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Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12 month period



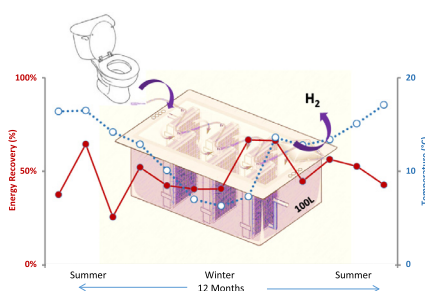
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HIGHLIGHTS

- A 100-L MEC (microbial electrolysis cell) was operated for a 12 month period.
- It produced hydrogen gas from raw domestic wastewater.
- The reactor worked at ambient temperatures ranging from 1 °C to 22 °C.
- Gas production rates declined with time but not with low temperatures.
- Average energy recovery was around half that needed for energy neutrality.

GRAPHICAL ABSTRACT



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ABSTRACT

A 100-L microbial electrolysis cell (MEC) was operated for a 12-month period fed on raw domestic wastewater at temperatures ranging from 1 °C to 22 °C, producing an average of 0.6 L/day of hydrogen. Gas production was continuous though decreased with time. An average 48.7% of the electrical energy input was recovered, with a Coulombic efficiency of 41.2%. COD removal was inconsistent and below the standards required. Limitations to the cell design, in particular the poor pumping system and large overpotential account for many of the problems. However these are surmountable hurdles that can be addressed in future cycles of pilot scale research. This research has established that the biological process of an MEC will to work at low temperatures with real wastewater for prolonged periods. Testing and demonstrating the robustness and durability of bioelectrochemical systems far beyond that in any previous study, the prospects for developing MEC at full scale are enhanced.

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1. Introduction

In 2005 the discovery was made that a microbial fuel cell can be turned into a microbial electrolysis cell (MEC) by adding a small supplement of electricity at the cathode to produce products such as hydrogen gas (Liu et al., 2005; Rozendal et al., 2006). This technology has led to considerable optimism about the potential of MECs to provide a sustainable means of treating waste organics, while converting the energy locked in wastewater

(Heidrich et al., 2011) into a more valuable form. Substantial progress has been made towards enabling the implementation of this technology: low cost durable alternatives to expensive components have been developed, such as platinum cathodes being replaced with stainless steel (Call et al., 2009a); alternative membrane materials have been trialled successfully (Rozendal et al., 2008); as have membrane-less systems (Call and Logan, 2008); anodes with greater surface areas have been found (Call and Logan, 2008); and methods to enhance the performance of these carbon anodes (Cheng and Logan, 2007). New cell architectures and configurations have improved performance of laboratory MECs from 0.0045 and 0.02 m³ H₂/m³ reactor/day (Logan, 2008) to 17.8 m³ H₂/m³

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reactor/day (Cheng and Logan, 2011) a 100–5000 fold increase in less than a decade.

However despite a large body of research, (5369 manuscripts with the search term “microbial fuel cell; microbial electrolysis cell; and bioelectrochemical systems” within Web of Science 01/04/2014), there is relatively little research into “real world” scenarios. If bioelectrochemical systems (BESs) are to fulfil their potential as full-scale wastewater treatment technology, then they need to operate under conditions found at real treatment plants. MECs need to: treat complex mixed waste with indigenous populations; operate throughout the year including low temperatures; work at scales typically treating millions/billions of litres of compositionally variable wastewater to an acceptable quality; and operate over substantial lifespans, perhaps decades. At present most laboratory studies are conducted with sterile acetate at 30 °C in a 10–100 mL reactor for a few days or weeks. There is a need to bridge this gap taking concept closer to application or reality.

The ability of MECs to digest a number of different complex substrates has been proven, e.g. domestic wastewater (Ditzig et al., 2007), piggery wastewater (Jia et al., 2010), potato wastewater (Kiely et al., 2011) and end-products of fermentation (Wang et al., 2011). However the performance levels achieved with these substrates falls well below that of simple substrates (Pant et al., 2010). It has also been shown that even with simple substrates performance and biology can vary significantly (Chae et al., 2009). A simple sterile substrate is not a realistic proxy for domestic wastewater. Using the power densities and Coulombic efficiencies of such studies at even pilot scale would lead to substantial under-design.

