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Microbial fuel cells for inexpensive continuous *in-situ* monitoring of groundwater quality

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ABSTRACT

Online monitoring of groundwater quality in shallow wells to detect faecal or organic pollution could dramatically improve understanding of health risks in unplanned peri-urban settlements. Microbial fuel cells (MFC) are devices able to generate electricity from the organic matter content in faecal pollution making them suitable as biosensors. In this work, we evaluate the suitability of four microbial fuel cell systems placed in different regions of a groundwater well for the low-cost monitoring of a faecal pollution event. Concepts created include the use of a sediment/bulk liquid MFC (SED/BL), a sediment/sediment MFC (SED/SED), a bulk liquid/air MFC (BL/Air), and a bulk liquid/bulk liquid MFC (BL/BL). MFC electrodes assembly aimed to use inexpensive, durable, materials, which would produce a signal after a contamination event without external energy or chemical inputs. All MFC configurations were responsive to a contamination event, however SED/SED and BL/Air MFC concepts failed to deliver a reproducible output within the tested period of time. BL/BL MFC and SED/BL MFCs presented an increase in the average current after contamination from -0.75 ± 0.35 μA to -0.66 ± 0.41 μA, and 0.07 ± 0.2 mA to 0.11 ± 0.03 mA, respectively. Currents produced by the SED/BL MFC (SMFC) were considerably higher than for the BL/BL MFCs, making them more responsive, readable and graphically visible. A factorial design of experiments (DOE) was applied to evaluate which environmental and design factors had the greatest effect on current response in a contamination event. Within the ranges of variables tested, salinity, temperature and external resistance, only temperature presented a statistically significant effect ($p = 0.045$). This showed that the biosensor response would be sensitive to fluctuations in temperature but not to changes in salinity, or external resistances produced from placing electrodes at different distances within a groundwater well.

1. Introduction

Shallow groundwater wells, are the main source of drinking water in many rural and peri-urban communities (Schmoll et al., 2006). The quantity and variety of shallow wells located in such communities make them more readily accessible than private or government operated deep boreholes, but shallow wells are more susceptible to faecal contamination, which is often due to leaching pit latrines (Schmoll et al., 2006). For this reason, online monitoring of water quality in shallow wells, in terms of faecal pollution, could dramatically improve understanding of acute health risks in unplanned peri-urban settlements. More broadly, inexpensive online faecal pollution risk monitoring is also highly relevant in the context of managed aquifer recharge via the infiltration of either stormwater or treated wastewater into the subsurface for aquifer storage and recovery (Vanderzalm et al., 2013; Page et al., 2015).

Determining groundwater faecal pollution is conventionally achieved by monitoring the presence of thermotolerant coliforms or faecal coliforms, which requires incubation of samples in off-site laboratories. Conventional chemical tests may also give an indirect indication of faecal pollution through measuring the dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen

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demand (COD), organic and inorganic nitrogen and carbon content, turbidity or conductivity of water. Commercially available kits for water monitoring such as DelAgua, WaterSafe, Pro-Lab, LaMotte are reliable, accurate and may be powered by batteries or solar cells, making them suitable for most places. However, analyses performed using kits introduces other drawbacks such as high costs or hazardous chemical waste generation. In addition reagents are not always available, and the requirement of trained personnel onsite limits their usability for water quality testing by water users. Other methods available for detecting microbiological quality of water; i.e. faecal sterols, microbial source tracking, and other indicators such as *Clostridia*, or bacteriophages, present accuracy challenges (Murtaugh and Bunch, 1967; Tyagi et al., 2006). This is due to the high susceptibility of the coliforms to chemical disinfection and thus failing to correlate with other microorganisms that can cause diseases such as parasites, protozoan, and enteric viruses (Tyagi et al., 2006). Additionally, these indicators cannot simultaneously detect organic matter contamination which should be expected in faecal pollution. Thus, the development of affordable continuous measurement tools providing early warnings for significant pollution events and supplementary *in-situ* information on the quality of groundwater at low cost and low maintenance, by creating a signal from the water pollution itself, without external chemical or power input, is extremely challenging and of paramount importance.

Microbial fuel cells (MFC) are systems that consists of an anode and a cathode electrode connected by a resistance. In a traditional MFC system the cathode and anode are separated by an ionic exchange membrane. Electrons are transferred to the anode electrode, from the anaerobic dissimilative respiration of electroactive microorganisms. Electrons then flow through a circuit generating current and merge in the cathode electrode facilitating the reduction of electron acceptors, e.g. oxygen. As electroactive microorganisms in the anode are able to degrade organic matter, the magnitude of electrical current generation has been proven to be related to the organic matter content in wastewater (Yang et al., 2015), opening up new perspectives for their application as biosensors. MFC biosensors reported in the literature (Chang et al., 2004; Feng et al., 2008; Quek et al., 2015; Yang et al., 2015) consist of a closed reactor system. The anode electrode is kept in a vessel full of bulk liquid under anoxic/anaerobic conditions to enable anaerobic respiration by microorganisms. The cathode is usually under aerobic conditions, so that the cathodic electrode acts as electron sink, with oxygen applied as an electron acceptor. The cathodic electrode may be in direct contact with oxygen from air (not requiring a vessel).

