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ARTICLE

A microbial fuel cell sensor for unambiguous measurement of organic loading and definitive identification of toxic influents

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Microbial Fuel Cells can be used as sensors to measure organic load (measured as Biochemical Oxygen Demand; BOD) in aqueous wastestreams, but electrcial current output can also be affected by the presence of toxic compounds. Just as BOD is a non-specific measure of organic loading the toxic response is not differentiated for specific toxicants and thus enables a generalised toxicity level to be monitored. To date combined BOD and toxicity sensing with a MFC has not been possible without comparison with reference feeds of synthetic solutions, as a decrease in signal caused by a decrease in BOD could not be distinguished from those caused by the presence of a toxicant in the waste stream. In this work, an innovative solution using a three-stage MFC sensor configuration was developed to explicitly distinguish BOD-related and toxicity-related signal decrease. A decrease in BOD led to an initial responses in the third, followed by the second and first stage MFC. For a decrease from 360 to 60 mg/L O_2 BOD_5 the decrease in normalised current density was 59%, 82% and 94% in the first, second and third MFC respectively. In studies using 4-nitrophenol as a model toxic compound, increasing 4-nitrophenol concentrations resulted in an approximately equal decrease in current in all MFC in the MFC array (63%, 66% and 74% respectively when exposed to medium containing 150 mg/L 4-nitrophenol). Thus the magnitude and ordering of the response allowed a decrease in signal from decreasing BOD to be distinguished from the presence of a toxic compound. Furthermore, the MFCs were capable of recovering to previous performance levels within hours of acute exposure to toxicity. The multi-stage MFC configuration therefore enabled truly combined BOD and toxicity sensing with enhanced detection capabilities without the need for a defined or synthetic reference feed.

Water impact

Online microbial fuel cell-based toxicity sensors could protect biological treatment plants by early detection of harmful toxic influents without being selective to specific toxicants. Conventional MFC sensors cannot distinguish a decrease in signal caused by toxicity from a decrease in BOD. Our multi-stage MFC-based sensor elegantly solves this problem, and offers definitive online toxicity detection using bacteria enriched from wastewater.

1 Introduction

To ensure environmental and health security for downstream water users real-time water quality monitoring is becoming a necessity. Increasing water consumption worldwide and incoming regulations such as those from the European Union[1] and Indian Central Pollution Control Board[2], will drive demand for real time monitoring technologies. Organic loading (e.g. Biochemical Oxygen Demand; BOD) and toxicity are important water quality parameters which are difficult to measure on line and conventional measurement methods are labour-intensive and time-consuming which prevents their use for real-time monitoring.

The faradaic current or charge generated from a Microbial Fuel Cell (MFC) coupled to consumption of labile organic carbon by the anodic biofilm can be correlated to the concentration of substrate consumed. This recent technological advance has led to development of MFC-based sensors for detection of several environmental factors[3,4] including BOD, specific substrates, dissolved oxygen and microbial activity.[5–8] Conversely, inhibition of the electrical current in MFCs (by external influences) has enabled sensors for toxicity and specific heavy metals.[9,10]

MFCs have shown potential for application as low-cost, real-time water quality sensors for estimation of BOD and toxicity and offer the possibility of monitoring both parameters with a single sensor.[11–14] A major issue for combined monitoring systems is that in order to accurately detect a change in one

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parameter (e.g. toxicity) the other is required to be fixed (e.g. BOD) and vice versa[15]. This has led to development of complex sensors which require frequent or parallel feeding with synthetic feeds (e.g. containing a known BOD concentration) to attribute response specifically to one or the other of the measured parameters, detracting from the low-cost and real-time monitoring benefits offered by MFC-based sensors. Stable detection of Cu(II) has been demonstrated in a system where BOD levels were artificially supplemented with glucose and acetate[16,17], however Tan et al.[17] noted that this reduced bioavailable toxicant and thus reduced sensor sensitivity.

We have reported previously that using multi-stage hydraulically-connected MFCs can extend the sensing range for organic material/BOD[18]. In the present study, we demonstrate an innovative approach to combined BOD and toxicity monitoring in a single sensor using multi-stage MFCs. This allowed explicit differentiation of signal decrease due to the presence of a toxic substance from signal decrease due a drop in organic load without requiring a separate reference feed with defined organic load or amendment with the toxic substance being measured.

2 Methods

2.1 Synthetic Wastewater Medium

The feed used to test the sensor was a phosphate buffered medium containing glucose and glutamic acid (GGA)[18]. This uses the same carbon source as the standard medium used to calibrate and test conventional BOD_5 assays[19]. In order to change the organic loading, the GGA concentration was varied between 100–2000 mg/L (60–1199 mg/L O_2 BOD_5). For toxicity tests, 10–150 mg/L 4-nitrophenol (4-NP; Sigma-Aldrich), as a model toxic compound, was added to 360 mg/L O_2 BOD_5 (600 mg/L GGA) medium before making up to the required volume with deionised water. 4-NP is commonly used as a model compound to test a range of toxicity assays[20].

