in Figure 6. Following the enrichment period when the SCMFC biosensors were fed with AW containing 1000 ppm COD, and no inoculum, the peak currents obtained was 0.12 ± 0.009 mA.

The response of the two SCMFC biosensor configurations to changes in feed COD, under a fixed external load of 50 Ω was investigated and compared (Figure 7). As shown, the dynamic response of the biosensor improved: the lower HRT of the small volume sensor resulted in a considerably faster response of the system for an up-shift but also for a down-shift in COD concentration. In particular, in the case of an up-shift, the system required only 40 min to reach a stable current which contrasts with the 4 hours required with the larger volume SCMFC biosensor. When the COD

concentration was reduced from 250 to 70 ppm the response time was in general longer but still the small reactor was faster with a response of 2 hours compared to the 8.8 hours required in the larger volume SCMFC biosensors.

The slower response for a down-shift in the COD concentration has been previously reported for a two chamber MFC (Moon et al., 2004), and may be explained by a high level of residual reducing equivalents in the anode biofilm community following feeding with a high level of electron donor. In this case the residual reducing power and the electrons from the added dose of COD both must be dissipated leading to an increase in response time of the biosensor.

Table 4 reports the performance of the small volume SCMFC biosensor in terms of COD removal and Coulombic efficiency, and compares it with the large volume SCMFC biosensor. As shown, the reduced spacing between the electrodes in the small volume SCMFC biosensor resulted in a better performance with a Coulombic efficiency of 56% in contrast with Coulombic efficiencies of 6% obtained with the 50 cm^3 reactor.

The SCMFCs exhibited very good stability over an operational period of 7 months. Figure 8 shows the value of the steady state currents obtained under a fixed inlet COD of 200 ppm, over this 7 month period of operation. As shown, the total variation observed was of the order of 15%.

3.7. Performance of the SCMFC biosensor with real wastewater

To investigate the applicability of the SCMFC biosensor in a treatment plant, real wastewater was used to test the relationship between current generation and COD concentration.

This study was conducted with the small volume SCMFC biosensor since it gave the best performance in terms of response time and Coulombic efficiency when AW was used as fuel.

The SCMFC biosensors were fed in continuous mode with several dilutions of the wastewater. Figure 9A reports the current output observed for a continuous feed (flow rate: 0.46 cm^3 min^{-1}) of wastewater of different dilutions. Figure 9B demonstrates a linear calibration between peak current and COD for the real wastewater. As for AW, the system showed a good relationship between COD concentration and current output, thus demonstrating applicability of this system to real treatment plant effluents.

Moreover, when wastewater dilutions of unknown COD concentration were considered, the system gave good correspondence between the COD measured chemically and the value given by the biosensor with respect to the calibration curve plotted (Table 5).

3.7. Effect of the operational temperature

All work previously reported on MFC-based BOD sensors was performed in temperature controlled system, at temperatures of 30°C or higher. In this study the tests were performed at room temperature, which was approximately 22 ± 2°C.

The operating temperatures of 20°C and 25°C, which simulated approximately the lowest and the highest temperature in the laboratory, were compared with a temperature of 30°C. Table 6 reports the average current outputs observed at each temperature for two SCMFCs (volume 12.6 cm^3, carbon cloth as anode) fed with real wastewater. The percentage COD removal and Coulombic efficiencies were also compared (Table 6). An operational temperature of 30 ^oC not surprisingly gave the

highest current (0.55 A) with an increase of approximately 72% compared to that at 25°C (0.32 A), and of 96% compared to that at 20°C (0.28 A). The COD removal was also improved at higher temperature. Higher temperatures would accelerate the hydrolysis of complex compounds and explain the decreased response times with increasing temperature: at 30°C it approached the HRT (27min). As shown in Table 6, by reducing the temperature from 25 to 20°C no substantial variations in response time and Coulombic efficiency were observed.

4 Discussion

Rapid and accurate monitoring of biochemical oxygen demand (BOD) is of great importance in the water industry, environmental treatment processes, and environmental regulation, since it allows the waste loading to treatment plants to be measured and evaluation of treatment efficiency (Eaton et al., 2005).

As an alternative to the traditional BOD_5 test, a single chamber microbial fuel cell (SCMFC) with an air cathode was for the first time tested as biosensor of labile organic carbon in wastewater.

The system performance was previously tested with artificial wastewater (AW) and the optimal flow rate and external resistance, corresponding to the fastest response, were investigated. At an external resistance of 50 Ω and for a flow rate of 0.46 cm^3 min^{-1} the biosensor exhibited a good linear correlation ($r^2 = 0.96$) between the AW BOD concentration and the steady-state current, thus demonstrating its applicability. Since an air cathode MFC does not require catholyte pumping, nor air purging at the cathode side, this new biosensor was simpler and more compact than two chambered-MFC type sensors previously developed for online BOD measurement (Chang et al., 2004, Gil et al., 2003, Kang et al., 2003, Kim et al., 2003a, Moon et al., 2004).

As a consequence of direct contact with air, better oxygen supply was provided at the cathode, thus increasing the dynamic range of the SCMFC-type biosensor by approximately 133% when compared with the range obtained with two chambered-MFC sensors. A linear response was in fact observed up to a BOD concentration of 350 ppm, in contrast with a maximum of 150 ppm COD found by Kim et al., (2003a). This range increased till a COD concentration of 500 ppm, when total charge generated where considered.

In a wastewater treatment plant (WWTP) the quality of the effluent in terms of COD content after the primary clarifier is of vital importance. In a typical treatment plant in the UK the COD value for the primary clarifier effluent is typically around 300 ppm. The SCMFC biosensor considered in this study would therefore be applicable to conditions typical of UK domestic wastewaters. In the case of WWTP effluents, characterized by COD values after the primary clarifier higher than 500 ppm, dilution of the wastewater could be performed prior to feeding to the SCMFC-type biosensor.

The SCMFC-based BOD sensor, had good reproducibility with a coefficient of variation of 0.53 %, considerably better than the conventional BOD_5 test where in general ± 15% is permitted (Eaton et al.,1995). The reproducibility was also superior to that observed in previous MFC-type biosensors, characterized by up to ± 10% of standard deviation (Chang et al., 2004, Kumlanghan et al., 2007), and was better than the reproducibility of other biosensors in general (Liu and Mattiasson, 2002).

The response time of the SCMFC- type biosensor was, however, longer than responses obtained for a two chamber MFC-type biosensor, by Chang et al., (2004) e.g. ~ 60 minutes and Moon et al. (2004) decreased the response time of their sensor to 5 min by reducing the reactor void volume.

