

A single chamber packed bed microbial fuel cell biosensor for measuring organic content of wastewater

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ABSTRACT

This study reports an investigation of the effect of the anode surface area on the performance of a single chamber microbial fuel cell (SCMFC) based biosensor for measuring the organic content of wastewater. A packed bed of graphite granules was used as the anode. The surface area of the anode was changed by altering the granule bed thickness (0.3 cm and 1 cm). The anode surface area was found to play a role in the dynamic response of the system. For a granule bed thickness of 1 cm and with an external resistance of 500 Ω , the response time (defined as the time required to achieve 95% of the steady-state current) was reduced by approximately 65% in comparison to a SCMFC biosensor with a carbon cloth anode.

Key words | biosensor, BOD, graphite granules, microbial fuel cell, wastewater

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INTRODUCTION

Biochemical oxygen demand (BOD) is a measure of the amount of biodegradable organic compounds in water and wastewater and it is most widely applied to measure the organic load and treatment efficiency in wastewater treatment plants (Eaton *et al.* 2005).

The conventional BOD measurement method known as BOD₅, which requires five days incubation at 20°C, has limitations of questionable accuracy, poor precision, labour intensive and time consuming (Kim *et al.* 2003a; Chang *et al.* 2004, 2005). It is therefore not suitable for process control and real-time monitoring where rapid feedback is desirable. Rapid determination of BOD could potentially be achieved by biosensor-based methods and this has been a subject of several reviews (Liu & Mattiasson 2002; Lei *et al.* 2006). Most of these biosensors are whole-cell-based biofilm devices and are all based on the respirometric principle: the response is typically a change in concentration of dissolved oxygen (DO) or other phenomena, such as light emission. A physical transducer is used to monitor changes

in an electrical or optical signal which are amplified and correlated to the biodegradable material measured.

These sensors have a short response time, and offer the possibility of on-line monitoring and process control. They have, however, the disadvantages of the intrinsic low oxygen solubility in aqueous solution, short term-stability due to the lysis of immobilized bacteria and complicated maintenance (Liu & Mattiasson 2002).

A promising alternative is a microbial fuel cell (MFC) based BOD sensor which can be used to detect and estimate organic material concentration in wastewater samples.

A microbial fuel cell (MFC) is a device that directly transforms the chemical energy, typically contained in an organic compound, into electrical energy via electrochemical reaction (Bullen *et al.* 2005). Once the enrichment and adaptation of the electrochemically active bacteria has occurred, the current generated by an MFC depends on the concentration of organics in the wastewater (Kim & Kwon 1999).

Several types of MFC-based BOD sensor have been developed. These include MFCs with chemically mediated electron transfer, (Pasco *et al.* 2004; Chang *et al.* 2005) and mediator-less MFCs, (Gil *et al.* 2003; Kang *et al.* 2003; Kim *et al.* 2003a; Chang *et al.* 2004; Moon *et al.* 2004).

The operational stability of MFC-based BOD sensors is well established. They have given up to 5 years stable performance with minimum maintenance, (Kim *et al.* 2003a,b; Chang *et al.* 2004, 2005) and the instruments' response correlated well with conventional BOD₅, thus demonstrating the validity of using a MFC-type biosensor for real wastewater.

Recently, we demonstrated the efficiency of a single chamber MFC (SCMFC) with an air cathode, as a biosensor of wastewater strength (Di Lorenzo *et al.* 2009). Since an air cathode MFC does not require catholyte pumping, nor air purging at the cathode, this new biosensor is simpler and more compact than previously described two chambered-MFC sensors.

The SCMFC-based BOD sensor, had good reproducibility with a coefficient of variation of 0.53%, considerably better than the conventional BOD₅ test where reproducibility of $\pm 15\%$ is considered acceptable (Eaton *et al.* 2005). The reproducibility was also superior to that observed in previous

MFC-type biosensors, characterized by standard deviations up to 10% (Chang *et al.* 2004; Kumlanghan *et al.* 2007).