MFCs have demonstrated a correlation between the electrical signal produced and organic matter present in wastewater as chemical oxygen demand (COD) (Feng et al., 2008), biological oxygen demand (Chang et al., 2004) or a pure substrate such as acetate (Modin and Wilén, 2012; Quek et al., 2015). Lately, improvements on biosensor performance have been achieved with micro-sized MFCs (Lee et al., 2015) or the use of specific types of bacteria (Quek et al., 2015). Most of these systems still require inoculation of the anode, a vessel, a membrane and the pumping of water through to the anode which increase their cost and maintenance. Logroño et al. (2016) reported the use of a terrestrial MFC biosensor which consisted of a single chamber reactor full of Adean Sediment where anode and cathodes were embedded in soil and kept far apart at open circuit. No membranes were used in the system but it still relied upon water pumping. It was found that at open circuit potential, the reactor responded quickly and accurately to low organic matter concentrations.

All the previously published systems rely on the anode being inoculated with bacteria in an anaerobic vessel. In this work, we propose to construct a MFC biosensor by embedding electrodes in different regions of the environment, representative of a shallow groundwater well, and not using a reactor or vessel or membrane, thus providing realistic templates of an in-situ MFC biosensor. The installation of carbon electrodes in environments such as the ocean has already been reported as sediment MFCs (SMFC) (Zabihallahpoor et al., 2015). The key advantages of these systems include the use of electroactive microbial species that inhabit sediments or aquatic environments (rivers, oceans) to produce an electrical current. For the first time, this paper evaluates the use of a SMFC for water quality monitoring, and proposes three novel ways of generating a galvanic system for monitoring a faecal pollution event (Fig. 1). MFCs were created using inexpensive, long-term, durable materials that will neither require maintenance nor energy input to generate a signal current which will decrease continuous monitoring costs and minimise maintenance. MFC devices are envisaged to be able to detect a pollution event in groundwater wells, which may then be further investigated using more conventional methods. To achieve this, different parts of the bulk liquid and/or sediment surrounding a groundwater well were used to evaluate applicability. The main disadvantage of having electrodes embedded in a natural system, is that they can be responsive to several variables and not just faecal pollution. For example, SMFC current and cell potential outputs have been reported to vary widely, not being able to reach a fast steady state (Mitov et al., 2015). Response to other factors is therefore evaluated here by selecting three variables: salinity (as conductivity), temperature and external resistance and their effect on the SMFC cell potential response. The first objective of this work was to observe a change in voltage/current production after the addition of faecal pollution on different MFC concepts embedded in a groundwater system. Following on from the initial concept screening, a factorial design of experiments (DOE) was then applied to evaluate how environmental parameters affect the bioelectrochemical response for the best of the previously tested designs. Hence, the second objective was to observe whether a change in other environmental variables significantly altered the previously observed MFC biosensor response to pollution. Finally, a third objective was to observe the performance of the MFC biosensor in the field.

Field tests were conducted in Dar es Salaam, the largest city and commercial centre in Tanzania. Dar es Salaam is one of the fastest-growing cities in Africa, after Bamako and Lagos (CityMayors, 2016). In 2011, about four million people lived in densely populated unplanned settlements. Many of these areas are located near river valleys, flood-prone areas and hill slopes. Servicing these areas is difficult due to the nature of the terrain, population density and layout of the settlements (WorldBank, 2013). The most common sanitation systems for households in unplanned settlements are pit latrines followed by septic tanks. It has been found that these are built and maintained by the owner of the house, or by a landlord. On-site sanitation is based on self-provision with regulation by the health departments of the municipal councils. The essential maintenance practice of these systems is faecal sludge emptying which is extremely difficult in unplanned settlements. As a result, some residents resort to unhygienic practice of emptying pit latrines and dispose of the contents in unacceptable manner. Other residents use the floodwater during the biannual rains to help empty the top part of their latrines. Both practices reintroduce sewage and pathogens into the immediate environment (BPD, 2006). This has compromised the quality of groundwater which is used for drinking; contributing to cholera outbreaks that have frequently caused significant mortalities in Dar es Salaam (Penrose et al., 2010; WorldBank, 2013). Experience has shown that waterborne disease outbreaks particularly in Dar Es Salaam City are usually triggered during the rainy season. This is thought to be caused by the washing of faecal matter from pit latrines into nearby