When a new reactor was tested, with carbon cloth as anode and a 75% lower volume, the response time obtained was as short as 40 min. The results was in accord with observations made with a two chambered MFC (Moon et al., 2004). Moon et al. (2004) treated the MFC as a plug-flow reactor in which current responded in a linear fashion following changes in fuel concentration, assuming that microbial and electrochemical effects were negligible. He found that scaling down the MFC reduced the non-ideal flow and therefore supported the hypothesis that plug flow occurred in the reactor. Consequently, through manipulation of the fuel-feeding rate, it was possible to reduce the response time and a steady-state current output was attained in a period very close to the HRT.

With the new configuration tested here the Ohmic losses inside the reactor were minimized: due to the shorter distance between the electrodes, the internal resistance of the small volume SCMFC biosensors was 20 Ω, considerably less than the 138 Ω characteristic of the larger volume SCMFCs used in this study. It was previously shown that the electrode distance represents a key factor for the overall performance of a MFC (Cheng et al., 2006).

The correspondence between energy recovery and COD removal was also improved in the new configuration, resulting in higher Coulombic efficiency (Table 4). High Coulombic efficiency values are important for an MFC-based biosensor: if alternative electron acceptors of higher redox potential, such as nitrate and oxygen, are present in the system, the signal is reduced and therefore the overall biosensor performance deteriorates. In a previous study, in order to obtain accurate BOD measurements, the effects of alternative electron acceptors in the system were removed by adding respiratory inhibitors to the sample, such as azide and cyanide (Chang et al., 2005). The work of Chang et al., (2005) was performed using a two chamber MFC biosensor.

In the case of an air cathode it is very likely that significant oxygen diffusion into the anode chamber will occur, even with a polymeric membrane such as Nafion used here (Liu and Logan, 2004). Therefore it would be very interesting to determine the effect of inhibitors of aerobic respiration on the performance of the SCMFC-based biosensor.

When the SCMFC-based biosensors were fed with real wastewater the system showed a linear relationship between COD values and current output (Figure 9B) and a good correlation between the COD values obtained from the standard method and the values obtained with the SCMFC biosensor (Table 5).

These results are encouraging and demonstrate the possibility of the practical application of this biosensor.

In a wastewater treatment plant (WWTP) the SCMFC-based biosensor could be installed between the clarifier and the aeration tank to determine the quality of the effluent in terms of COD content after the primary clarifier. The SCMFC biosensor would give an on line information about the organic content of the wastewater with the possibility of detecting immediately a problem in the efficiency of the treatment process.

4. Conclusions

This study demonstrated that a SCMFC, with an air cathode, has potential to be used as a biosensor for labile organic carbon. The biosensor optimization was carried out by considering AW as fuel. Subsequently the optimized biosensor was tested with real wastewater.

- With an external load of 50 Ω, the measuring range of the SCMFC was larger than previously reported for two chambered MFC biosensors: linearity was observed up

to 350 ppm COD when calibrated against current and up to 500 pm COD when calibrated against charge.

- Repeated measurements conducted at the same time were highly reproducible (0.53% coefficient of variation) and measurements made over a prolonged (approximately 7 months), were also stable with a total variation of only 15%
- When the reactor volume was reduced from 50 cm^3 to 12.6 cm^3, a response time close to the HRT was observed (i.e. 40 min). The smaller reactor also gave Coulombic efficiencies 9 times higher than the larger reactor with a carbon cloth anode.
- When real wastewater was tested with the biosensor a good correlation between COD concentration and current output was obtained, demonstrating the applicability of this system to real treatment effluents.
- Even if the optimal operational conditions were observed at 30°C, this study demonstrated good performance of the system also when operated at room temperature (~ 20°C), which would allow greater simplicity of the system and lower operational costs.

Acknowledgements.

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Legends to Figures

Figure 1 Schematic diagram of the SCMFC biosensor system. A) single chamber microbial fuel cell. A: cathode side; b: gasket; c: inlets; d: anode side; e: connection wire (titanium). B) System layout, not to scale. Feed tank (A); fuel cell (B); peristaltic pump (C); tube outlet (D).

For cell tests nitrogen was purged into the feeding tank (A). The AW was fed to the microbial fuel cell (B) in up-flow mode using a peristaltic pump (C). When the feed pump was stopped (starvation) the tube end (E) was connected to the outlet (D) to allow liquid recirculation. The MFCs were connected to an external resistor and a voltmeter, and the cell potential was monitored continuously during operation.

Figure 2. **Variation of current with time for electrochemically-active bacterial enrichment of the SCMFCs**. Data are the average from two reactors, 3% accuracy. Arrows indicate the points of reactor batch feeding. Only the first 6 batches were performed with anaerobic sludge as inoculum (0.5% by volume) and AW (1000 ppm COD). The batch indicated with ** was performed with AW containing 1000 ppm as COD, while the following batches AW contained 200 ppm as COD. Anode area: 12.5 cm^2. External resistance: 500 Ω.

Figure 3. **SCMFC response to COD concentration with an external load of 50 Ω.** A: Current generated under different COD concentrations. The SCMFCs were fed with AW containing different concentrations of COD, until a steady-current was generated. Afterwards, the cells were starved until a current of 0.015 ± 0.05 mA was obtained and the MFC sensors were then fed with fresh feed containing a different concentration of COD. B: Steady-state current in relation to the inlet COD. C: Calibration curve. Flow rate: 0.46 cm^3 min^{-1}. Data are the average from two reactors.

Figure 4. **Correlation between charge generated and COD concentration**. For each COD value the charge generated was calculated by integrating the current output

over the time from the starting point of the batch-feeding to the time where the current decreased to 5% of its maximum. Flow rate: 0.46 cm^3 min^{-1}. R_{ext}=50 Ω. Data are the average from two reactors.

Figure 5. SCMFC biosensor reproducibility. The system was fed in continuous mode with AWs containing the COD concentrations indicated (in ppm). Feeding rate: 0.46ml/min. Anode material: carbon cloth. External resistance: 50Ω. Data are the average from two reactors.

Figure 6. **Variation of current with time for electrochemically-active bacterial enrichment of a small volume SCMFC biosensor.** Arrows indicate the points of reactor batch feeding with fresh AW. The batch-feedings performed during the first 250 hours did not cause any current increase and therefore are not shown in the graph. Data are the average of two reactors with a coefficient of variation of 5%.

Reactor volume: 12.6 cm^2. Anode material: carbon cloth. External resistance: 50 Ω.