In this study, we investigated the effect of increased anode surface areas on the performance of the SCMFC-type biosensor. In particular, the surface area was increased by means of a packed bed of carbon graphite granules, used as anode material. Data is compared with that obtained with an MFC using a carbon cloth anode. Graphite granules represent a low cost option for MFCs. The first use of graphite granules as an MFC anode was reported by (Rabaey *et al.* 2005) and since then they have been used both as an anode and cathode (Aelterman *et al.* 2006; Heilmann & Logan 2006; Rabaey *et al.* 2006;).

MATERIALS AND METHODS

Microbial fuel cell and its operation

The single chamber, microbial fuel cells (SCMFC) were made from polyacrylate tube, 12.5 cm inner diameter, 4 cm length, with an open anode chamber volume of 50 cm³, as shown in Figure 1. The cathode was a carbon supported platinum catalyst (0.3 mg Pt cm⁻²; 60% on Vulcan, E-tek),

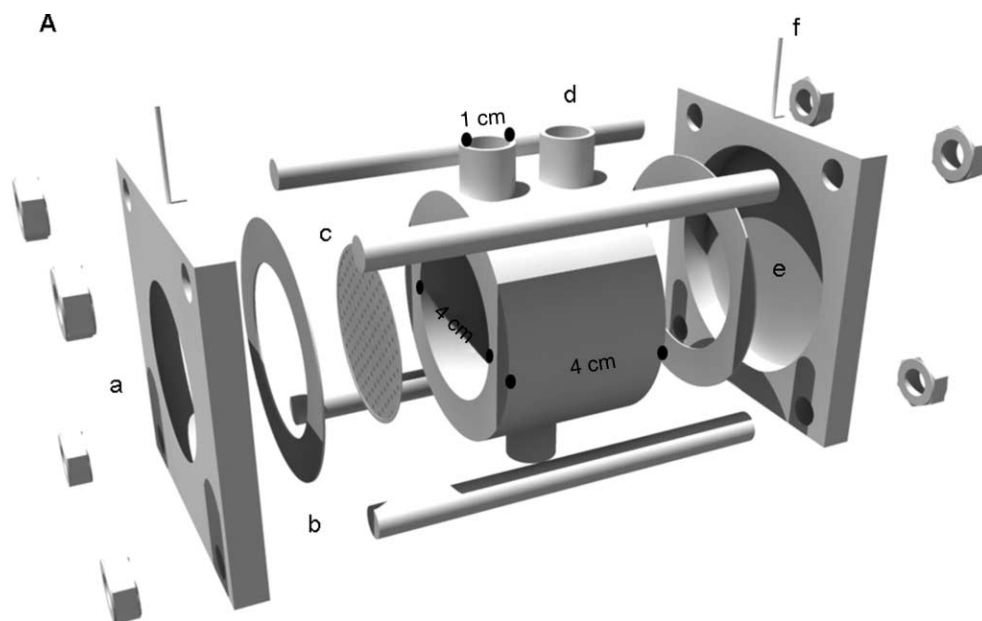


Figure 1 | Schematic diagram of the SCMFC biosensor system. Single chamber microbial fuel cell. a: cathode side; b: gasket; c: titanium net; d: inlets; e: anode side; f: connection wire (titanium).

deposited on wet proofed (20 wt % Teflon) Toray carbon paper from E-tek. The cross section area was 12.5 cm^2 . The cathode was exposed to air on one side (position a, in Figure 1) and separated from the anode by a cation exchange membrane (Nafion® 117, DuPont). A circular piece of titanium net (c) was used to separate the carbon granule anode from the membrane and to provide an electrical connection. By adjusting the position of the titanium net, it was possible to fill the reactor anode chamber with graphite granules of different thickness to provide anodes with a range of surface areas.