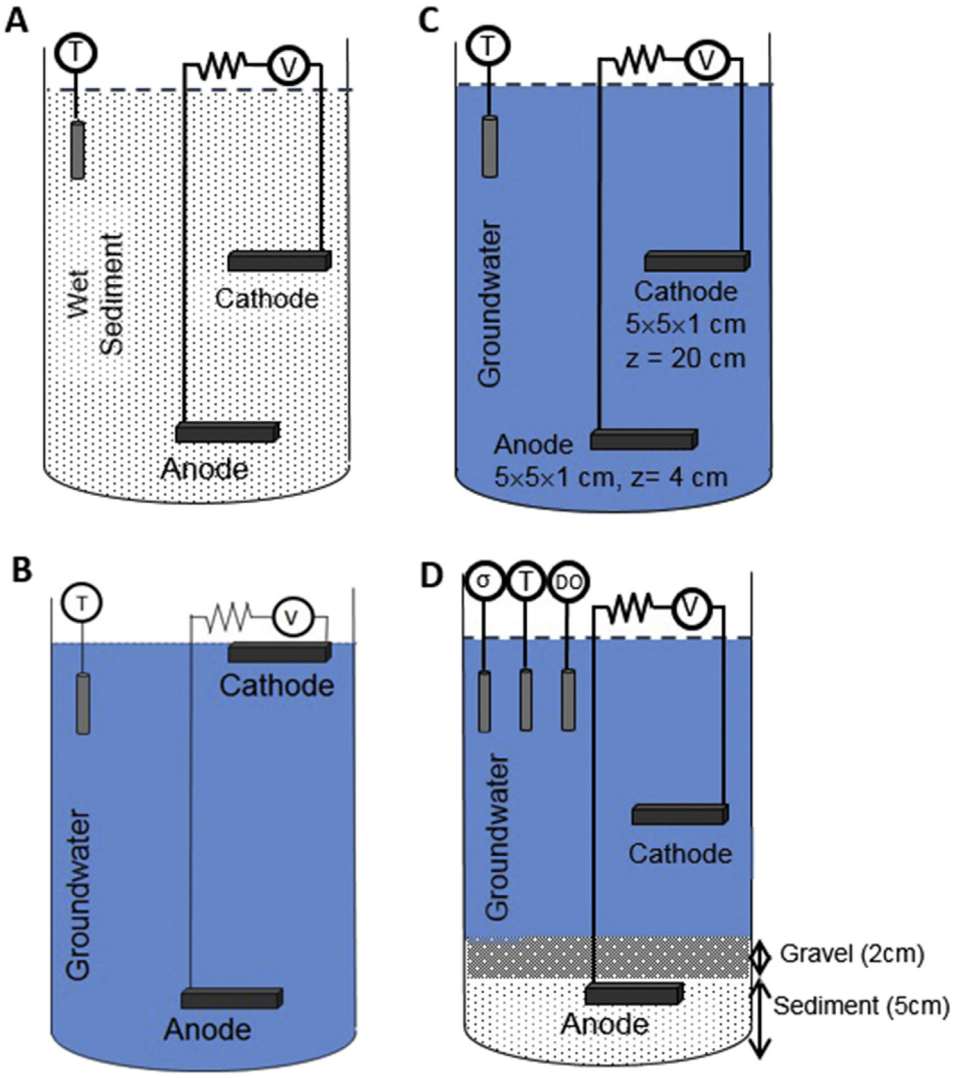

Fig. 1. MFC reactor concepts. A) SED/SED MFC (sediment/sediment MFC); B) BL/Air MFC (bulk liquid/air MFC); C) BL/BL MFC (bulk liquid/bulk liquid MFC); D) SED/BL MFC, SMFC (sediment/bulk liquid MFC). Sensors used to record data were T: temperature sensor; DO: dissolved oxygen sensor; σ: conductivity sensor; V: voltage sensor.

groundwater drinking water sources such as shallow wells, which has been noted in other countries (Furlong and Paterson, 2013), in this context, inexpensive groundwater quality monitoring equipment could help raise public awareness of waterborne hazards in shallow wells.

2. Methodology

2.1. Sediment collection and synthetic groundwater preparation

Sediment was collected from the Ouseborne River located in Jesmond Dene (54.9708°N 1.5883°W) and sieved (1.18 mm aperture) in order to remove large particles (e.g. stones, branches). The sediment collected had a pH of 6.6, an electrical conductivity of 246.4 ± 1.28 $mScm^{-1}$ and a Total Organic Carbon (TOC) of 7.93 ± 0.14 mg/L. Groundwater was made up to resemble the physicochemical parameters of unpolluted groundwater located in a chosen region of Tanzania where communities use several *in-situ* shallow and deep groundwater wells. Samples were taken from three groundwater wells of the region at different depths. Parameters from groundwater in Tanzania were used as a reference to determine the synthetic groundwater composition for the experiments (see Table 1).

2.2. MFC biosensor concepts

Alternative ways on how a MFC system could be incorporated into a groundwater well were analysed and four concepts derived. MFC cathode and anode electrodes were made of graphite felt (10 mm thickness, Olmec, UK), using a stainless steel mesh as the current collector. The two electrodes were connected using a resistor box set to 50 Ω, a low resistance that enables electron transport. Parameters such as pH was measured manually using a portable probe (Omega, UK). Conductivity, temperature and Dissolved Oxygen (DO) were continuously recorded using calibrated probes (Metter Toledo, UK). The four templates shown in Fig. 1 and summarised in Table 2, are described as follows: A) Sediment/Sediment MFC concept (SED/SED), where the cathode electrode was located in the soil close to the surface associated to the water inlet of the well, while the anode electrode was placed at the bottom of the sediment, associated with the sediment further away from the aquifer under increased anaerobic conditions; B) Bulk

Table 1
Groundwater characteristics. Initial parameters were collected from boreholes located in Dar es Salaam. A minimum 30 m depth was chosen to represent unpolluted groundwater, as shallower groundwater in the area is heavily impacted by faecal pollution. Reported range values were based on previously reported data (Bakari et al., 2012; Sappa et al., 2015).

	Borehole Depth			Reported Range	Synthetic groundwater
	80 m	30 m	60 m		
pH	5.90	6.80	6.60	5.9–7.4	7.2
Electrical Conductivity (μS/cm)	1177	1217	984	982–3300	1430
Ca^{2+} (mg/L)	52	70	44	18.2–380	28
Mg^{2+} (mg/L)	24	26	22.36	7–78	6
Na^{+} (mg/L)	185	193.5	179.4	179–450	71
K^{+} (mg/L)	3.72	3.76	1.54	1.5–52.4	1.6
Cl^{-} (mg/L)	245	240	185	185–463	151
HCO_3^{-}(mg/L)	35	47	61	35–1000	34
SO_4^{2-} (mg/L)	46.6	88.8	55.2	2.1–250	21
NO_3^{-} (mg/L)	1.3	1	1	1–208	0

Table 2
Experimental conditions for the MFCs concepts.