Figure 7. **Influence of reactor volume on SCMFC biosensor response time to step-wise changes in COD concentration**. A and B refer respectively to a down-shift in COD concentration and an up-shift in COD concentration. A COD concentration of 70 ppm was used for the down-shift and a COD concentration of 250 ppm for the up-shift. The fuel feeding rate was 0.46 cm^3 min^{-1} and the external resistance was 50 Ω. The response time was defined as the time required to reach 95% of the steady-state current. Average data from two reactors (error bars). Standard deviations refer to three replicates of the experiment

Figure 8. **Long term stability of the SCMFC biosensor**. Steady state current obtained during each month of SCMFC operation. The SCMFC was fed in continuous mode with AW having an inlet COD of 200 ppm. External load: 50 Ω. Data are the average from two reactors.

Figure 9. **Variation in current in an SCMFC biosensor in response to different dilutions of effluent from a wastewater treatment plant**. A: The SCMFCs were fed in continuous mode with several dilutions of sewage wastewater at a flow rate of 0.46 cm^3 min^{-1}. The COD of the undiluted wastewater was 175 ± 50 ppm. The dilutions were performed by using tap water. Arrows indicate the steady-state conditions obtained at a particular wastewater dilution while numbers refer to the inlet COD values expressed in ppm. B: Calibration curve of wastewater COD and current. Anode: carbon cloth. External resistance: 50 Ω. Reactor volume: 50 cm^3. Average data from two reactors

Legend to Tables

Table 1 Characteristic of the wastewater utilized

Table 2. Effect of feeding rate on current generated and Coulombic efficiency. Artificial wastewater was used as fuel. The influent COD was fixed at 100 ppm. The solution was fed into the cell at a specific feeding rate until a stable current was generated, the flow rate was further increased in a stepwise manner. Loading rates refer to anode area (12.5 cm^2). External resistance: 500 Ω. Average data from two reactors. Effluent COD mean data from at least three replicates. HRT = hydraulic retention time.

Table 3. Effect of the external resistance on the dynamic response of the SCMFC biosensor. The cells were fed under an external load ranging from 50-500 Ω by means of a resistor box. The AW feed was stopped once a steady-current output was reached and the MFC sensor was starved prior to being fed with AW with a different COD concentration. Feeding rate: 0.46 cm^3 min^{-1}. Fuel: AW with 100 ppm COD. Data are the average from two reactors. For the COD removal rate and Coulombic efficiency values, the data represent the mean from at least three replicates

Table 4 Comparison of the performance of SCMFC biosensors in relation to reactor volume. Artificial wastewater was used as feed. The influent COD was fixed at 100 ppm. The flow rate was 0.46 cm^3 min^{-1} giving a loading rate of 0.221 mg COD h^{-1} cm^{-2} of anode cross sectional area. Data are the average from two reactors. COD removal rates are the mean from at least three replicates. HRT = hydraulic retention time. AV: anolyte volume, expressed in cm^3.

Table 5. Comparison of COD values measured by the SCMFC biosensor and the standard method. Fuel: wastewater with unknown COD concentration. The COD of

the samples was determined either by the standard method described by (Eaton et al., 2005) either by feeding the samples into the SCMFC biosensor and determining its COD concentration by correlating the current output with the calibration curve reported in Figure 8B. Average on three replicates.

Table 6. Effect of temperature on an SCMFC- biosensor.

Data are the mean from two replicates. Anode material: carbon cloth. Reactor volume 12.56 cm^3. Flow rate 0.46 cm^3 min^{-1}. External resistance: 50 Ω. The response time was defined as the time required to reach 95% of the steady-state current.

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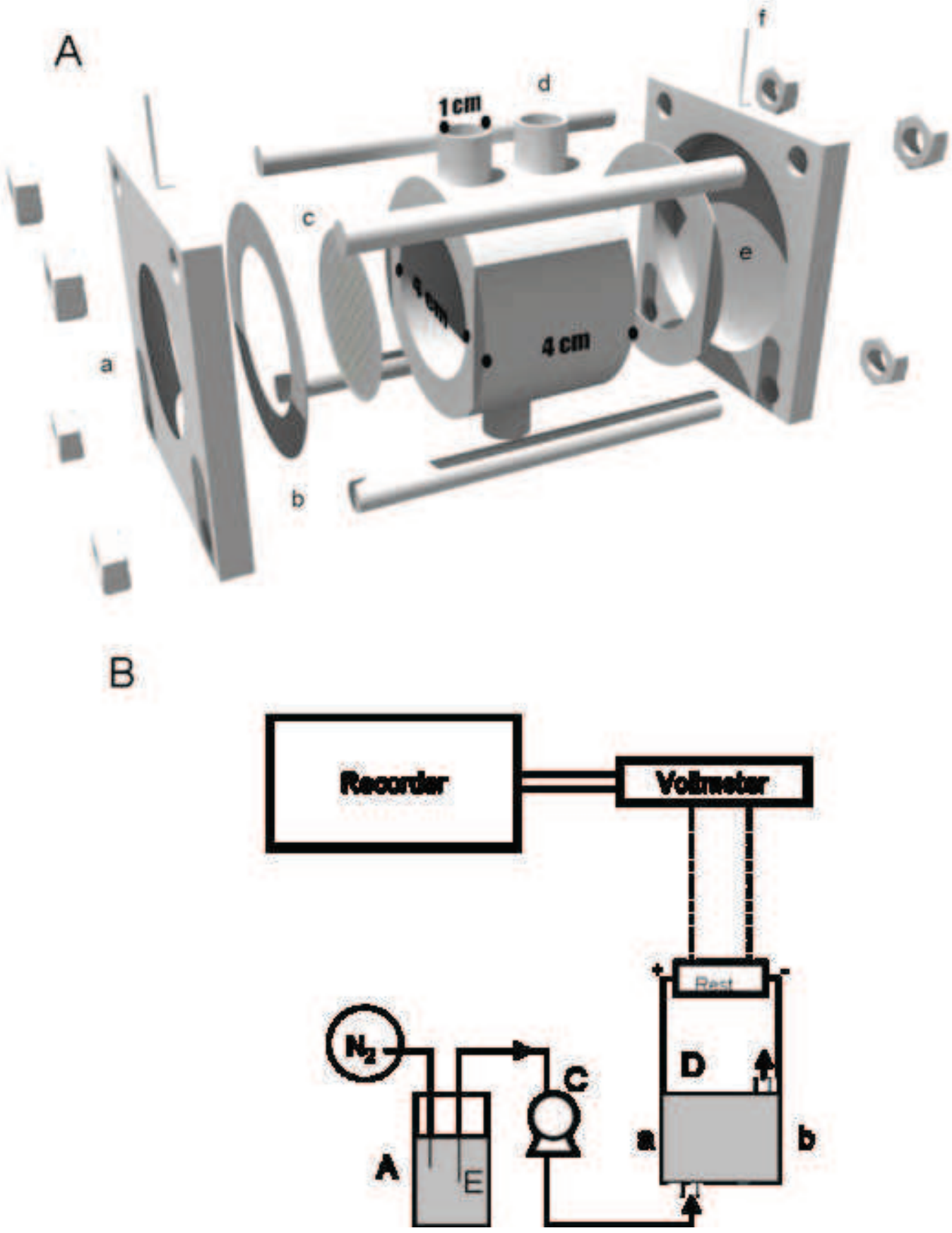