2.2 Microbial Fuel Cells

10 mL single chamber MFCs were assembled with a carbon cloth anode (effective area (A_{Eff}) of exposed electrode not covered by gasket = 4.91 cm^2), 0.5 mg/cm^2 Pt gas diffusion cathode and Fumapem F-930 cation exchange membrane (FuMA-Tech), operated with an external resistance (R_{Ext}) of 305 Ω. The cell voltages were recorded using a NI-USB 6225 datalogger and LabVIEW SignalExpress data acquisition software (National Instruments). The current was calculated from $I = V/R_{Ext}$ and current density by dividing by A_{Eff}.

2.3 Single-pass, continuous flow system

A three-stage array of MFCs was constructed in triplicate in the same configuration as described in detail in our previous study.[18] Briefly, each feed line comprised a five-litre medium bottle containing up to 5600 mL synthetic wastewater medium (without 4-NP) leading to a sterile drip chamber, a peristaltic pump and then past a UV lamp (to minimise upstream contamination). Medium was pumped at maximum 1.24 mL/min into the three-stage MFC array and to a waste collection vessel in single-pass mode. In this study, three-way valves were installed upstream of the peristaltic pump (Figure S1, position 3-W M; ESI) to allow the medium feed to be switched to a feed with different BOD levels or containing toxicant, in order for the sensor response to acute BOD changes and toxic shock events to be tested. Valves were also installed downstream for sampling after the final MFC (Figure S1, 3-W 3; ESI).

2.4 Assessing inhibition due to very high BOD levels

The MFCs response to substrate inhibition was assessed as it is a well-known microbiological phenomenon involving the inhibition of bacteria at excessive substrate concentrations[21–23]. To assess the effect at high levels of glucose and glutamic acid (e.g. 1199 mg/L O_2 BOD_5) the medium was changed from a low BOD concentration (e.g. 150-300 mg/L BOD_5) to a high BOD concentration using the procedure described in section 2.3.

2.5 Simulation of an acute toxic event

For testing of inhibition events due to the presence of a toxic chemical, the BOD_5 was maintained at 360 mg/L O_2 BOD_5 (600 mg/L GGA) in the five-litre medium bottle and events were simulated by switching the peristaltic pump inlet feed using the 3-W M valve (Figure S1, ESI) from the 5-litre bottle to a 500 mL bottle containing the toxic compound to be tested. Acute exposure tests were conducted with medium containing 4-NP pumped for 90 minutes before reconnecting the feed with no 4-NP present and monitoring the recovery of current generation. Experiments were conducted starting with the lowest concentration of 4-NP to ensure that the maximum data could be amassed in the event of total biofilm death due to exposure to the toxic compound. In all tests one of the replicate MFCs arrays (feed line B, Figure S1, feed line B ESI) was used as a control and the feed was not switched from the non-toxic 360 mg/L O_2 BOD_5 medium fed from the five litre bottle. The exact same procedure was used for testing the response to acute decreases in BOD from 500 mL bottles containing medium with different levels of BOD without 4-NP. It should be noted that feed B was used as a control for experimental purposes only and is not required to distinguish toxicity from a drop in BOD in this system.

2.6 Analytical Methods

BOD_5 tests were performed following the APHA standard procedure[19]. From four 1/250-diluted replicates of each sample, a single initial dissolved oxygen (DO) measurement and three final DO measurements were taken after incubation at 20 °C for 5 days. A conversion ratio of 0.599 BOD_5/GGA was measured and used to calculate BOD_5 values from known medium GGA concentrations.

Chemical Oxygen Demand (COD) was determined from samples digested at 148 °C with potassium dichromate for 2 hours using a photometric test kit according to the manufacturer's instructions. Absorbance values at 605 nm were measured

using a Spectroquant Pharo 300 spectrophotometer (Merck Millipore).

4-nitrophenol was determined from samples adjusted to pH 3–4 by addition of 4 µl HCl (35%) to 1 mL sample and measuring its absorbance at 320 nm with a Spectrostar Nano spectrophotometer (BMG Labtech) in comparison to a calibration constructed using external standards.

2.7 Current Density Normalisation

The current density generated by each MFC was normalised to 100% to permit comparisons between cells irrespective of electrochemical performance (i.e. caused by cathode degradation[18]). For each medium cycle, the average stable current density ($\bar{I}$) was determined as the average current density during the period in which the first derivative (dI/dt; which approached zero as the current stabilised) was below 3% of the peak current density. Normalised, average stable current density ($\bar{I}/\bar{I}Max \times 100\%$) was thus calculated from the maximum, average stable current density ($\bar{I}Max$). Limit of detection[24] was calculated ($3.3 \times SD_{Res}/m$) from the residual standard deviation (SD_{Res}) about fitted (current vs concentration) calibration lines and the linear regression slope (m = sensitivity[11]).

3 Theory

Current density sensing principles must be established in order to identify water quality events based on the response of a MFC. With MFC sensing an increase in current can be generally attributed to increases in BOD, however it has been shown that presence of respiratory inhibitors can result in an increase in MFC current density when competing terminal electron accepting compounds are also present (as only the electrode can be utilised).[25] Prior to this study, an approach to distinguish decreases in current caused by decreases in BOD from those caused by the presence of toxic compounds without controlling one of these parameters had not been established.

As with any biosensor, various environmental factors (e.g. non-optimal pH or temperature, lower conductivity and higher dissolved oxygen levels) can also result in changes in the response (e.g. MFC current) which are not associated with the target analytes (e.g. BOD or toxicity). Yet these parameters are readily measured online using conventional electrochemical probes and can therefore be accounted for in calibration models.