There is limited understanding of long-term performance of BES technology, with many papers not even stating the duration of experiments. There are studies, mainly with acetate, which indicate there is long term applicability though with a decline in performance (Lee and Rittmann, 2010; Zhang et al., 2013). Understanding the reasons for this decline, and engineering solutions for it will be a major issue in application of this technology, thus more studies of this nature are needed. Low temperatures have been studied in a few BES manuscripts, with varying conclusions. Cheng et al. (2011) found power was produced at 15 °C but not at 4 °C, Larrosa-Guerrero et al. (2010) operated reactors at 4 °C and 35 °C using a mixture of domestic and brewery wastewater, observing a decline in performance at the lower temperature. By contrast Jadhav and Ghangrekar (2009) operated an MFC's in a temperature range of 8–22 °C and found lower temperatures performed better, probably due to a reduction in methanogenesis;

Lu et al. (2012) also observed reduced methanogenic activity increasing performance at low temperatures. Wastewater treatment plants in temperate regions are designed to work down to 5–8 °C. However the majority of BES studies are still conducted in laboratories at a constant temperature of 30 °C.

The full scale application of MEC technology requires us to overcome numerous scale up issues including resistance, distance between electrodes, membrane placement, and overpotentials having a significant impact on performance (Hamelers et al., 2010). A small alteration in any of these factors can make an energetically viable system become not viable. Successful demonstration of MFC technology at scale has yet to be shown (Janicek et al., 2013), and attempts to scale MECs have resulted in low performance levels (Cusick et al., 2011; Heidrich et al., 2013).

If MECs are to be a viable and sustainable wastewater treatment option for the future then we need to gain an understanding of their long-term performance with real wastewaters at larger physical scales, longer temporal scales and realistic ambient temperatures. The aim of the research conducted here was to test the feasibility of this technology under these realistic conditions. A 100-L reactor was built and run for a 12 month period fed on raw domestic wastewaters at ambient temperatures in Northern England. By examining performance under these conditions, insight can be gained into the areas that need to be addressed to take BES technology from a laboratory concept to industrial reality.

2. Methods

2.1. MEC design

The MEC reactor configuration consisted of a 120-L polypropylene tank containing 6 separate MEC cell cassettes that function as individual electrolysis cells, all cells were placed in series within the wastewater tank as in Fig. 1. With the MEC cassettes in place there was a total working volume of the reactor of 88 L. The top of the reactor was sealed using a Perspex lid to maintain anoxic conditions within the wastewater/anode compartment. The individual cathode compartments within each MEC cassette had no headspace and were connected with a 50 mm length of 3 mm ID Polyvinyl chloride tubing (VWR International, UK) to a Tedlar™ 5-L gas bag to collect the hydrogen gas (Sigma Aldrich, UK).

Each cassette had two carbon felt anodes measuring 0.2 m wide by 0.3 m high and 10 mm thick (Olmec Advanced materials Ltd., UK), one on each side of the cathode compartment giving a total anode electrode surface area for the whole reactor of 16.4 m²/m³.

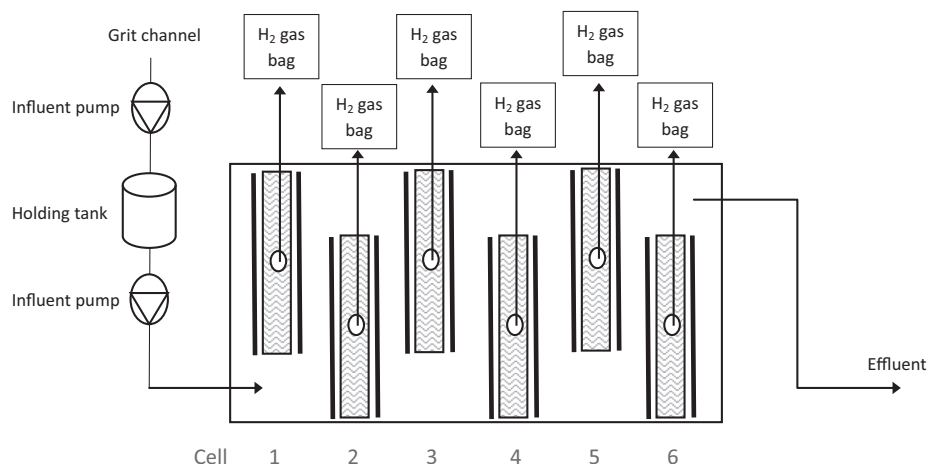


Fig. 1. Schematic diagram of the reactor set up.