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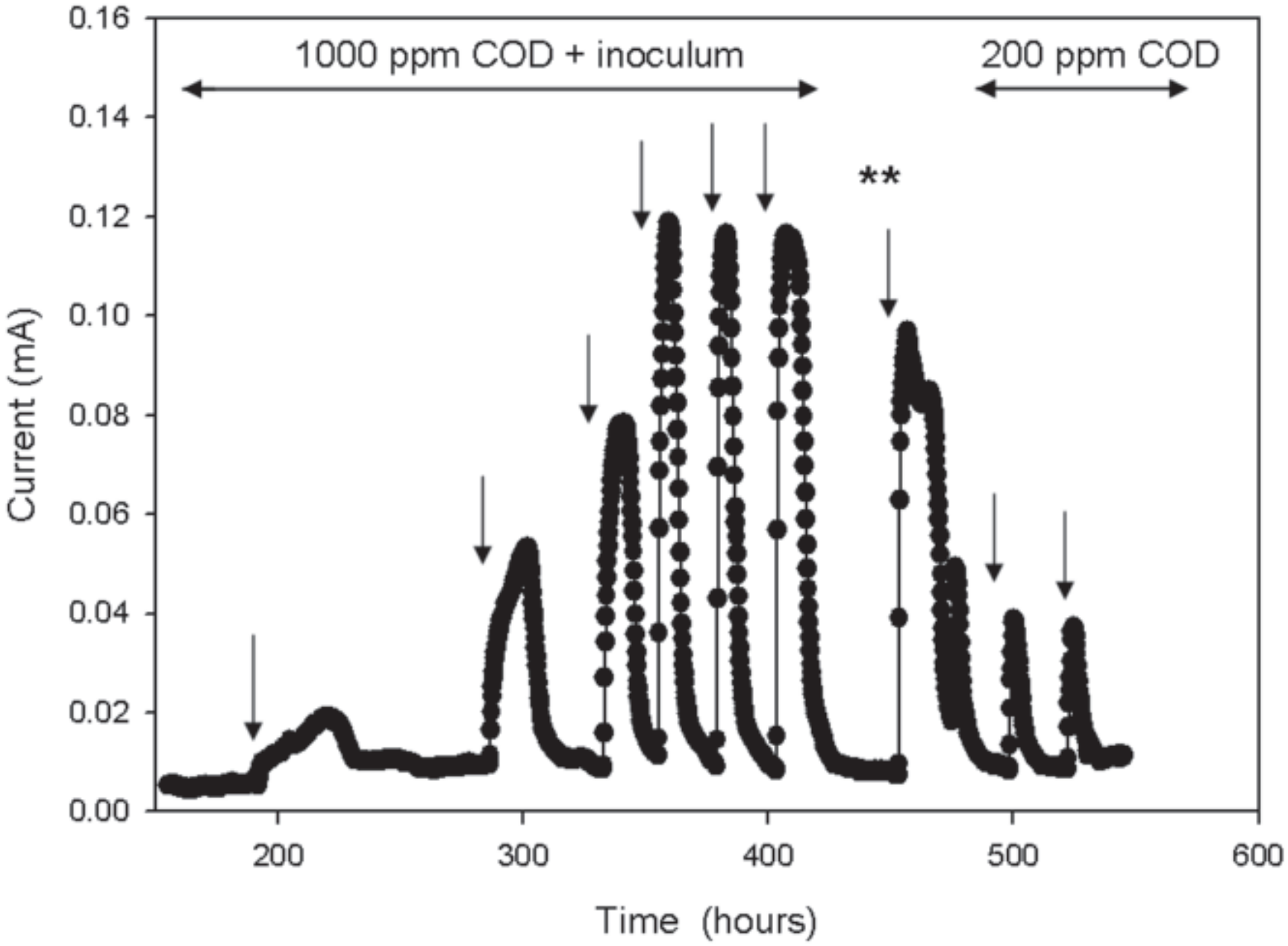

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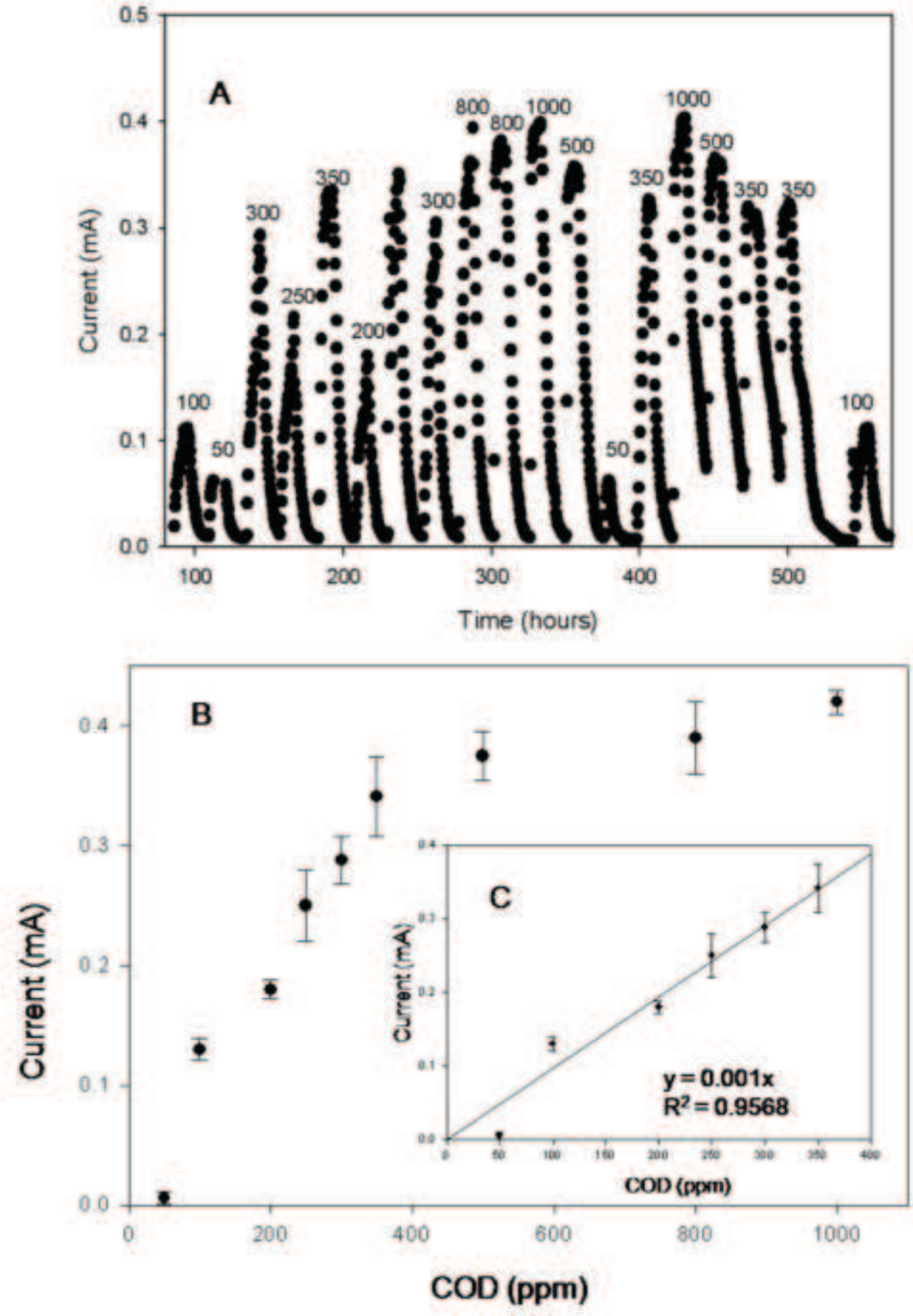