Under typical operation of a three-stage MFC-based BOD sensor with non-toxic media, current generation was observed initially in the first-stage MFC[18]. This was followed by the second and third-stage MFCs as BOD was increased, the first-stage biofilm saturated and maximum current was reached; delivering partially-degraded effluent to the downstream MFCs (Figure 1a/b/c). It is this ordered response which proffers opportunities for sensing applications.

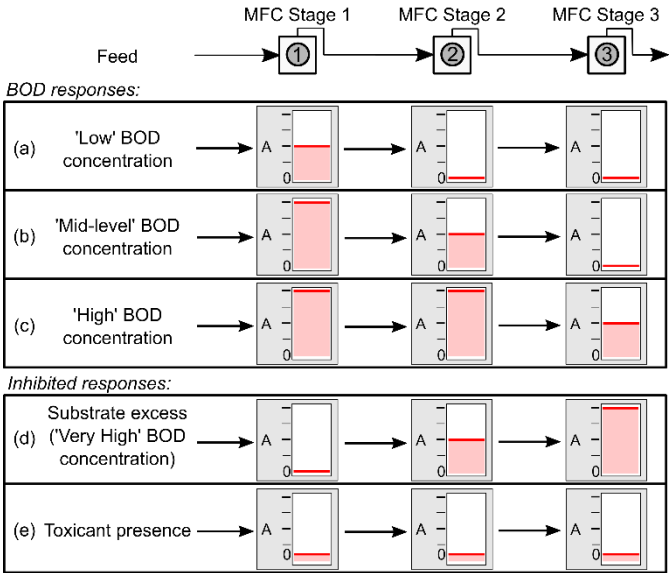

Figure 1: Schematic diagram of scenarios regarding BOD sensing and inhibition leading to decreases in current to be addressed with multi-stage MFCs. Examples are given for (a) 'low', (b) 'mid-level' and (c) 'high' BOD concentrations and inhibition events of (d) substrate excess and (e) toxicant presence. The relative, hypothesised electrical current (– in amperes (A)) response is indicated for each MFC in a three-stage hydraulic array.

3.1 Current response to increases in BOD

As BOD concentrations in a feed to a multi-stage MFC increase to levels above which the anodic biofilms can operate maximally, current decrease has been observed due to inhibition caused by substrate excess.[18,23] Here, the substrate acts as a biodegradable toxicant and inhibits electrogenic activity by anodic bacteria. As the first stage MFCs receive the highest dose of medium they would be expected to respond (be inhibited) the most. When substrate is consumed non-electrogenically (fermented) in the upstream MFCs, the BOD level would decrease to non-inhibitory levels and thus less inhibition would be expected in the downstream MFCs. If the downstream MFCs were not saturated they would exhibit a current increase due to increased levels of BOD in the feed received. The current output observed during substrate excess events could therefore be characterised by current in stage 1 < stage 2 < stage 3 (Figure 1d).

3.2 Current response to decreases in BOD

Decreases in BOD of a feed to a multi-stage MFC configuration result in an ordered decrease in current with the initial and greatest current decrease observed in the final stage MFC (Figure 1c to 1b to 1a) i.e. current decrease in the order 3 > 2 > 1. This is likely because the third-stage cell 'starves' first as it receives the degraded effluent of the second and first-stage MFCs. Therefore, once BOD is lowered below the saturation concentration of the second and first stage MFCs, the current decreases in those cells as well (Figure 1b to 1a).

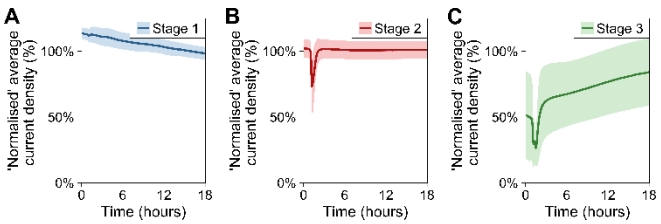

Figure 2: Average normalised current density response of the (a) first, (b) second and (c) third stages of MFCs to a BOD increase from 300 (0-1 hour) to 360 (1-18 hour) mg/L O_2 BOD_5 (estimated from GGA concentration) showing saturation in stages 1 and 2 and an increase in current in the third stage MFC. The inflection point at approximately 1 hour was when the medium replacement occurred. Shaded bands are ±SD from triplicate cells.

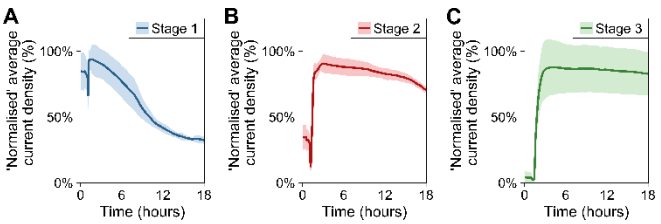

Figure 3: Average normalised current density response of the (a) first, (b) second and (c) third stages of flow-mode MFCs to a BOD increase (medium bottle replacement) from 150 (0-1 hour) to 1199 (1-18 hour) mg/L O_2 BOD_5 (estimated from GGA concentration) showing current decrease in first stage response. The inflection point at approximately 1 hour was when the medium replacement occurred. Shaded bands are ±SD from triplicate cells.