MFC Concept	Sediment	Bulk Liquid	Response	Specifications
SED/SED	1 L Wet River Sediment	N/A	Change in current produced	Reactor fully filled with sediment, the anode is buried at the bottom of the reactor, and the cathode is buried half way up the reactor.
Air/BL	N/A	1 L Synthetic Groundwater	Increase in current produced	Reactor filled with electrolyte, the anode is submerged in the electrolyte and the air cathode is floated on its surface, contacting both the air and electrolyte.
BL/BL	N/A	1 L Synthetic Groundwater	Increase in current produced	Reactor filled with electrolyte, and 2 electrodes are placed either side of the reactor.
SED/BL	0.5 L River Sediment	0.5 L Synthetic Groundwater	Change in current produced	Reactor half filled with sediment, half filled with electrolyte. The anode is buried in the sediment and the cathode submerged within the electrolyte.

liquid/air MFC concept (BL/Air) had the anode electrode placed at the bottom of groundwater and the cathode floated on the groundwater's surface to reduce oxygen within air, with this it was expected that no current will be produced until a pollution event occurs; C) The bulk liquid/bulk liquid MFC concept (BL/BL) had both electrodes submerged; D) Sediment/bulk liquid MFC concept (SED/BL) operated as a conventional SMFC where the anode was placed in the sediment and the cathode in groundwater; it was hypothesised that one electrode would be selected by microorganisms as cathode or anode to produce a current and allow bacteria energy gain. Each experimental system set-up was given a period of time to acclimatise. Acclimatisation was said to be reached once each biosensor concept produced a base current. After this period, the systems were contaminated with activated sludge to make a COD of 300 mg/L in the synthetic groundwater. A COD of 300 mg/L was selected as a plausible scenario for significant infiltration of leachate emanating from pit latrines into nearby shallow groundwater wells. Sludge in pit latrines has a reported COD exceeding 20,000 mg/L (WELL, 2007). A concentrated sample of activated sludge was sampled from a local wastewater treatment plant and a small volume was spiked to the well to make up the desired concentration (the volume added was less than 5% the total volume in all cases). After contamination, the change in voltage was recorded between 4 and 6 days. The biosensor response was taken as a change in MFC average currents obtained before and after a sudden contamination. The stated null hypothesis was a no significant difference between the initial and final average currents after a sudden contamination. The null hypothesis was analysed using the Minitab® 17 statistical analysis toolbox using a confidence interval of 95% for two-tailed t-tests.

2.3. Full factorial DOE

A 2^3 full factorial design was conducted in duplicate using SMFCs (Table S1 in supplementary information). The three factors investigated were a combination of SMFC (external resistance) and environmental (temperature and electrical conductivity) parameters. The response variable was measured as the mean change in cell voltage output after contamination. Each factor was investigated over two levels, a high and a low level. Temperature ranges were selected as 20 °C (low) and 30 °C (high), based on temperature fluctuations according to meteorological data in Tanzania (TMA, 2015). Salinity (as conductivity) was selected to reflect sea water intrusion to which many Tanzania wells are subject, especially during the decrease in aquifer levels in dry seasons. The number of ions were increased ten times from the synthetic groundwater based value, obtaining a change of conductivity from a low value of 1.43 ± 0.07 to a high value of 4.70 ± 0.45 mS s^{-1}. A small increase in external resistance was evaluated to determine if there would be response effects on changes from the wiring length required to connect electrodes together. The longer the wire the higher the external resistance, for this 100 Ω was taken as the low value and 200 Ω as the high value. Duplicate experiments were conducted by different researchers, however full randomisation was not achievable as the two temperature levels were systematically divided into blocks, per availability of one water bath. All single experiments were conducted using fresh sediment, groundwater as a synthetic electrolyte and clean autoclaved electrodes. Results obtained from the experiment were analysed using a parametric and non-parametric analysis in Minitab® (Minitab 17 Statistical Software). For the parametric test, interactions among factors and residuals were evaluated.

2.4. Field tests

Initial field tests were conducted in Tanzania as it is a low income country with a rapidly increasing population. One SMFC and one BL/BL MFC sensors were placed in a family shallow well located nearby Mogo Street – Majumbasit Dar Es Salaam airport (Julius Nyerere International Airport) connected using a resistance of 50 Ω. The shallow well has a depth of 3 m and receives different amounts of groundwater according to the period of the year. Two control experiments (One SMFC and one BL/BL MFC sensors) were also set-up nearby in vessels that resembled the well outline and conditions. In the control experiments a contamination event was simulated by adding wastewater to a concentration of 300 mg/L of COD. The performance of sensors was initially monitored for a period of 60 days (July to September, 2016).

3. Results and discussion

3.1. MFC concepts

Groundwater initial conductivity and pH did not have a significant change after contamination ($p > 0.05$). During tests, pH stayed between 6.6 and 7.5 which was a suitable range for bacterial viability and catalytic performance. There was no significant change in conductivity although the mean increased from 1.51 ± 0.23 to 1.67 ± 0.19 after contamination ($p = 0.216$). DO levels were lower after contamination, however they remained above 2 mg/L indicating that oxygen was still available to be reduced at the cathode of MFCs.