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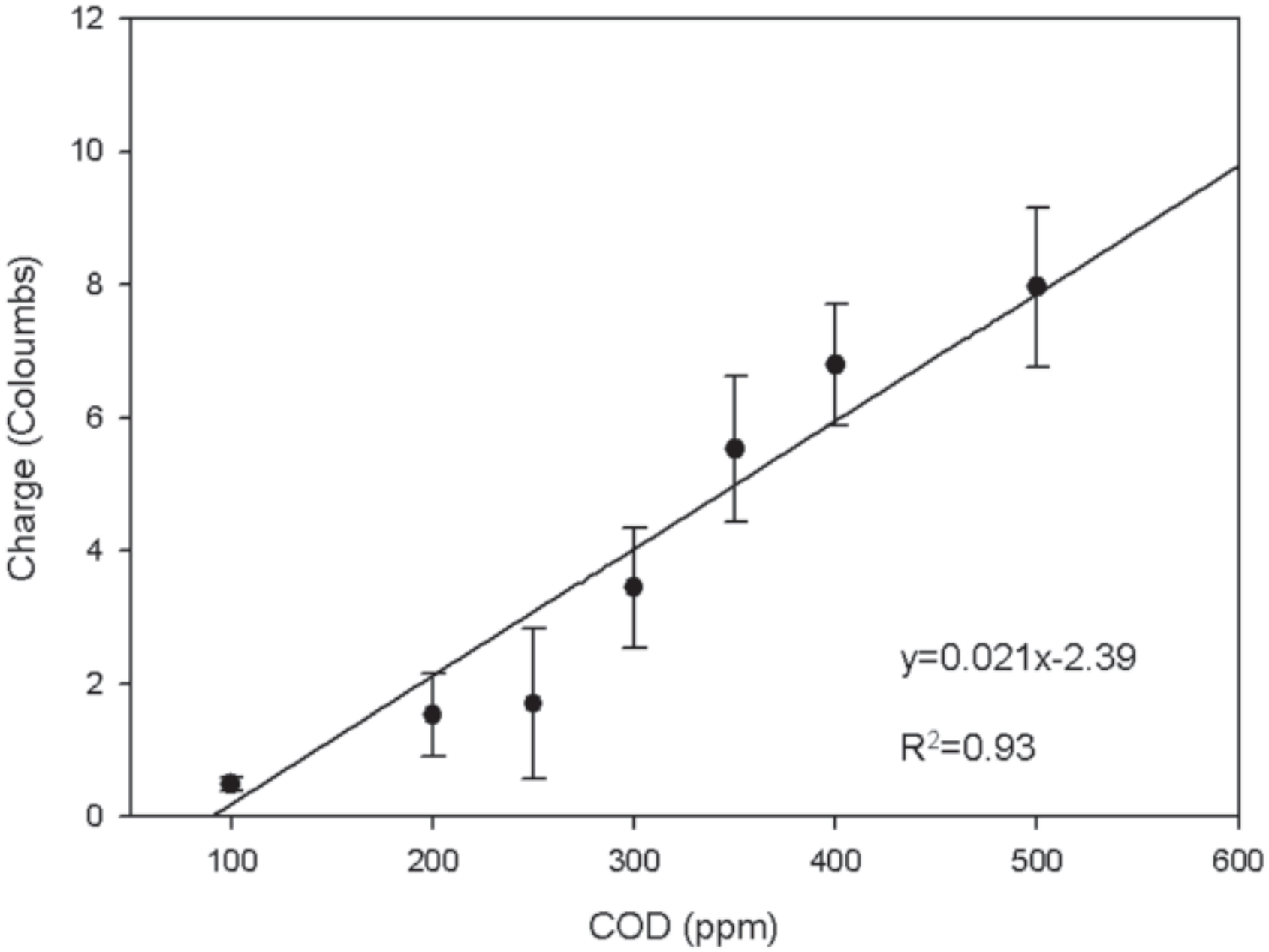