3.3 Presence of toxic compounds

When a toxic compound is present in the feed, the first-stage MFCs would receive the highest dose. However, for non-readily biodegradable toxicants no consumption would occur and the toxicant concentration would not decrease between the first and the final MFC. Consequently, the downstream MFCs would be expected to be inhibited to the same degree, resulting in a decrease in current more or less simultaneously, depending on the hydraulic retention time, in all MFCs in the hydraulically connected array. Inhibition caused by a non-biodegradable toxicant could therefore be identified by approximately equal decrease in current in each MFC, i.e. 1=2=3 (Figure 1e). Thus, the pattern of signal output from the three stages of the sensor distinguishes different types of toxicity event from a decrease in BOD (Figure 1d/e vs Figure 1c-b-a).

4 Results and discussion

4.1 Microbial Fuel Cell setup and operation

All MFCs were set up in a triplicate three-stage hydraulic array (Figure S1, ESI) and were inoculated on day 0 with effluent from batch-fed MFCs, initially inoculated with activated sludge from a wastewater treatment plant (Tudhoe Mill, Northumbrian Water Ltd). Following a 14-day batch-mode enrichment MFCs achieved peak current densities of 246.7 ± 12.3, 245.4 ± 21.6 and 265.7 ± 11.1 μA/cm^2 in the first, second and third stages respectively. At this time operation was switched from batch to continuous single-pass operation fed from sterile medium bottles. The experiments reported here were conducted following 126 days of operation with GGA medium (not containing any toxicants). During this 126 days of operation the sensor was tested for its ability to measure BOD5 at higher concentrations than a single-stage sensor[18]. Following this initial development of the sensor for BOD measurement we tested its utility as a toxicity sensor. The ability to operate the sensor for toxicity detection is reported here.

4.2 Multi-stage MFCs response to BOD increases

With a step-up change in BOD_5 from 300 to 360 mg/L the first and second stage MFCs were observed to remain at approximately 100% of the normalised current density (Figure 2A, 2B; 0-1 hour = 300 mg/L, 1-18 hour = 360 mg/L). The response of the third stage MFCs increased from 54% (± 37%) with 300 mg/L O_2 BOD_5 to 76% (± 24%) normalised current density with 360 mg/L O_2 BOD_5 (Figure 2C). The greatest degree of variability was observed with unsaturated MFCs (operating at BOD concentrations below levels that gave maximum MFC output). This could be attributed to replicate MFCs having small performance differences and larger relative variation with respect to low absolute values of output from the cells. Performance differences may have arisen during preparation (i.e. cathode ink application affecting MFC performance) or due to small flow rate differences (i.e. flow rate was measured as 1.09-1.24 ml/min) across channels A-C.

A feed concentration of 360 mg/L O_2 BOD_5 was chosen as a starting condition for current decrease tests (section 4.4 and 4.5). Preliminary tests determined that it was sufficient to saturate the first and second stage MFCs and give capacity in the third stage MFCs to increase or decrease current output based on inhibition and starvation treatments (Figure 2).

4.3 Multi-stage MFCs response to substrate inhibition at high BOD

The current density of the three-stage MFCs was also assessed in response to a medium concentration step-up change from 150 to 1199 mg/L O_2 BOD_5 (250 to 2000 mg/L GGA; Figure 3). At the starting BOD_5 of 150 mg/L O_2, the first stage MFCs were close to the upper limit of linearity and were almost saturated with an average normalised current density of 85% (±8%). The second and third MFC stages were not saturated at this concentration and had average normalised current densities of 36% (±10%) and 4% (±4%) respectively.

The upper limits of linear response for each stage had been previously determined (as 180, 300 and 420 mg/L O_2 BOD_5 respectively; Figure S2, ESI). It was therefore reasoned that medium containing 1199 mg/L O2 BOD_5, would be in excess of the capacity of each MFC individually or all three combined and would be expected to output the maximum current density at this concentration (100%). Immediately after medium replacement the normalised current densities in each stage were observed to increase close to 100% (Figure 3). However, despite the increase in concentration, a steady decrease in normalised current density was observed in the first stage MFCs to 32% (±2%) (Figure 3A, 18h). The normalised current density in the second stage of MFCs fell to 68% (±4%)(Figure 3B, 18h) and the third stage MFCs remained at a normalised current density of 83% (±13%) (Figure 3C, 18h).