Fig. 2-A shows the current obtained before and after contaminating SED/SED MFCs. Previous tests indicated that the system only produced a positive current when the sediment was wet, therefore a layer of groundwater on top of the sediment was kept to ensure a flow of electrons. A biosensor response to contamination was expected as a change in the average current output. MFC replicates responded to contamination but with a varied response. After contamination, SED/SED MFC2 showed a higher average current output (from 14.6 ± 10 μA to 35.5 ± 8 μA, $p = 0.000$), however this trend was starting before contamination, so the contamination effect was unclear. In contrast, SED/SED MFC1 had a significant decrease in average current output after contamination from 28.9 ± 14 μA to 17.5 ± 5 μA ($p = 0.000$). The contrasting effects of SED/SED MFCs indicated that variations produced due to contamination were not consistent for the tested period of time. In the experiments reported by Logroño et al. (2016) using a similar type of configuration, open circuit potential were maximised and stabilised after 15 days of feeding wastewater to the system. Wastewater used in their work to obtain a steady state current output, would not be appropriate in biosensors used for groundwater monitoring, however a longer period of time could have helped to stabilise the biosensor response and should be further evaluated.

Air/BL MFCs response to contamination was expected to start a flow of electrons shown as positive current (Fig. 2-B). As expected, no positive current was observed before a contamination event, as the synthetic groundwater neither had organic matter nor microorganisms. As soon as the contaminant was introduced in groundwater then faecal matter and microorganisms became available. Results obtained between replicates were contrasting. Air/BL MFC1 did not present significant differences in the average current output before and after contamination (mean of −0.394 μA, $p = 0.64$) while Air/BL MFC2 presented a significant statistical difference shown as an increase in mean current after contamination from −0.266 μA to 0.072 μA ($p = 0.000$). In MFC2 contamination provided microbes with substrate that may have helped catalyse organic matter oxidation and a flow of electrons. The response time for the Air/BL MFC2 was defined as the time taken to

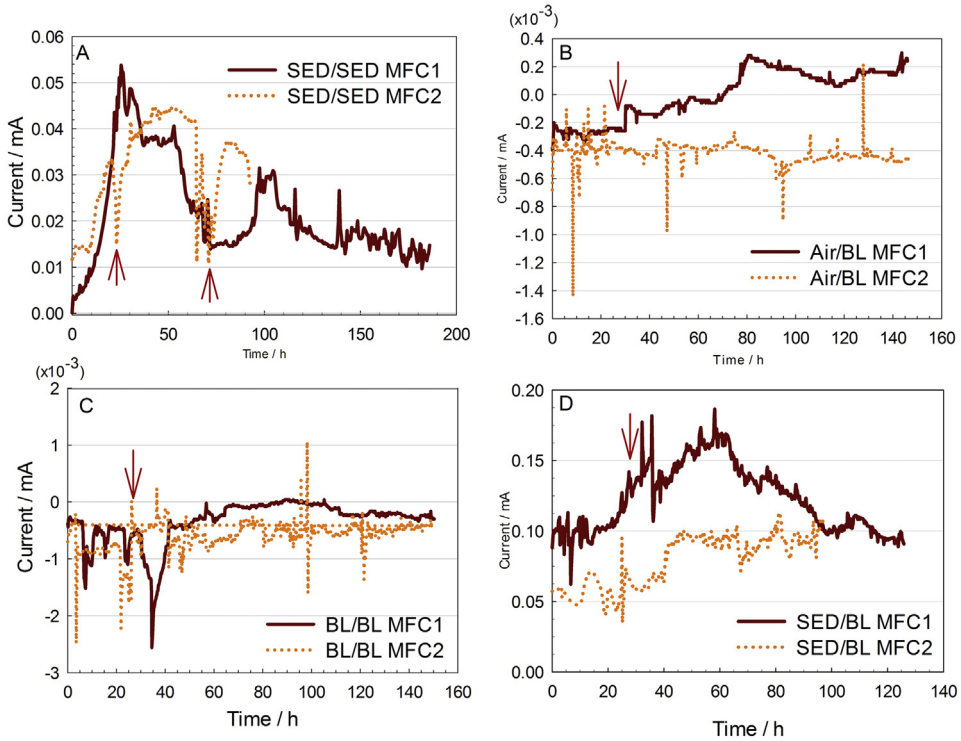

Fig. 2. Current response obtained from four distinct MFC biosensor concepts. Experiments were run at 20 °C, arrows indicated when the system was contaminated. MFC1 and MFC2 are duplicate experiments of the same system. Arrows indicate when the MFC system was contaminated, this was after 30 h for most cases, except in SED/SED MFC2 where contamination was done twice at different timings.

record a measurable current from the system, which would be 30 h. The failed reproducibility and low current output produced by Air/BL MFCs indicated that the contamination effect on this type of biosensor was inconclusive for the tested period of time.

BL/BL MFCs presented significant statistical differences in the average current ($p = 0.000$) after contamination despite an unclear response from a subjective impression (Fig. 2-C). BL/BL MFC1 mean currents changed from -0.91 ± 0.3 μA to -0.5 ± 0.1 μA, while for BL/BL MFC2 mean currents changed from -0.60 ± 0.3 μA to -0.28 ± 0.36 μA after a contamination event. Here both electrodes were exposed to similar oxic bulk liquid conditions (synthetic groundwater) with dissolved oxygen concentrations between 2 and 6 mg/L. As there was no clear anode or cathode for the system, the flow of electrons was unpredictable after contamination which was the possible reason for negative currents. The low currents obtained indicate that microorganisms close to the anode may have also used the available and competing oxygen as electron acceptor.