The graphite granules were of diameters between 0.2 and 0.3 cm, from Carbon International, London, UK. The granules were packed inside the anode to occupy a defined fraction of the reactor. In this work, the SCMFC-biosensor was filled with granule thicknesses of either 0.3 cm or 1 cm. This gave total granule volumes in the anode compartment ($V_{a_{\text{tot}}}$) of 3.75 cm^3 and 12.5 cm^3 respectively. The anode cross sectional area was 12.5 cm^2 whilst the total anode surface area ($S_{a_{\text{tot}}}$) was calculated by approximating the area of a single granules with the surface of a sphere having an average diameter of 0.3 cm and by multiplying the surface of one granules by the total number of granules, i.e. 45 for the 0.3 cm layer and 248 for the 1 cm layer. With this assumption, the estimated total anode surface areas were of 90 cm^2 for a 0.3 cm layer of granules and 496 cm^2 in the case of a 1 cm layer. This approximation did not consider the contribution of the granules roughness to $S_{a_{\text{tot}}}$ nor its porosity. The total anolyte volumes were 46.25 and 37.5 cm^3 for the 0.3 cm and the 1 cm thick configuration respectively.

The anode and cathode were connected through an external resistance to polarise the cell. Cell voltages were measured across the resistor using a voltmeter and data logger (Pico® data logger) to monitor the current variation under closed circuit conditions. The external resistance was controlled by a resistor substitution box (RS 500, 1% accuracy, Elenco electronics). Cell polarization curves were recorded using a potentiostat (Gillac, ACM Instruments) at a voltage scan rate of 1 mV s^{-1} starting from open circuit potentials, over a 4 hour period.

In operation of the cell, wastewater was fed through the injection port at a flow rate of $0.46\text{ cm}^3\text{ min}^{-1}$ using a peristaltic pump (Watson-Marlow, 520S) equipped with Marprene II tubing (0.14 cm internal diameter).

The MFCs were operated at room temperature which was approximately $21 \pm 2^\circ\text{C}$. Each experiment was performed in duplicate.

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°C for 10 min prior to use.

Enrichment

The enrichment and adaptation of the electrochemically-active bacteria in the SCMFCs was performed in a batch mode under a fixed external load of $50\ \Omega$, and was carried out over a period of approximately four weeks.

A chemical oxygen demand (COD) of $1,000\text{ mg dm}^{-3}$ (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 1,000 ppm and no inoculum.

The system response time was tested with an AW with a COD of 100 ppm, while the test regarding the response to step-wise changes in the COD were performed with 70 ppm and 250 ppm concentrations.

Analyses

In this work we based the analysis on the chemical oxygen demand (COD) since it was more conveniently measured than BOD_5 . For the artificial wastewater used the COD was equal to the BOD and was correlated to the glucose concentration.

The chemical oxygen demand (COD) was determined using a standard method with chromate as the oxidant as previously described (Eaton *et al.* 2005). All samples were filtered through a $0.22\ \mu\text{m}$ pore diameter membrane filter (VWR International) prior to COD measurements.

The Coulombic efficiency (fractional), at the steady-state was calculated with formula (1):

$$\varepsilon_c = \frac{MI}{FzQ\Delta\text{COD}} \times 100\% \quad (1)$$

where F is Faraday's constant ($96,485 \text{ C mol}^{-1}$); $M = 32$ 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 ($\text{dm}^3 \text{ s}^{-1}$) the flow through the system, ΔCOD (g dm^{-3}) the difference in the influent and effluent COD.

Conductivity measurements were performed with a conductivity meter provided by Hanna instruments (HI 8633, measuring range: $199.9 \mu\text{S}$ to 199.9 mS). The internal resistance was measured by electrochemical impedance spectroscopy using a potentiostat (Gillac, ACM Instruments). 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.

RESULTS

Investigation of the effect of the anode surface area to reactor volume ratio on the system response time

Previously a SCMFC-type biosensor was investigated using a carbon cloth anode (12.5 cm^2 in area and 0.3 cm thickness) with a anode chamber volume of 50 cm^3 (Di Lorenzo *et al.* 2009). One limitation of this biosensor was its relatively long response time, defined as the time required to achieve 95% of the steady-state current. With an external resistance of 500Ω , the steady-state current was, in fact, only reached after a period greater than 13 hours. Several factors will have influenced the system response time, including electrode material, external resistance, and cell design. Increasing the anode surface area, whilst maintaining the same reactor configuration, could improve the anode biofilm contact with the bulk solution and, therefore, reduce the time required to reach steady-state conditions. Consequently, graphite granules were tested as the anode material for the SCMFC-based biosensor, and the performance compared with the SCMFC with a carbon cloth anode.