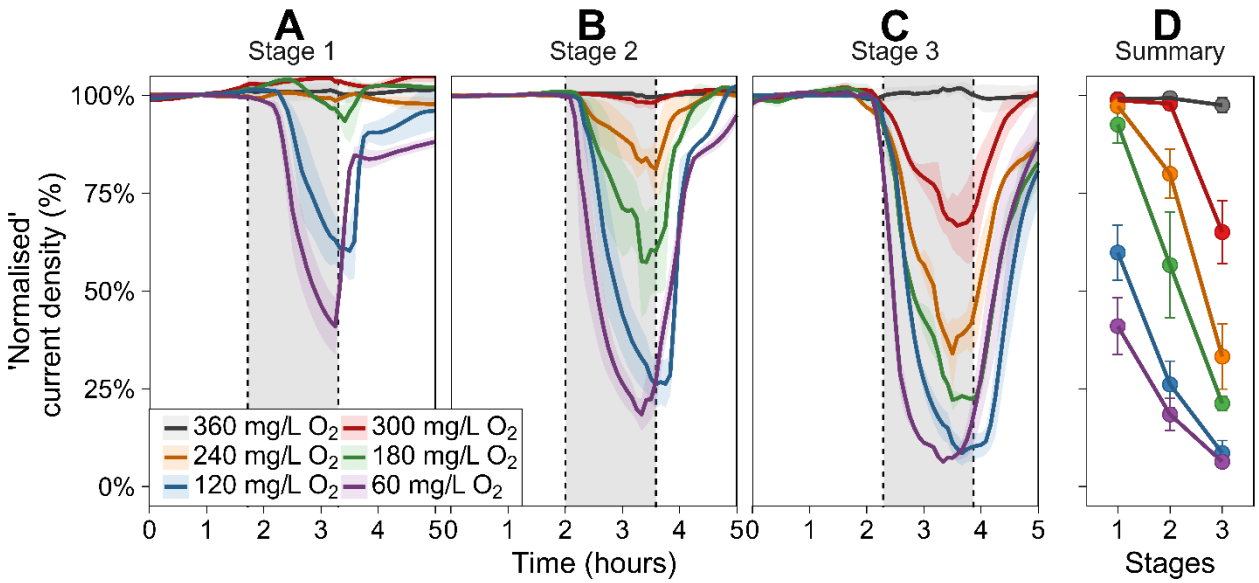

Figure 4: Normalised average current density response of three stages of MFCs to decreases in BOD_5 from 360 to 60 mg/L O_2 (estimated from GGA concentration). The dashed line-enclosed region is the period during which the low-BOD medium was fed to MFCs and current decreases were observed. Coloured, shaded bands and error bars are the range of values from duplicate MFCs in flow channels A and C (channel B is black control fed 360 mg/L O_2 BOD_5). Summary plot shows minima values from each current decrease test cycle at each MFC stage.

The trend in normalised current densities (32% < 68% < 83%) showed an increase down the hydraulic series which was concurrent with the theoretical prediction (Section 3.2, Figure 1d) with current in stage 1 < stage 2 < stage 3 (Figure 1d). This behaviour of the three-stage MFCs suggested that substrate inhibition occurred in the upstream MFCs but due to the biodegradability of the substrate, sufficient substrate had been removed to reduce the degree of inhibition in the downstream MFCs.

4.4 Multi-stage MFCs response to BOD decreases

The response of the multi-stage MFCs to decreases in BOD was systematically assessed by diverting the medium feed using the 3-W M three-way valve (Figure S1, ESI) from a 360 mg/L O_2 BOD_5 medium bottle to a solution of lower BOD concentration. Five lower BOD_5 solutions were tested, each over a 90 minute period, before switching back to a 360 mg/L O_2 feed (Figure 4). With a 300 mg/L O_2 (Δ60 mg/L O_2) BOD_5 medium, no loss of response was observed in the first and second stages of MFCs (i.e. remained at 100% (±2%)) (Figure 4A and B). The third stage MFCs normalised current density decreased to 65% (±8%) (Figure 4C). As the BOD_5 concentration was lowered further, greater decreases in current density were observed with the third followed by the second and first stage MFCs. When medium containing 60 mg/L O_2 (Δ300 mg/L O_2) BOD_5 was passed through the cells the normalised current density in the first, second and third stage MFCs was reduced by 59% (±7%), 82% (±4%) and 94% (±0%) respectively. The limit of detection for changes in BOD_5 was a difference of 43.7, 55.5 and 59.6 mg/L in the first, second and third stage MFCs respectively and the average sensitivity was 0.4% of the normalised current density per mg/L change in BOD_5.

The trend observed (shown by the Summary plot in Figure 4D), followed the same order as step-up BOD increases due to the first stage MFCs receiving the highest concentration of substrate, followed by the second and then third stages of MFCs. Thus the third stages responded first ('starved' first) as they were fed last. The trend in current decrease (e.g. 59% < 82% < 94% lost) showed greater decrease in output down the hydraulic series which was concurrent with the predicted order hypothesised earlier (output stage 3 < 2 < 1; Figure 1c-b-a). A calibration curve fitted to the Hill equation was produced by transforming the current density to current density loss (%) and the medium BOD_5 to the change in BOD_5 (ΔBOD_5; Figure S3, ESI). The BOD decrease current loss calibration was a reflection of the response observed with current generation.[18] With current loss (due to BOD decrease), the third stage MFCs responded first as the smallest decreases resulted in those cells receiving the least substrate.

4.4.1 Analysis of effluent from MFCs during BOD decrease events

Immediately following the restoration of the 360 mg/L O_2 BOD_5 feed samples were taken from each flow channel at the 3-W 3 three-way sampling valve (Figure S1, ESI). The flow path took 59 minutes at 1.24 mL/min to reach the 3-W 3 valves (passing through the first, second and third MFC stages), therefore after 90 minutes of testing low BOD solutions the effluent would have been flowing past this sampling point for 45 minutes. Samples were also taken of the fresh low-BOD medium prior to feeding to the MFCs (Figure 5).