A stainless steel mesh of each size to the anode (mesh size) was attached to each side of the anode to act as a current collector. The cathode compartment constructed using 10 mm thick plastic sheeting with a membrane at each of the flat sides, it had an internal size of 0.280 m by 0.200 m by 0.048 m deep, giving a volume 2.6 L. This cathode compartment was in the centre of each MEC cassette sandwiched between the two anodes separated on either side with a low cost membrane called Rhinohide® (Entec Ltd., UK). This membrane is just a separator, and not ion selective, it is likely there was some transfer between the catholyte and the anodic liquid.

The cathode electrode itself was composed of 20 g of stainless steel wool (Merlin Ltd., UK) giving a projected cathode surface area of 3.4 m²/m³. The wool was packed into the cathode compartment and a 0.8 m length of stainless steel wire wound into the wool and out of the side of the cathode to fix the wiring to. This gave an anode to cathode ratio of 5:1. The cathode compartment had a total reactor volume of 2.6 L. This was filled with 50 mM of sterilized (121 °C, 15 min) phosphate buffer, pH 7.0 at the start of the experiment and was not refilled during its operation. The cassettes were snaked through the reactor, acting as baffles to provide efficient and turbulent hydraulic flow to minimise settling and maximise anode contact time as per Fig. 1. Further details of this design can be found in [Supplementary information](#) and (Heidrich et al., 2013).

2.2. MEC operation

Power to cells 1–6 was provided using two bench top adjustable multichannel DC power supplies (PSM 2/2A, Caltek Instruments, Hong Kong), adding voltage to each cell. The voltage of each cell was measured across a 0.1 Ω fixed resistor (Farnell, UK) using ADC-16 Pico high resolution data loggers (Pico Technology, UK) every 30–60 min, and saved onto a computer. Using the voltage and resistance, current could be calculated using ohms law as previously reported (Heidrich et al., 2013).

Wastewater was pumped from the grit chamber at the wastewater treatment site using a Watson Marlow 520s peristaltic pump (Watson Marlow, UK) into an initial holding tank. The wastewater was then fed into the MEC reactor using another 520s peristaltic pump at 0.07 mL/min giving a hydraulic retention time of 1 day.

The cathodic gas was measured volumetrically on site twice weekly using a 1-L gas tight syringe (SGE Analytical, Australia) through manual withdrawal and normalised to standard temperature and pressure. Samples were also taken back to the laboratory within the gas bags for concentration analysis. Anodic gas was captured under the lid of the reactor. Anodic gas samples were taken for concentration analysis from gas sampling ports in lid and stored in 3-mL Vacutainers (Labco, UK) prior to analysis back at the laboratory.

During the acclimatisation period of the reactor set up, the applied voltage was increased in steps from 0.6 V to 0.9 V then finally 1.1 V, which was the voltage at which gas was produced and so was used throughout the duration of the study. Current densities during these changes increased also, and when plotted against input voltage indicate that within the reactor the inherent overpotential is around 0.6 V. The added voltage of 1.1 V used in the study actually equates to a potential difference of around 0.5 V between the electrodes, well below that required for abiotic hydrogen evolution from water of 1.23 V at 25 °C.

2.3. Reactor site

The reactor was located at Howdon wastewater treatment plant (Northumbrian Water Ltd., UK), which treats approximately 250,000 m³ of primarily domestic wastewater daily from the city

of Newcastle upon Tyne, UK. Wastewater for the reactor was taken directly from the end of the grit chambers, prior to primary clarification. This location was selected because of the practical and safety constraints of the site.

2.4. Analytical methods

Aqueous samples were taken twice weekly from influent and effluent ports located exterior to the reactor. All analysis was undertaken in duplicate within two hours of samples being taken. Total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) were measured using commercially available colorimetric COD test kits (25–1500 mg COD/L, Merck & Co. Inc., USA) on a Spectroquant Pharo 300 according to manufacturer's instructions (Merck & Co. Inc., USA). The soluble component was obtained by centrifugation at 4000 rpm for 10 min in a Sigma 3-16p centrifuge (Sigma Aldrich, UK) then filtered through a 0.22 μm Polyethersulfone membrane syringe filter (VWR International, UK). Conductivity in influent and effluent was measured using a EC300 portable conductivity probe (VWR International, UK) calibrated using two prepared NaCl calibration standards. Volatile Fatty Acids (VFAs) were determined using an Ion Chromatograph (IC) Dionex ICS-1000, equipped with an Ionpack ICE ASI column, using heptafluorobutyric acid as the eluent and tetrabutylammonium hydroxide as the regenerant. Anions were measured using an Ion Chromatograph (IC) Dionex ICS-1000 (Dionex, UK) equipped with an Ionpack AS 14A column, with carbonate as the eluent.