Figure 2 shows the current generation during the period of enrichment of the anode biofilm with electrochemically active bacteria, for SCMFC biosensors containing 0.3 cm and 1 cm thick graphite granule anodes. The 1 cm thick anode gave a higher peak current than the 0.3 cm thick anode. The differences between the peak currents obtained for the two reactors increased with the number of batches used. In the case of the first batch, only a difference of 0.02 mA was observed, whilst after the 7th batch the difference was 0.095 mA . For the 1 cm layer anode the current output after the 7th batch was more than twice that for the 0.3 cm layer anode. The peak current stabilised after being fed with 6 batches of AW for both of the SCMFC biosensors. Following the enrichment period, when the SCMFC biosensors were fed with AW containing $1,000 \text{ ppm}$ COD and no inoculum, the peak currents were: 0.136 mA and 0.063 mA with 1 cm layer and 0.3 cm layers of granules respectively. This compares with a current of 0.09 mA , obtained when the SCMFC with carbon cloth anode was fed with an inlet COD of $1,000 \text{ ppm}$, after the enrichment (Di Lorenzo *et al.* 2009). The responses of the 0.3 cm thick graphite granule bed and carbon cloth were similar. Using a 1 cm thick graphite bed increased the current response by

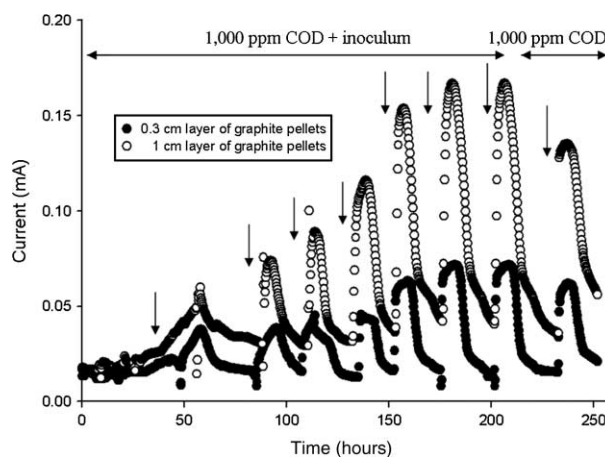


Figure 2 | Variation of current with time for electrochemically-active bacterial enrichment of the SCMFCs with graphite granule anodes. Two thicknesses of granules were used, 0.3 cm and 1 cm , giving a total anolyte volume of 46.25 and 37.5 cm^3 respectively. Data regarding both graphite granules filling are the average from two reactors, 3% accuracy. Arrows indicate the points of reactor batch feedings. Only the first 7 batches were performed with anaerobic sludge as inoculum (0.5% by volume) and AW ($1,000 \text{ ppm}$ as COD). The 8th batch was performed with AW containing $1,000 \text{ ppm}$ COD and no inoculum. Anode material: graphite granules. External resistance: 500Ω .

around a factor of 2, although the thickness and available area were increased by a factor of 3.3 and 5.5 respectively. This would indicate that due to current distribution and substrate mass transport effects, the total area of the bed may not have been fully utilised. This in part is influenced by the hydrodynamics of the MFC where flow across the face of the anodes was laminar with a Reynolds number (Re , typically < 1.0) as estimated below:

With a volumetric flow rate of $0.46 \text{ cm}^3 \text{ min}^{-1}$, an equivalent diameter of 0.3 cm and a cross-section area for flow at the inlet of 0.3 cm^2 and a viscosity for water of $10^{-2} \text{ cm}^2 \text{ s}^{-1}$

$$Re = ud/\nu = (1.5/60)0.3/10^{-2} = 0.75$$

The two graphite granule anodes led to only a small difference in the internal (Ohmic) resistances; 128Ω and 104Ω , respectively for a 0.3 cm layer and 1 cm layer of graphite granules respectively. Higher current output was therefore related to higher anode surface area.