With a decrease to 60 mg/L O_2 BOD_5 (102 mg/L O_2 COD), the effluent COD of the flow channels A and C was observed to decrease by 84 ±5%. The effluent COD reduced from 115 ±12 mg/L O_2 COD and 90 ±10 mg/L O_2 COD down to 24 ±1 mg/L O_2 COD and 19 ±7 mg/L O_2 COD in channels A and C respectively. The effluent from flow channel B, which was maintained as a control at a constant BOD_5 of 360 mg/L O_2 (613 mg/L O_2 COD), was quite variable between samples but remained approximately constant at 91 ±23 mg/L O_2 COD averaged over all treatments (Figure 5, inset).

Analysis of the effluent samples confirmed that the COD concentration was significantly lower in the outlet than the fresh medium bottle and therefore validated the theory that with decreases in feed BOD, the concentration of substrate received at the third stage of MFCs was less than in stage 1 and 2 as biodegradable substrate decreased through the hydraulic array. This led to the observed order in current decreases as the third stages of MFCs became depleted of substrate (starved) first and thus generated the least current (see Figure 1c-b-a).

4.5 Multi-stage MFCs response to toxicant presence

The response of the multi-stage MFCs to toxicant exposure was assessed with 4-nitrophenol (4-NP) present in the anolyte feed. The same method as testing BOD decreases was employed. Five toxicant-doped medium solutions were tested each over a 90 minute period each before switching back to a 360 mg/L O_2 feed to enable biofilm recovery (Figure 7).

No loss in current response was detectable with 4-NP concentrations of 10 and 25 mg/L in the three-stage MFCs. As the 4-NP concentration was increased to 75 mg/L and above in subsequent cycles, decreases in current density were observed in all MFC stages. When a 150 mg/L 4-NP-doped medium was passed through the normalised current densities of cells in the first, second and third stage MFCs were reduced to 37% (±29%), 34% (±15%) and 26% (±6%) respectively. There was no ordered trend observed along the hydraulic series (shown by approximately equal current decreases (inhibition) in each MFC stage in the Summary plot in Figure 7D). Thus, the decrease in current density response was distinct from BOD related current decreases (both substrate excess inhibition and BOD decreases).

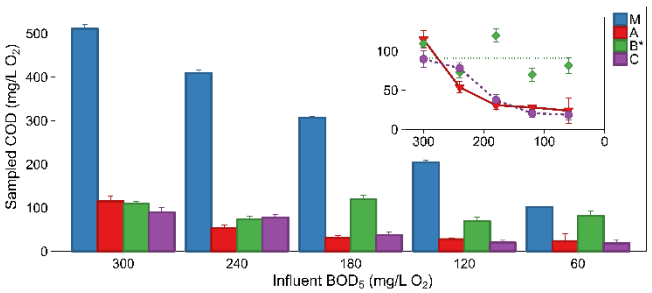

Figure 6: COD measured at each sampling point after 90 minutes of BOD decrease from 360 mg/L O_2 BOD_5 (estimated from GGA concentration; M = Fresh medium sample; A, B & C = Final effluents from 3-W 3 valve in each flow channel). Inset shows COD of effluents from each channel in a line graph. Error bars are the range from duplicate measurements. *Channel B was maintained at 360 mg/L O_2 BOD_5 as a control.

The limit of detection was 67.1, 89.6 and 76.4 mg/L 4-NP in the first, second and third stage MFCs and the average sensitivity was 0.5% of the normalised current density per mg/L 4-NP.

This observation suggested that the toxicant was not consumed within the MFCs and each stage received approximately the same dose of toxicant, resulting in the same degree of inhibition (as hypothesised for non-/slowly-biodegradable toxicants (see Figure 1e).

After the non-toxic medium was restored, the current density recovery following the 150 mg/L 4-NP toxic exposure was monitored (Figure S4, ESI). 11.5 hours after the 360 mg/L BOD_5 medium feed had been restored, the inhibited MFCs recovered to the same normalised current density as the control MFC (in flow channel B). This recovery period was much greater than the recovery times of up to four hours observed for the exposure tests with 4-NP concentrations below 112.5 mg/L (Table S1, ESI). Kim et al.[26] also observed increased recovery times following increased levels of toxicity (up to 8 hours following a 1 mg/L cadmium dosing). Additionally, this long response time was only observed with the first stage MFCs, this mirrors the recovery response following exposure to inhibitory substrate concentrations but in reverse order compared to the BOD decrease response (with low BOD the stage 3 MFC which is most 'starved' takes longest to recover). The fact the biofilm completely recovered to pre-toxic current density showed the resilience of the MFC-based sensor.

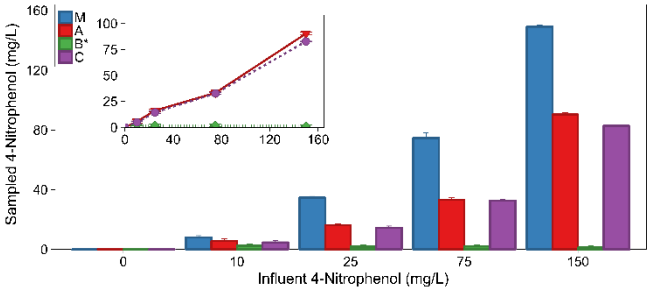

Figure 5: 4-Nitrophenol (4-NP) concentrations measured at each sampling point after 90 minutes of toxicant exposure (M = Fresh medium sample; A, B & C = Final effluents from 3-W 3 valve in each flow channel). Inset shows effluents from each channel in a line graph. Error bars are the range from duplicate measurements. *Channel B was maintained with no 4-NP as a control.