The pH and dissolved oxygen (DO) were measured continuously in the influent and effluent throughout the duration of the experiment using ProcessProbes (Broadley James, UK). Both probes were connected to a Model 30 Transmitter (Broadley James, UK) with the output voltage from the transmitter logged using Pico EL 037 convertor and EL 005 data loggers (Pico Technology, UK). Values were recorded every 30 min. Both probes were calibrated regularly according to the manufacturer's instructions (Broadley James, UK).

Hydrogen concentration was measured initially using a membrane inlet mass spectrometer (MIMS, Hyden Analytical, UK), then using a Thermo fisher trace ultra GC (ThermoFisher Scientific, UK) equipped with a Restek Micropacked 2-m column (Restek, UK), Internal diameter 1 mm, 1/16" outer diameter, with a thermal conductivity detector (TCD) using argon at 40 PSI as the carrier and reference gas. A series of hydrogen standards (Scientific Technical Gases, UK) with varying concentrations were first injected to provide a calibration curve using a 100-μL gas tight syringe (SGE Analytical, Australia) followed by samples from both the cathode and anode. All analysis was undertaken in triplicate.

Methane and carbon dioxide analysis was undertaken on a SRI 8610C GC (SRI instruments, USA) equipped with a flame ionisation detector (FID) and methanizer packed with a nickel catalyst for conversion of CO₂ to CH₄ prior for detection. The column was a 6-inch packed silica column, held isothermally at 80 °C for 200 min, with hydrogen as the carrier gas.

2.5. Performance calculations

The calculations for energy recovery, coulombic efficiency and substrate efficiency were performed as described previously (Heidrich et al., 2013).

2.6. Statistical analysis

Statistical analysis using a Pearson's correlation and Spearman's rank rho correlation was carried out using Minitab 15 (Minitab Inc., USA).

2.7. Molecular methods

Anode samples were taken for molecular analysis during the decommissioning process at the end of the year, using a sterile boring device into the anode felt. These samples were placed immediately in sterile filtered phosphate buffer and frozen. DNA extraction of the anode samples was carried out using BIO 101 FastDNA Spin Kit for soil (MP Biomedical, USA) according to manufacturer's instructions. A sterilized scalpel was used to slice the carbon felt into small pieces, the section was then added to a lysing matrix tube and the weight of each sample was recorded. Each tube contained 0.41 ± 0.02 g of anode. The manufacturer's instructions were followed for cell lysis, DNA isolation and purification. The purified DNA pellet was eluted in 50 μ L of DES (DNase/Pyrogen-Free Water) prior to polymerase chain reaction (PCR) amplification and analysis of products by gel electrophoresis. Bacterial and archaeal 16S DNA genes were amplified by PCR from DNA samples using a V4 oligonucleotide primer. Following amplification, all PCR products were checked for size and specificity by gel electrophoresis on 2.5% w/v agarose gel.

Prior to Ion Torrent sequencing, all amplicon types were assessed for DNA concentration using a Qubit Fluorometer (Life Technologies, UK). The amount of library required for template preparation was calculated using the Template Dilution Factor (TDF). All of the PCR products contained at least 3.60×10^3 ng/mL of DNA on Qubit, equating to 15,321.24 picomoles and therefore requiring significant dilutions. Size selection was performed with Ampure XP ensuring collection at 356 bp.

Template preparation was carried out on Ion OneTouch™ 2 System (Life Technologies, UK); comprised of the Ion OneTouch™ 2 Instrument and the Ion OneTouch™ ES (enrichment system). Clonal amplification, thermal cycling and centrifugation were carried out on the OneTouch Instrument, after which the templated Ion Sphere™ particles were recovered. Following this enrichment was carried out on Ion OneTouch™ ES, isolating template-positive Ion Sphere™ particles with magnetic beads. Finally, the spheres were loaded onto an ion semiconductor chip (Ion 314™ Chip Kit v2) ready for sequencing.