Figure 3 shows the polarization curve obtained with the 1 cm graphite granules anode (Figure 3A) and the curve obtained with the carbon cloth anode from our previous work (Figure 3B). In both cases the cell voltage fell with increasing current density and, in the case of carbon cloth, was characterized by a very slow fall in voltage up to a current density of approximately 0.005 mA cm^{-2} , followed by a region of relatively rapid voltage loss at higher current density. The cell voltage variation in the SCMFC with the graphite granule anode (Curve A) exhibited a first phase of rapid voltage loss, typical of activation polarisation at low current density; a second phase of linear voltage drop and a third phase of a relatively rapid voltage drop at high current.

Table 1 compares the response time of SCMFC biosensors, with respect to current generation, obtained with carbon cloth and graphite granule anodes. When the biosensors were fed with AW (COD of 100 ppm) and operated with an applied external resistance of 500Ω , the 1 cm thick graphite granule anode gave the greatest current and more rapid response time. In this case, the SCMFC reached a steady current of 0.11 mA after 4.5 hours , compared with 7 and 13 hours for SCMFCs with a 0.3 cm layer of graphite granule and carbon cloth anodes respectively. Overall the carbon cloth anode gave the longest

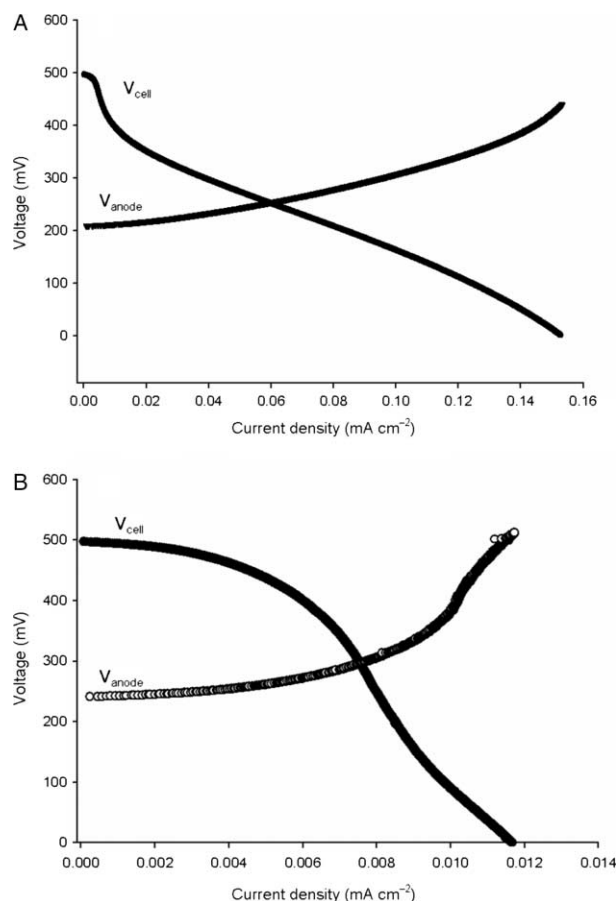


Figure 3 | Polarization curves from SCMFC biosensors with carbon cloth and graphite granules as anodes. (A) Anode: graphite granules. (B) Anode: carbon cloth. Current density refers to the anode cross sectional area: 12.5 cm^2 . The polarization curves were recorded using a potentiostat (Gillac, ACM Instruments) at a scan rate of 1 mV s^{-1} and a prior open circuit potential of over 4 hours .

response time, whilst the 0.3 cm thick graphite granule anode gave the lowest current. For an external load of 500Ω , the response of the SCMFC biosensors with graphite granule anodes was in general faster.