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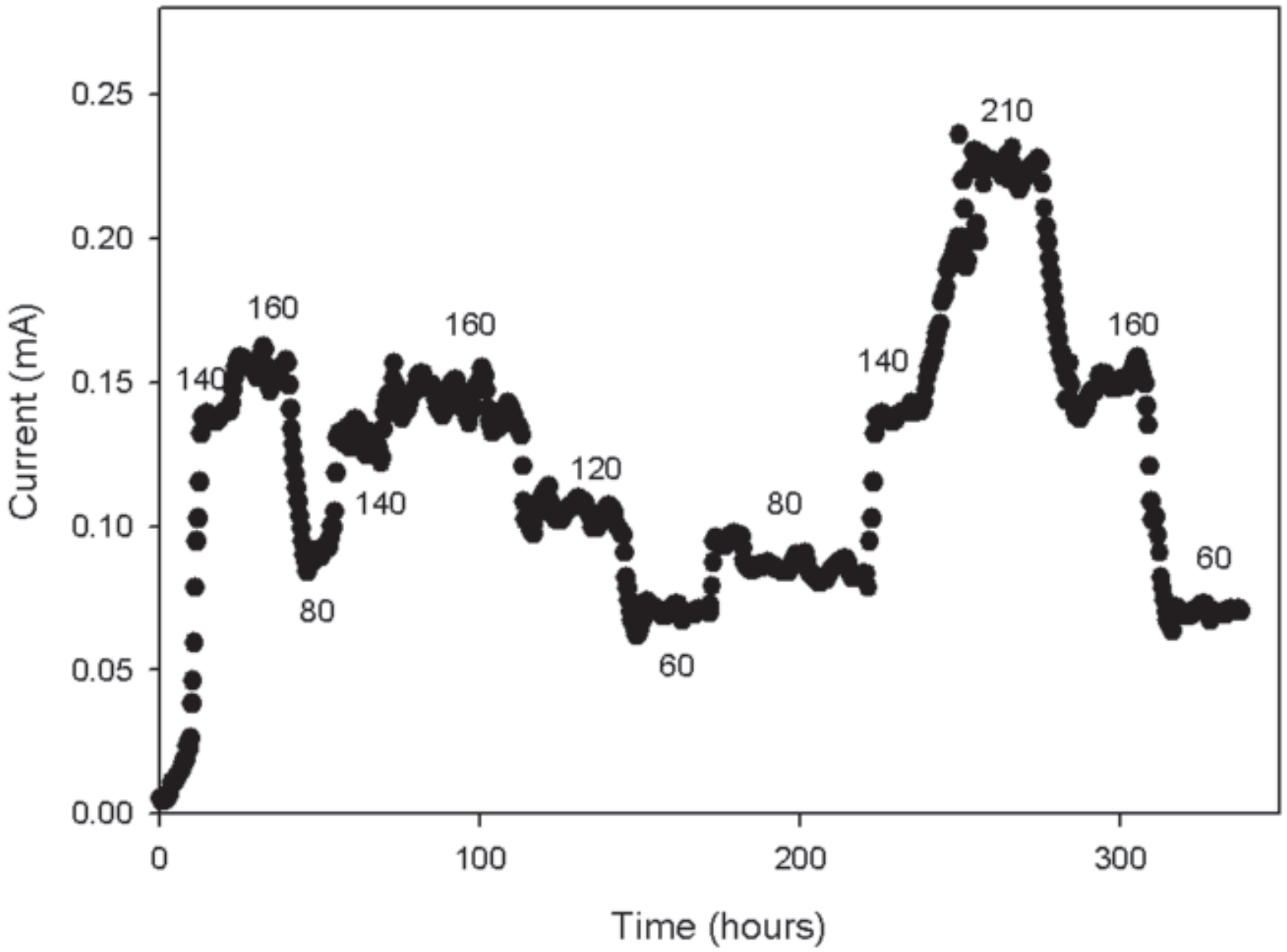

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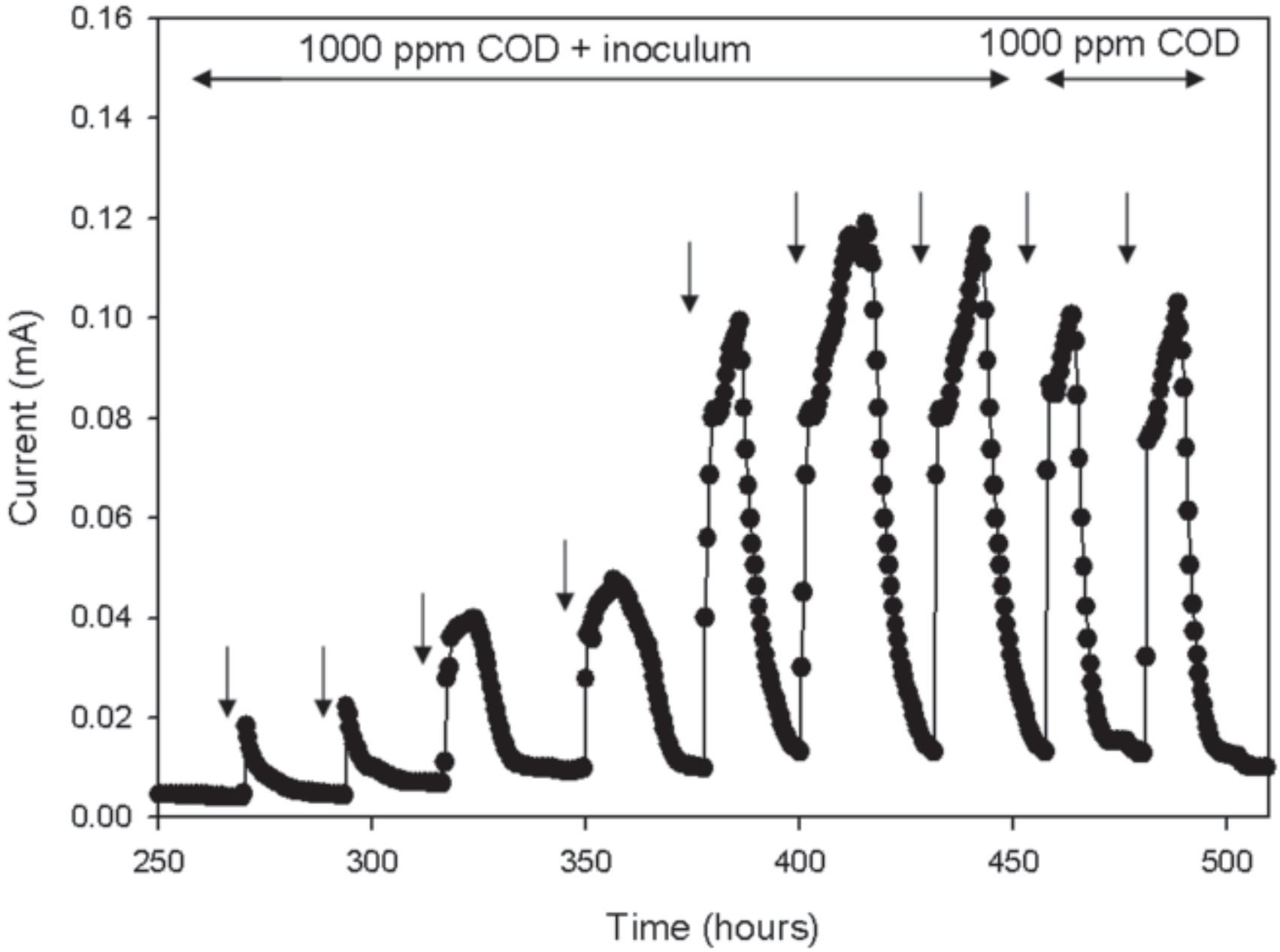