Currents produced by the SED/BL MFCs (SMFC) were the highest among all configurations tested (Fig. 2-D). Anode and cathode electrons in the SMFC were likely to behave as bio-electrodes as microbes present were able to colonize electrodes. The anode was in contact with soil microorganisms while the cathode was exposed to microbes through diffusion from the soil to synthetic groundwater. Microorganisms are likely to enhance the oxygen/reduction reaction occurring in SMFC electrodes. Holmes et al. (2004) demonstrated that biofilm growth on the cathode occurs from the diverse microbial community present in the aqueous environment. After contamination, both duplicate reactors had a significant positive change in average current produced from 0.07 ± 0.2 mA to 0.11 ± 0.03 mA ($p = 0.000$). The response time for both reactors to achieve a peak current was approximately 25 h. It could be that the already formed biocathode facilitated the use of different electron acceptors present in faecal pollution resulting in an increased current. It is well known that bacteria are able to facilitate the transport of electrons from electrodes for oxygen or metal reduction (Hasvold et al., 1997; Rhoads et al., 2005). In fact, algal microorganisms have long ago been shown to benefit the cathodic reaction by supplying oxygen to it (Berk and Canfield, 1964). The mechanisms of microbial electron transfer for this biosensor response should be further explored in the future. Currents produced by the SED/BL MFC (SMFC) were considerably higher than for the BL/BL MFCs, making them more responsive, readable and graphically visible. For this reason a full factorial design was applied to this concept in order to evaluate its response to environmental variables.

3.2. Evaluation of changing variables on the response to contamination by the SMFC

Currents and voltages produced were higher for reactors conditioned at 30 °C than for the ones at 20 °C (Fig. 3 and Fig. 4). From Fig. 3, it was also observed that, after contamination, SMFCs at 20 °C had a linear increase in current production for most cases whilst SMFCs at 30 °C had an immediate decrease in current after contamination followed by a slow increase. At 20 °C, the increase in SMFC current production after groundwater contamination (Fig. 3-a), was also reflected in an increase in the mean cell voltage (Fig. 4) and was statistically significant for most cases (p-values reported in Table S2). In contrast, changes in current production after contamination were not as evident for SMFCs current outputs at 30 °C (Fig. 3-b) but still cell voltage changes can be seen in the obtained mean and median cell voltage (Fig. 4) and were statistically significant (Table S2). Comparison of mean and median SMFC cell potential produced from reactors at 30 °C indicated a decrease

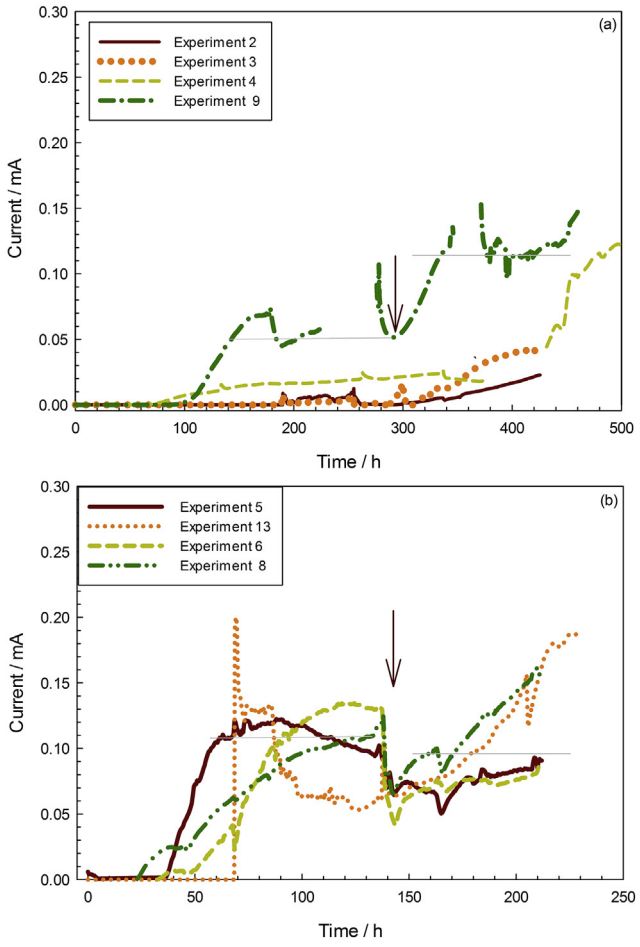

Fig. 3. Response of SMFC to different operating conditions. (A) SMFC ran at low temperature, (B) SMFC reactors ran at high temperature. Arrows indicate when the MFC systems were contaminated. Current response curves from all experiments can be found in the supporting information.