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). Thus, the effect of external resistance on the dynamic response of the SCMFC-biosensor was investigated and Table 1 reports the results obtained with a R_{ext} of 50Ω . The steady-state currents generated by the carbon cloth and the 1 cm thick graphite granule anode were, in this case, similar. The response time with the graphite granule anode was around

Table 1 | Response time of SCMFC biosensors with graphite granules and carbon cloth anodes

Anode	Internal resistance Ω	Steady-state current at $R_{\text{ext}} = 500 \Omega$ mA	Steady-state current at $R_{\text{ext}} = 50 \Omega$ mA	Response time at $R_{\text{ext}} = 500 \Omega$ h	Response time at $R_{\text{ext}} = 50 \Omega$ h
Carbon cloth*	138	0.084 ± 0.002	0.140 ± 0.012	13 ± 0.6	5.7 ± 0.4
Graphite granules, 0.3 cm layer	128	0.033 ± 0.004	0.052 ± 0.003	7.2 ± 0.5	4.2 ± 0.1
Graphite granules, 1 cm layer	104	0.108 ± 0.011	0.145 ± 0.005	4.5 ± 0.1	4 ± 0.3

Note: Feeding rate: $0.46 \text{ cm}^3 \text{ min}^{-1}$; Feeding solution: AW; Inlet COD = 100 ppm; Cross sectional anode area: 12.5 cm^2 ; Standard deviations refer to three replicates of the experiment.
 *Di Lorenzo *et al.* (2009).

4 hours, whilst for the carbon cloth it had fallen to around 5 hours. Decreasing the external load to 50Ω , therefore, gave for the 1 cm thick graphite granule anode, a variation of only 10% in the output current with respect to the case of R_{ext} equal to 500Ω . This result suggests that other factors still limited current generations and the response time of 4 hours, which corresponded to approximately twice the hydraulic retention time (HRT) of the reactor, may be the minimum that could be obtained with the reactor configuration considered. In conclusion, for a graphite granule anode, the external resistance had only a minor effect on the current generated.

Response to varying COD concentration: comparison between graphite granule and carbon cloth anodes

The response to changes in feed COD, under a fixed external load of 50Ω , of the SCMFC biosensors with 1 cm thick graphite granule anode is compared in Figure 4 with the case of the carbon cloth anode previously reported (Di Lorenzo *et al.* 2009). Two reactors were tested and each shift was performed three times. The response times in the case of a step up in COD, from 70 ppm to 250 ppm, was in general shorter than for a step down in COD, with a decrease of 62% for the case of carbon cloth and 75% for graphite granules. 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.

In particular, the SCMFC with carbon cloth reached a stable current with a step down in COD after 8.8 hours which was 63% of the corresponding response time of the SCMFC biosensor with a graphite granule anode (Figure 4). An explanation could be that, since a low flow rate was used, the glucose concentration in the volume between the anode granules decreased slowly, due to slow internal diffusion, leading to a long response time.

Figure 4 also shows that at higher COD concentrations, when a step increase in COD was imposed, a decrease in the response time of the SCMFC biosensors was obtained, irrespective of the type of anode. For an inlet COD of 250 ppm, the response times were 3.5 hour for the SCMFC biosensor with a graphite granule anode and 4.5 hours for the SCMFC with a carbon cloth anode; lower than the response times reported in Table 1.

DISCUSSION

This study was aimed at investigating the effect of the anode surface area on the performance of the SCMFC-based biosensor previously developed (Di Lorenzo *et al.* 2009). The anode chamber was filled with graphite granules as the electrode, and two different granule bed thicknesses were used. To compare carbon cloth and graphite granules, a layer of 0.3 cm, equal to the carbon cloth thickness, was used. The effect of increasing surface areas was subsequently analysed with a thicker layer of granules (1 cm).