4.5.1 Analysis of Effluent from MFCs Exposed to 4-Nitrophenol

Samples were taken from the 3-W 3 valve (Figure S1, ESI) in each channel immediately following the restoration of the non-inhibitory GGA feed (360 mg/L O_2 BOD_5). Samples were also taken of the fresh 4-NP-doped medium prior to feeding to the MFCs (Figure 6).

The effluent 4-NP concentration of flow channels A and C was observed to decrease compared to the initial 4-NP-doped medium concentrations of up to 150 mg/L 4-NP.The 4-NP concentrations in effluents from channels A and C decreased on average by 47 ± 10% (Figure 6). The effluent from flow channel B, which was maintained as a control at a constant BOD_5 of 360 mg/L O_2 and no 4-NP, showed no absorption at 320 nm indicating that the values obtained for channels A and C could be attributed to 4-NP presence.

Analysis of 4-NP showed that there was a decrease in 4-NP concentration through the hydraulic array of MFCs. 4-

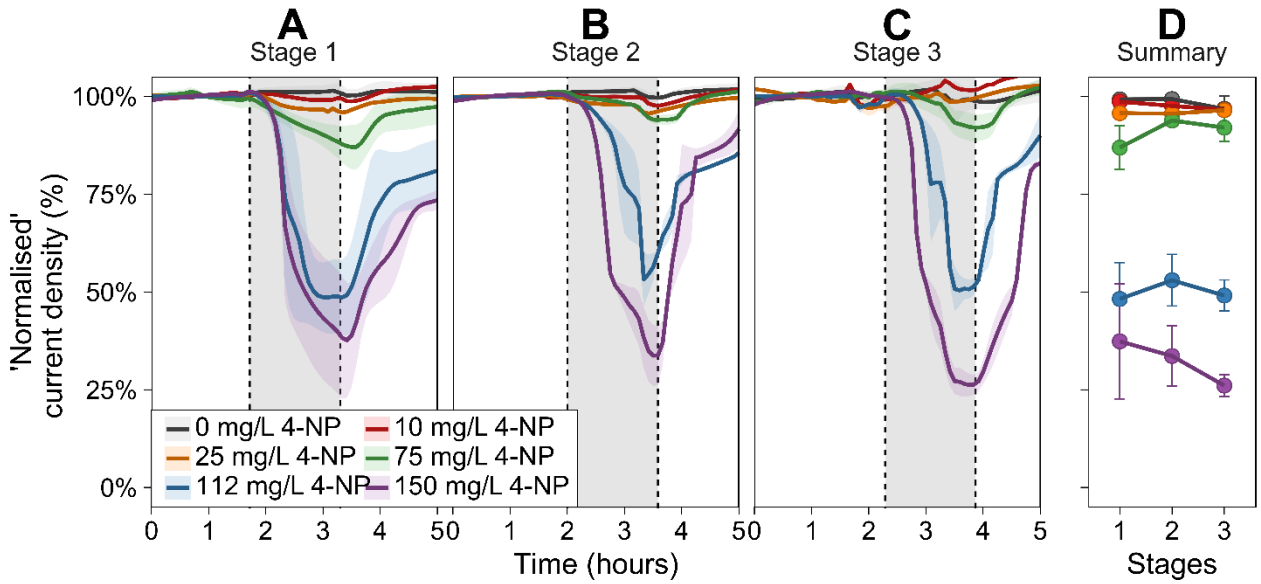

Figure 7: Normalised average current density response of three stages of MFCs to increasing concentrations of 4-nitrophenol (4-NP) from 0 to 150 mg/L. The shaded region enclosed by dashed lines is the period during which the toxicant-doped medium was fed to MFCs and current decreases were observed. Coloured, shaded bands and error bars are the range of values from duplicate MFCs in flow channels A and C (channel B is black control fed 0 mg/L 4-NP). Summary plot shows minima values from each current decrease test cycle at each MFC

nitrophenol is biodegradable in aerobic environments by bacteria which are able to completely convert 4-NP to carbon dioxide and water using the carbon and nitrogen for growth.[27] Bacteria and archaea in anaerobic methanogenic bioreactors that have previously exposed to 4-NP for long periods, showed degradation of up to 100 mg/L 4-NP after days to weeks of exposure.[28] However, the tests in the present study were conducted over the course of 90 minutes which was not expected to be enough time to select for 4-NP-degrading bacteria (by the time the feed had reached the 3-W 3 valve and sampling was performed after only 45 minutes of exposure had elapsed).

It is therefore thought that the decrease in 4-NP occurred due to non-biological processes such as dilution due to the hydraulic retention time (HRT), adsorption within the hydraulic array or the 4-NP may have been reduced to 4-aminophenol under anoxic condition[29]. When the 4-NP reached each 10 mL MFC chamber it is possible that the toxicant may have become mixed with non-toxic medium and thus diluted. With the HRT to reach the 3-W 3 valve approximately 45 minutes, and only 90 minute toxicity tests being conducted (for operational and biofilm recovery reasons), it is likely that steady-state mixing and equilibration of the toxic medium across the hydraulic array had not occurred at the time of sampling (at least 3HRT = 135 minutes would theoretically be required to ensure complete mixing). 4-NP persisting for longer within the hydraulic array would explain the prolonged recovery time of the MFCs with high concentrations of 4-NP. The observed current response of the MFCs did not appear to show that the 4-NP was being consumed/degraded, as an equal degree of inhibition was observed in each stage. For toxicants which are degraded as medium passed through the hydraulic array the response would be expected to be similar to the substrate excess where the first stage MFCs receive the greatest dose. Further investigations, including lengthier exposure periods and sampling after each stage of MFCs would be required to confirm the fate of 4-NP in the MFC-based sensor.