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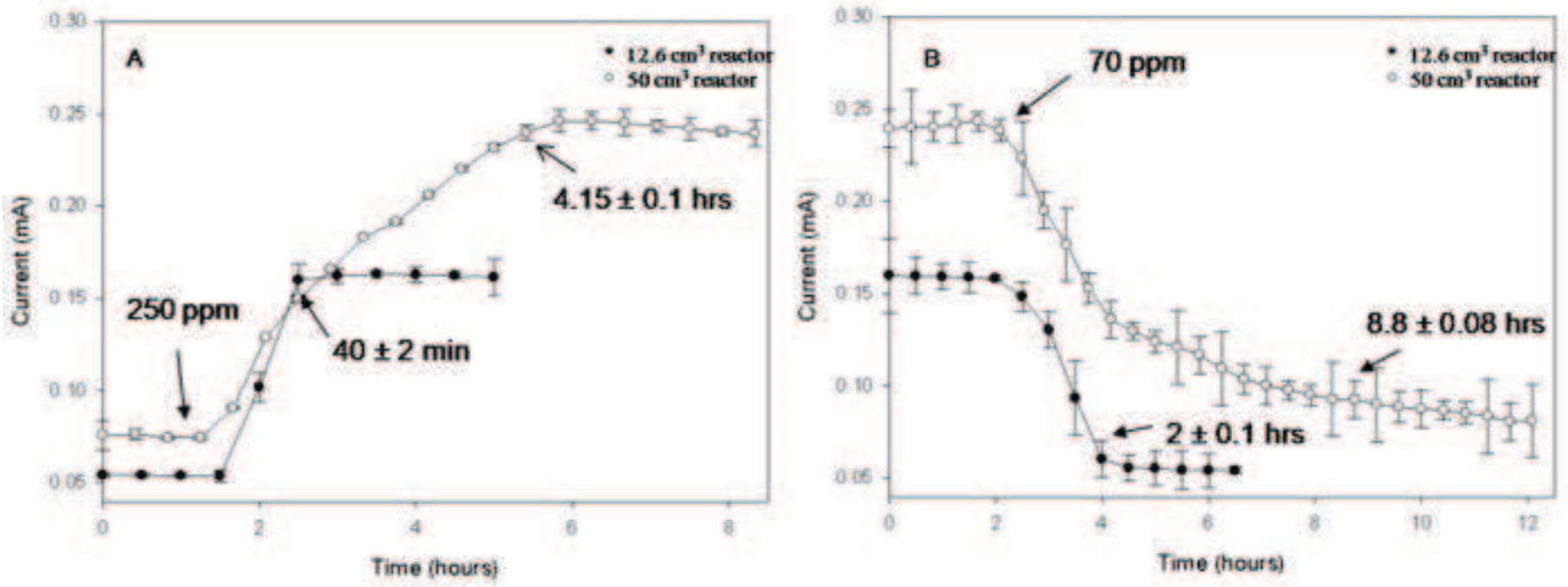

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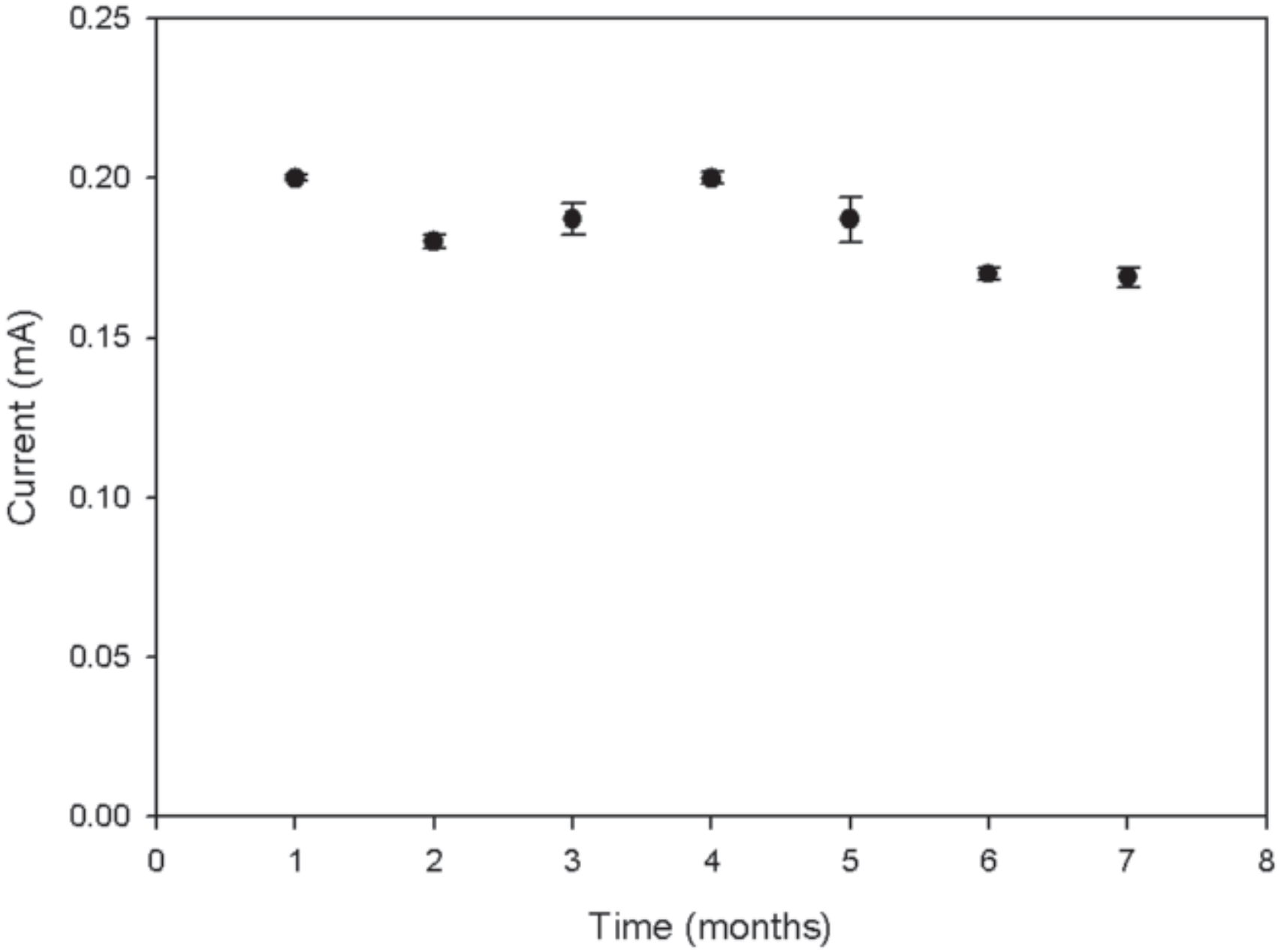