The cell voltage and anode potential vs. current density behaviour of the carbon cloth at low current densities was different from that of the carbon granules which suggests that activation limitations were less important in the carbon cloth reactor. However another factor may be associated

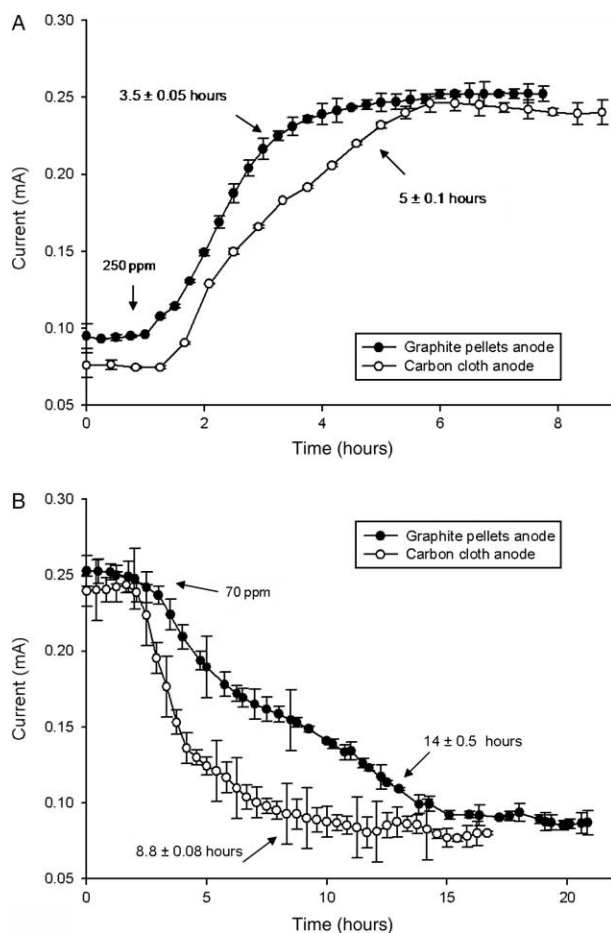


Figure 4 | Influence of the anode surface area on SCMFC biosensor response time to step-wise changes in COD concentration. Comparison between carbon cloth (Di Lorenzo *et al.* 2009) and 1 cm thick graphite granule anode. A) relates to an up-shift in COD concentration and B) to a down-shift in COD concentration. A COD concentration of 250 ppm was used for the up-shift and a COD concentration of 70 ppm for the down-shift. The feeding rate was $0.46 \text{ cm}^3 \text{ min}^{-1}$ and the external resistance was 50Ω . 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.

with the fact that the carbon cloth anode was not operated with flow through of feed through its structure and biofilm was predominantly formed on its outer surface.

Approximate calculations based on the polarisation curve (Figure 3A, with area = 12.5 cm^2) give the value of resistance near 200Ω . This value is greater than that measured by impedance spectroscopy and thus indicates that both Ohmic and electrode kinetic (and some mass transport) polarization losses are significant in the SCMFC.

Diffusion limitations were observed for both cells but the two cells showed different characteristics. The data for

the anode polarisations showed that mass transport limitations arise with both electrodes at higher current densities but were more significant with cells using a carbon cloth anode. The more rapid increase in voltage (anode overpotential) at higher current density observed with the carbon cloth anode, indicates mass transfer limitation of electron transfer/fuel oxidation at the anode. These data suggest that the graphite granule anode helped to alleviate mass transfer limitation at higher current densities.

The 0.3 cm thick graphite granule anode exhibited a response time faster than that of the carbon cloth anode (with a decrease of 46%), thus demonstrating that the anode surface area played a role in the biosensor dynamic response. However the graphite granule anode gave a current output 60% lower than that observed with carbon cloth. The differences observed may be due to a combination of the anode characteristics (bacterial activity and growth) and the effective surface area of the anode exposed to the feed. When a thicker layer of granules was considered, the biosensor response time was the shortest (4.5 hours) and in this case the current output was higher than for the carbon cloth: $\sim 0.11 \text{ mA}$ compared with 0.08 mA . In these examples the SCMFC biosensor was operated with a 500Ω external load, nevertheless, when the electrodes were connected through a 50Ω external load, the current output for the carbon cloth anode and the 1 cm layer graphite granule anode were the same ($\sim 0.14 \text{ mA}$). Moreover, the response time for a 1 cm layer of graphite granules did not change significantly when the external load was reduced to 50Ω (Table 1). Thus, it appears that for an inlet COD of 100 ppm, 4 hours was the lowest response time that could be obtained for this cell configuration and the flow rate used.