Sequencing was undertaken using a 314 chip, providing a maximum of 400–550 thousand reads per run. The Ion Sequencing Kit v2.0 for 200 bp was used following the recommended protocol. Data collected as FASTQ files from the Torrent Server were then processed through QIIME 1.7 (Caporaso et al., 2010). Reads shorter than 100 bp and longer than 1000 bp were rejected, along with homopolymers longer than 6 bp. The pipeline selected OTUs based on open reference to Greengenes 13_8 using Uclust and default parameters. The reads were aligned, and then the alignment was filtered for gaps or failures from the OTU table. Chimeric sequences were identified using ChimeraSlayer and these were then filtered from alignment and OTU table. Finally, a phylogenetic tree was built using defaults and 'core diversity' was run to establish the alpha and beta diversity, assigning taxonomy using RDP and Greengenes 13_8.

3. Results and discussion

Following an acclimatisation period of 64 days (Heidrich et al., 2013), the MEC produced hydrogen gas continuously for a 12 month period, and was still working when it was decommissioned. Routine technical problems were encountered (for example occasional power failures and pump blockages). This research has tested the robustness and durability of MECs to a greater extent than any other published research. The 100-L MEC produced hydrogen from domestic wastewater at low temperatures continuously for 12 months.

3.1. Effect of temperature on performance

The MEC continued working through the winter in Northern England with sustained periods where the water temperature in the reactor was between 1 and 5 °C, testing the technology to lower, and more fluctuating temperatures than UK systems generally experience. Typically wastewater treatment systems in the UK are designed for 5–8 °C. The ability to function at such low temperatures is surprising. Methanogenic wastewater treatment systems, which are likely to share some of the hydrolytic and acetogenic steps with MECs, are limited by low temperatures (Bowen et al., 2014). This bottleneck may be due to the difficulty of adapting mesophilic sludges to lower temperatures. In MECs the seeding of the reactors seems also to be critical. In MEC studies reporting failure at low temperatures the seed has been taken from a higher temperature reactor (Cheng et al., 2011). Those using seed from ambient or low temperature environments tend to work (Heidrich, 2012; Jadhav and Ghangrekar, 2009). In the present study the autochthonous bacteria present in the wastewater were used; the bacteria thus selected were probably adapted already to the ambient temperatures.

Energy recovery ranged from 66.8% to 37.5% with an average of 48.7%, i.e. the reactor recovered around half of the electrical energy input into the system. There is no statistical trend in performance with either seasonality (and therefore temperature) or with time. Fig. 2 shows the relationship between energy recovery and temperature, and although there does appear to be a dip in performance over the winter, this was not significant (Pearson's correlation 0.306, *p* value 0.009).

The lack of significant temperature effect on performance is surprising. Low temperatures are associated with a slowing of metabolic pathways, which should then cause a reduction in the rates of production of the end products. Other studies have shown that low temperatures do cause a reduction in performance in BES (Larrosa-Guerrero et al., 2010). However Jadhav and Ghangrekar (2009) reported a rise in performance with lower temperatures probably caused by a reduction in methanogenesis. Methane was not detected at any time during our experiment, and temperatures remained sufficiently low at all times to discourage methanogenesis, and so we doubt that methanogens are masking a temperature effect in this study. It is probable that the general low levels of performance, and high variance in the data have masked any temperature trend that may have occurred. We anticipate observing a modest temperature effect in improved pilot scale MEC. However we do not anticipate that low temperatures in an MEC will be an overarching limiting factor in MEC performance.

The performance of the MEC was low, with an average energy recovery of 48.7% and Coulombic efficiency of 41.2%. The average cathodic hydrogen production rate of 0.007 m³ H₂/m³ reactor volume is well below all but the first MEC laboratory studies. However such studies are typically performed at scales of around 100 mL, and are run on acetate at 30 °C. The positive energy balance reported in a previous MEC pilot study (Cusick et al., 2011) was based on the anaerobic production of methane, not of electrogenic hydrogen. Laboratory scale reactors have seen a 100–5000 fold increase in hydrogen production rates through multiple iterations of design. Here energy recovery would need only to double to reach energy neutral wastewater treatment.