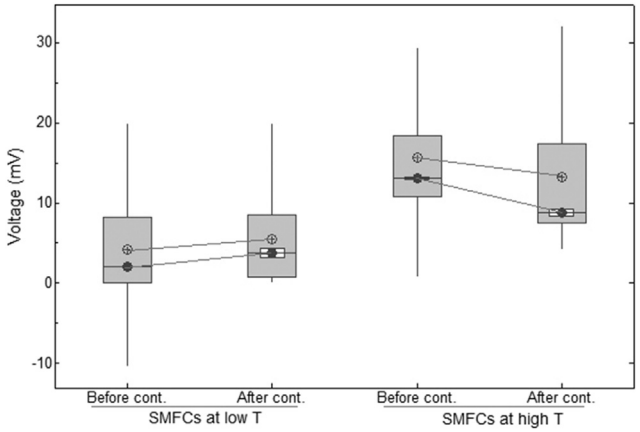

Fig. 4. Box plot showing the voltage medians and means from contaminated and not contaminated SMFC at different temperatures. Empty circles refer to mean values; dark circles are median values; white squares are the 95% confidence interval for medians. Grey squares are the range of values falling between the 1st and 3rd quartiles; vertical lines depict the range for all data. Each analysis for each condition used between 889 and 1560 individual voltage values.

after contamination (Fig. 4). The observed increases in current production may be either due, to bacteria colonizing the cathode as

a biofilm, facilitating electron transfer, or to the improved degradation of organic matter with a subsequent increase in DO levels. For the later, a persistent decrease in DO levels was recorded after contamination, but COD values were reduced by about 10 times after four days. This indicated that changes were more likely produced by bacteria facilitating electron transport than by an increase in DO availability. Therefore, the cathode electrode was likely developing a biofilm and behaving as a biocathode. The fact that SMFCs at 30 °C had a slower response than SMFCs at 20 °C could be due to the decrease in groundwater conductivity at high temperatures.

Table 3 depicts the significance that changing variables and interactions have on the average changes of voltage obtained by SMFC before and after an organic matter pollution event. Values used for the statistical test were taken from the delta changes in cell voltages obtained (Table S2). From the variables tested, only temperature presented a statistically significant effect on the response ($p = 0.045$). Residuals produced from the statistical test showed normal distribution with constant variance validating test assumptions and results (Fig. S2).

The significant effect of temperature found, was in line with previous literature for chambered MFCs where temperature change was analysed as a sole variable (Larrosa-Guerrero et al., 2010; Bakari et al., 2012; Li et al., 2013). Additional tested variables and their interactions did not represent a significant effect on the biosensor response to contamination for the selected ranges. This highlighted the primary role that temperature played in SMFC performance. Li et al. (2013) found that a decrease of temperature from 37 °C to 10 °C produced an increase in internal resistance of the MFC cell, resulting in a lower output current. As per Fig. 3, SMFC at 20 °C produced 10 to a 100 times less current than SMFC at 30 °C which can also be attributed to an increase in SMFC internal resistance. The increased cell resistance, was likely produced by the anode electrode; as groundwater conductivities decreased at high temperatures. This decrease would have negatively affected the cathode at high temperatures but this variable was not as significant as internal resistance triggered by the anodic side. There was also no significant effect in the biosensor response from changes in groundwater conductivity confirmed from the experimental design (Table S2). This again showed that anodic bacteria were likely producing increased cell voltages at high temperatures. Bacteria may have had an increased metabolic performance at 30 °C with an increased consumption of organic matter present in the sediment, an effect previously shown in MFCs using wastewaters (Larrosa-Guerrero et al., 2010).

In terms of biosensor performance, this study showed that the response would be very sensitive to fluctuations in temperature but not as sensitive to small changes in external resistance from placing electrodes at different distances within the groundwater well; or to changes in salinity within the ranges tested. Additionally, the current magnitude produced by the SMFC biosensor will also be dependent on the organic matter degradation in the anodic side, which may be constant once the anode electrode is buried in a specific soil.

Table 3
Response obtained from the factorial design.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	92.621	13.232	1.14	0.423
Linear	3	70.828	23.609	2.04	0.187
Temperature	1	64.947	64.947	5.61	0.045
Salinity	1	4.131	4.131	0.36	0.567
Resistance	1	1.75	1.75	0.15	0.707
2-Way Interactions	3	12.306	4.102	0.35	0.787
Temperature*Salinity	1	3.109	3.109	0.27	0.618
Temperature*Resistance	1	2.267	2.267	0.2	0.67
Salinity*Resistance	1	6.929	6.929	0.6	0.461
3-Way Interactions	1	9.487	9.487	0.82	0.392
Temperature*Salinity*Resistance	1	9.487	9.487	0.82	0.392
Error	8	92.534	11.567		
Total	15	185.155			

3.3. Field tests

Current outputs obtained from the two types of sensors installed in a family owned shallow well (Fig. 5-A) showed different results from laboratory tests. Here the BL/BL MFC1 biosensor and the SED/BL MFC2 biosensor produced both very low current outputs of $-1.81\ \mu A \pm 4.26$ and $0.21\ \mu A \pm 4.93$, respectively (Fig. 5-B). The reason for this could be derived from the different soil and water used on different locations. Even that water used in the laboratory simulated Tanzanian water parameters, it did not contain microbiota present in the well. Further characterization of the water and soil physicochemical and microbiota parameters would be required to fully explain differences between tests. Field observations showed that the performance of biosensors installed in shallow wells was also sensitive to water level fluctuations when the cathode electrode in the bulk of the groundwater was occasionally either exposed to air or submerged in the groundwater as the level of the groundwater receded or raised, respectively. In addition fluctuations were also observed during the extraction of water for domestic use. This should be considered on the final biosensor design. Regarding the control experiments (Fig. 5-B), a clear response can be observed when significant contamination occurs. This is shown as a marked increase in current production after contamination confirming laboratory observations. In this case, the BL/BL MFC4 produced a higher increase in current than the SED/BL MFC3. BL/BL MFCs were in contact with microbiota from groundwater while the groundwater used in lab-based BL/BL MFCs was synthetic. For this reason BL/BL electrodes located in the field tests could have been enriched with microorganisms, prompting an enhanced response to contamination. This shows the feasibility of the detection system for significant pollution events but also highlighted the need for more extensive field testing of the various proposed designs.