5 Conclusions

MFC-based sensors for measurement of organic load or toxic materials in wastewater are hampered by the inability to distinguish a decrease in current caused by a decrease in organic load from a decrease caused by the presence of a toxic material. If such a sensor was used for process control, the consequences of mischaracterising BOD and toxicity related incidents would be severe. Low BOD indicates that treatment process parameters (e.g. aeration rate) can be reduced, whereas high BOD effluents containing toxicants would require prompt notification of operators that higher levels of treatment are required or possibly intervention to protect the micro-organisms in biological treatment processes.

We have devised an MFC biosensor configuration that allows these two situations to be distinguished and furthermore distinguish substrate inhibition from inhibition caused by a poorly, or non-biodegradable toxicant. While the system hydraulic retention time (HRT) (from inlet of MFC1 to outlet of MFC3) is increased by having three MFCs hydraulically in series, the individual HRT of each cell is not impacted and thus the sensitivity and responsiveness remains the same as single-stage MFCs as the current from each cell is monitored individually.

If one considers that stage 1 MFCs behave exactly as a conventional single stage MFC-based sensor (Figure 8, top row only), there is no way to differentiate decreased current density

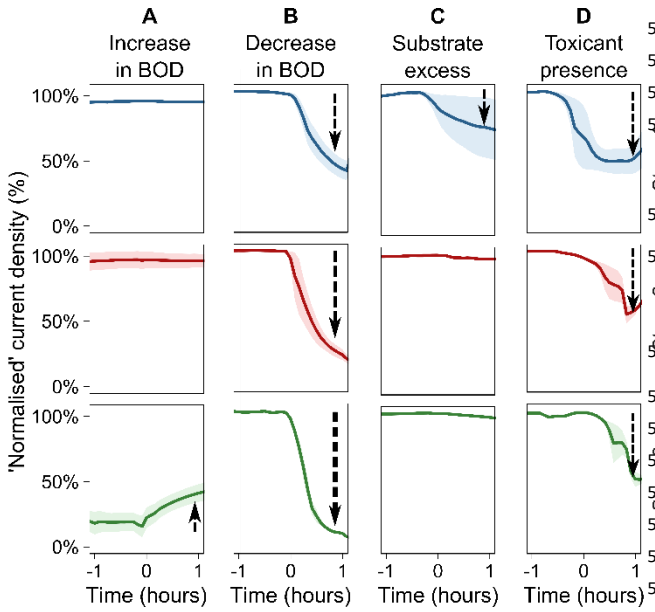

Figure 8: Representative examples of the average current densities (shaded region is ±SD) obtained from multi-stage MFCs to scenarios which would not be distinguishable with single-stage MFCs (i.e. row 1 only). (A) BOD increase can be detected by response of third stage MFCs (which are not at 100% saturation). It is also possible to distinguish between decreases in current density due to (B) BOD decrease (current decreases in stage 3 > 2 > 1 order), (C) substrate excess (current decreases in 1 > 2 > 3 order) and (D) toxicant (4-NP) presence (equal current decreases in all stages). t = 0 is the approximate point at which the event occurred.

caused by BOD decrease (Figure 8B) substrate excess (Figure 8C)or toxicant presence (Figure 8D). The multi-stage MFCs however exhibited an ordered response, which can be used to explicitly differentiate these three different types of event.

- Decreases in BOD_5 led to a decrease in current density with downstream (third stage) MFCs receiving the lowest concentration of substrate and therefore 'starved' first (confirmed by COD measurements) (Figure 8A).
- Increases in BOD_5 to excessive levels of substrate (e.g. 1199 mg/L O_2) resulted in inhibition and loss of current in upstream MFCs first as the 'strongest dose' was received there (Figure 8B).
- With 4-NP toxicant presence no ordered response was observed, with each stage of MFC being equally inhibited by the toxicant as the medium passed through the hydraulic array (Figure 8C).

MFC-based toxicity sensors were shown to be capable of full recovery to pre-toxic current densities, however recovery from highly toxic 'shock' events could take considerable time (e.g. 11.5 hours following a 150 mg/L 4-NP dosing) to recover to 100% normalised current density. We have a reported an innovative method for distinguishing toxicity and BOD-related responses using a MFC-based sensing system. Based on the ordered responses observed in the presence of a toxic substance (first stage MFC responds first) or with a change in BOD (last-stage MFC responds first) these different types of event are distinguishable using this framework. Nevertheless an important limitation is that the sensor would not be able to determine the presence of a toxicant under very low BOD conditions, as at low BOD there would be minimal electrical output from the sensor. The presence of acute toxicity presence during an acute change in BOD could also lead to complex outputs from the sensor that would require further systematic testing to resolve.

Conflicts of interest

There are no conflicts to declare.

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