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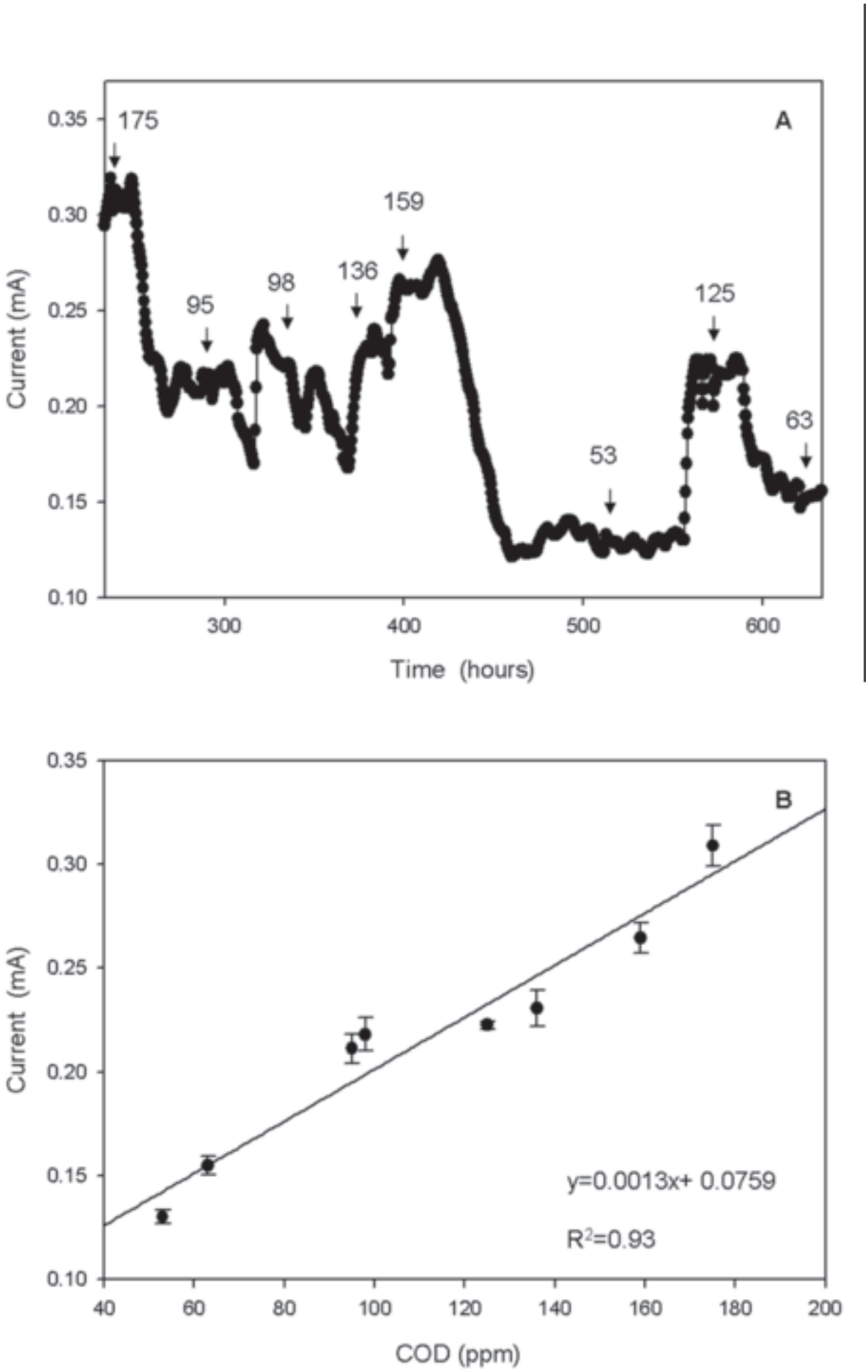

Tables
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Table 1

Parameter	Value
pH	7
Alkalinity, ppm	46 ± 3
Conductivity*, μS cm^{-1}	1570 ± 10
COD, ppm	175 ± 50
BOD, ppm	88 ± 5
TOC, ppm	98 ± 2
NH_3-N, ppm	21 ± 0.3
Phosphate, ppm	4.1 ± 0.9
Sulphate, ppm	49.4 ± 9
Total suspended solids, ppm	187 ± 18

* at 20.7°C

Table 2

Flow rate $cm^3\ min^{-1}$	**Loading rate** mg COD $h^{-1}\ cm^{-2}$	**Steady state current** mA	**HRT** min	**COD effluent** ppm	**COD removal rate** mg COD x h^{-1}	**Coulombic efficiency** %
0.12	0.058	0.063	417	34 ± 8.3	0.475 ± 0.06	4 ± 0.7
0.26	0.125	0.151	192.3	40 ± 0.25	0.936 ± 0.02	5 ± 1
0.38	0.182	0.181	131.6	39.9 ± 2.6	1.37 ± 0.1	4 ± 0.8
0.46	0.221	0.26	108.7	50 ± 1.8	1.38 ± 0.2	6 ± 1.2

0.56	0.267	0.27	89.3	52 ± 2.7	1.61 ± 0.8	5 ± 0.9

Table 3

R_{ext} Ω	Response time hours	Max Current mA	COD removal rate mg COD h^{-1}	Voltage mV
50	4.5	0.14	2.7 ± 0.9	7
120	5	0.126	3 ± 0.5	15.12
250	5	0.105	2.3 ± 1.2	26.25
500	13.75	0.084	2.37 ± 1	42

Table 4

Reactor volume cm^3	Steady state current mA	HRT min	COD removal rate mg COD h^{-1}	Coulombic efficiency %
12.6	0.12 ± 0.01	27	0.06 ± 0.007	56 ± 4
50	0.14 ± 0.007	108.7	1.2 ± 0.2	6 ± 0.5

Table 5

COD (ppm) SCMFC biosensor	Standard measurement
169 ± 9	175 ± 50
98.5 ± 2.1	100 ±9

122 ± 1	120 ± 10
61.5 ± 0.9	60 ± 5

Table 6

Temperature **°C**	**Steady state current** **mA**	**COD removal** **%**	**Coulombic efficiency** **%**	**Response time** **min**
20	0.28 ± 0.008	24 ± 6	10± 1.2	41 ± 2
25	0.32 ±0.007	28.7± 10	11 ± 0.8	40 ± 4
30	0.55 ±0.008	46 ± 8	28 ± 0.5	31 ± 2