4. Conclusion

This work provided an initial assessment and proof-of-concept of *in-situ* MFC sensors for the non-expensive monitoring of faecal pollution in groundwater. From the different MFC concepts analysed BL/BL MFCs and SMFCs provided a response as a change in current or voltage output after a significant faecal contamination event. Laboratory tests indicated that temperature was an external variable significantly affecting the biosensor response and it would have to be considered in the interpretation of the final output. Furthermore, field tests highlighted the importance to develop a system that can accommodate fluctuations in the well water level. To progress beyond the proof-of-concept of the working prototypes proposed and evaluated in this study, further optimisations and the mathematical refining of the current response interpretation would be essential. In-situ MFC sensors have the potential to provide an affordable continuous measurement tool for groundwater quality at low cost and low maintenance, by creating a current response to significant faecal pollution without the addition of chemicals or an external power input.

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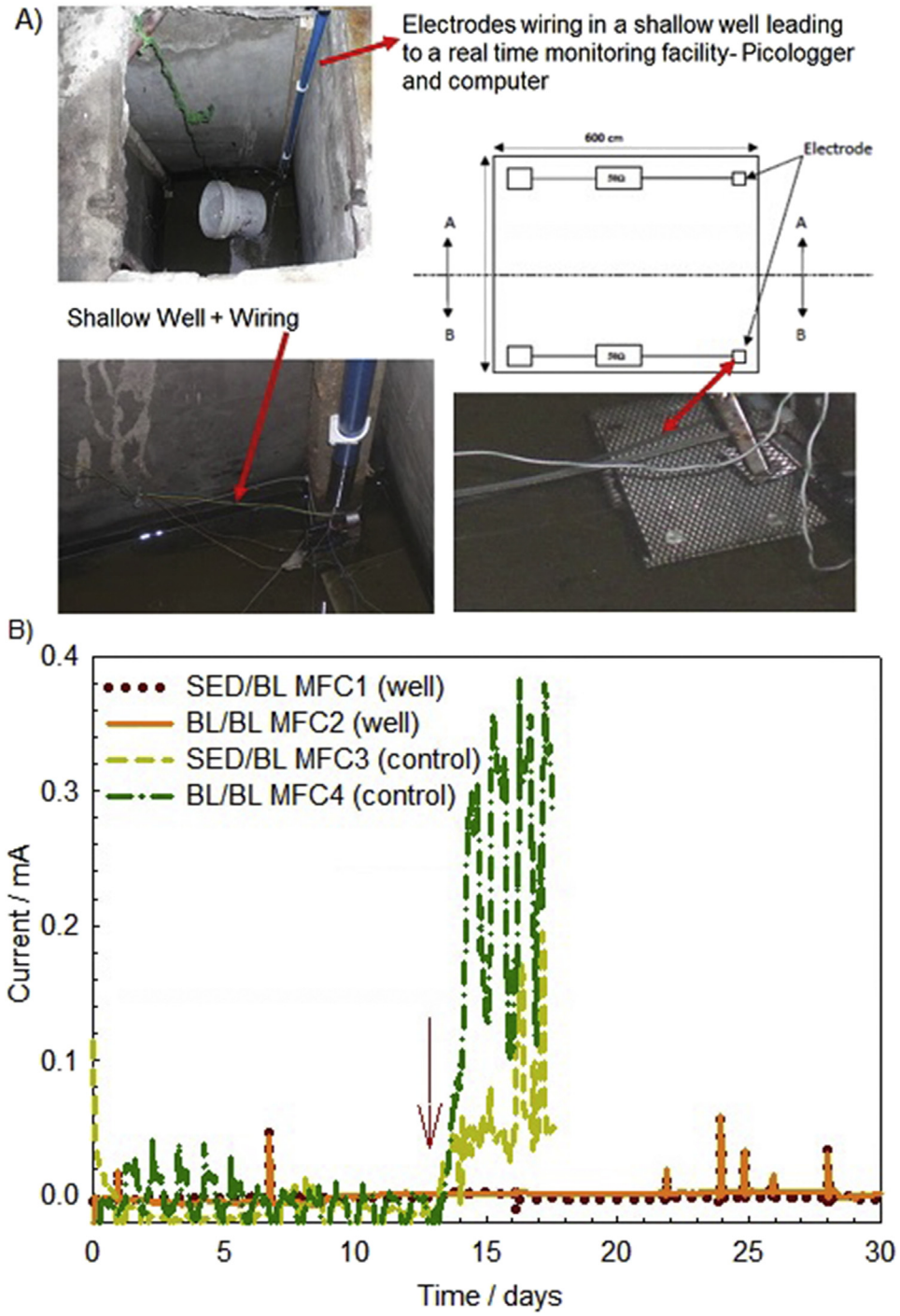

Fig. 5. Field test set-up and data output from the MFC biosensor monitoring. A) The diagram shows an aerial view of the system configuration and distance between sensing system and data collection system. B) MFC1 and MFC2 were biosensors placed on the well; MFC3 and MFC4 were control biosensors placed in a vessel simulating the groundwater well. MFC3 and MFC4 were located in a room close to the well and the arrow indicates when they were intentionally contaminated. Monitoring of the sensors contained in the well lasted for 60 days obtaining the same trend as for the period shown.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.03.040.

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