We have previously investigated the effect of the reactor configuration on the SCMFC-type biosensor performance (Di Lorenzo *et al.* 2009). The system reported in that study, had a carbon cloth anode and a reactor configuration similar to the present study, but with an anode chamber volume 75% smaller. In this way, for a feeding rate of $0.46 \text{ cm}^3 \text{ min}^{-1}$, the HRT was reduced to 27 min compared to 82 minutes in the SCMFC biosensor with a graphite granule anode. As summarized in Table 2, the lower HRT of the small volume sensor resulted in a considerably faster response of the system for an up-shift but also for

Table 2 | Comparison of performance of the SCMFC—type biosensor response time to step-wise changes in COD concentration according to anode material and reactor configuration

Anode material	Net anodic volume cm ³	Steady-state current* mA	HRT min	Down-shift response time hours	Up-shift response time hours	Coulombic efficiency %
Carbon cloth (small cell configuration) [†]	12.6	0.17 ± 0.01	27	2.0 ± 0.1	0.69 ± 0.01	56 ± 4
Carbon cloth [†]	50	0.23 ± 0.02	109	8.8 ± 0.1	5 ± 0.1	6 ± 0.5
1 cm thick graphite granules	37.5	0.25 ± 0.01	81.5	14.0 ± 0.1	3.5 ± 0.05	30 ± 0.3

Note: COD concentration used for the down-shift: 70 ppm; COD concentration for the up-shift: 250 ppm; The fuel feeding rate was 0.46 cm³ 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.

*For an inlet COD of 250 ppm.

[†]Di Lorenzo *et al.* (2009).

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 5 hours required with the larger volume SCMFC biosensor. When the COD concentration was reduced the response time was 2 hours compared to the 8.8 hours required for the larger volume SCMFC biosensors.

In general reactors with different volumes would have different response time even if they have the same catalytic activity. Designs with shortest HRT (small volumes) will see a pulse in a shorter time and therefore, they will show a faster response. Surface area clearly plays an important role in defining the performance of the sensors/reactors. The carbon cloth reactor showed in fact a faster response, compared with the 1 cm thick reactor, in spite of having a larger volume (Table 2).

This alternative configuration also gave a higher coulombic efficiency which represents an important factor for an MFC-based biosensor. This feature would be important if alternative electron acceptors of higher redox potential, such as nitrate and oxygen, were present, and then the signal would be reduced and therefore the overall biosensor performance poorer (Chang *et al.* 2005).

CONCLUSIONS

This study analyzes the utilization of a packed bed of graphite granules as anode of a SCMFC-based biosensor for the organic content in wastewater. Several factors influenced its performance, including electrode configuration and cell design. The anode surface area played a role in the dynamic response of the system, mainly due to improved

biofilm contact with bulk solution and the greater area of biofilm. When, a 1 cm layer of graphite granules was used as the anode, the response time decreased by approximately 65% in comparison to carbon cloth (with an external resistance of 500 Ω). An important factor overall will also be the biofilm surface area which itself will depend upon the specific area of the anode used.

Under conditions at which the reactors have the same activity, designs with the shortest HRT (small volumes) will experience a pulse in a lower time and therefore, they will show a faster response.

Graphite granules alone may, however, not be the best option for the SCMFC biosensor. It should also be combined with an appropriate reactor configuration, as demonstrated for the case of a carbon cloth anode when the internal losses were minimized by reducing the reactor volume from 50 cm³ to 12.6 cm³ (Di Lorenzo *et al.* 2009).

ACKNOWLEDGEMENTS

This work was supported by the European Union for Transfer of Knowledge award on biological fuel cells (contract MTKD-CT-2004-517215) and Northumbria Water.

We thank Northumbria Water for providing anaerobic sludge and wastewater from the primary clarifier from Cramlington WWTP.

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