3.2. Effect of time on performance

Energy recovery did not deteriorate with time; however there was a decline in performance. Hydrogen production decreased throughout the year, yet as the cells drew less current energy recovery per se did not decline. In a large scale system built to generate hydrogen over long design life this deterioration in current

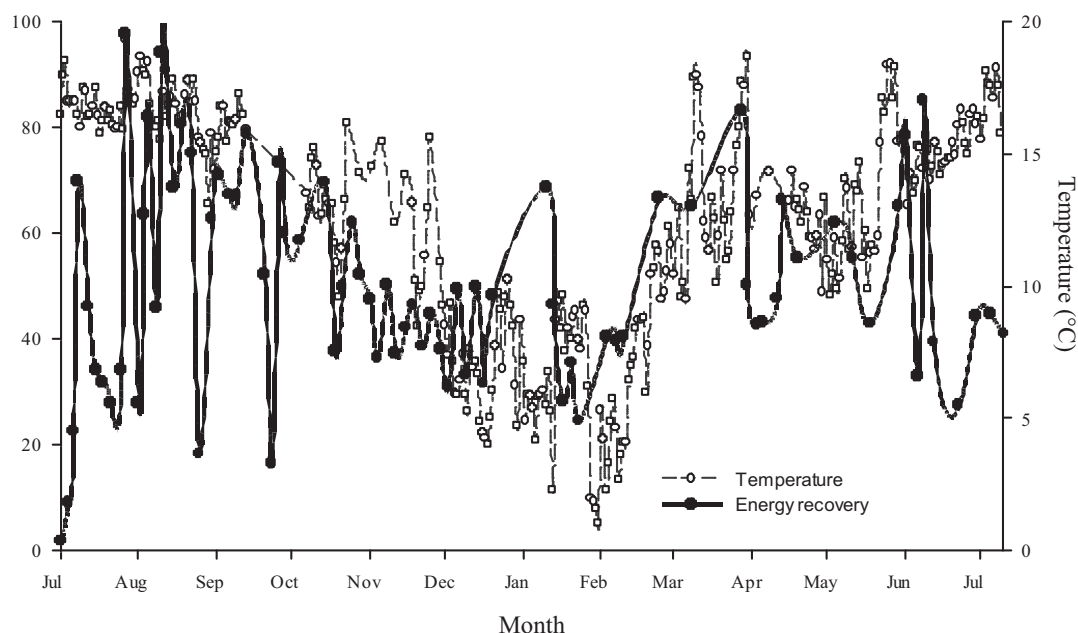


Fig. 2. Wastewater temperature (dashed line) and the energy recovery (solid line) trend for the MEC reactor throughout the year of operation, values represent monthly averages.

Table 1

Average monthly performance data for the MEC and recorded wastewater temperature within the reactor throughout the year period.

Month	Energy recovery (%)	Hydrogen production (L/day)	tCOD removal ^a (%)	Coulombic efficiency (%)	Wastewater temperature (°C)		
					Ave.	Min	Max
July	37.5	0.7	63.1	29.3	16.4	8.5	22.5
August	64.5	1.2	31.3(38.8)	51.1	16.5	12.0	23.4
September	60.8	0.9	31.7(36.7)	46.7	14.2	9.3	18.5
October	52.1	1.1	15.2(29.5)	46.1	12.9	8.0	16.4
November	42.4	0.7	1.8(50.3)	33.1	10.1	4.5	14.0
December	40.6	0.6	24.0(37.2)	35.2	7.0	4.0	10.3
January	40.7	0.3	13.5(65.6)	27.7	6.3	1.1	9.7
February	66.8	0.5	59.3	51.3	7.3	2.3	12.3
March	66.3	0.3	40.8	51.9	13.6	9.5	18.7
April	44.6	0.3	5.3(36.5)	36.0	12.8	9.2	17.4
May	56.4	0.5	12.6(37.4)	44.7	13.4	9.7	18.4
June	52.7	0.3	48.2	39.8	15.1	13.1	16.7
July	42.8	0.5	41.0	42.4	17.1	15.5	18.3
Average	48.7	0.6	29.8(44.5)	41.2	12.5	8.2	16.7

^a Frequent problems with pumping caused sporadic issues with concentrated sludge being pumped into the reactor causing negative COD removal values to be recorded. The figure given in brackets is the data with these negative values removed.

and gas production would be problematical. The initial reactor design was estimated to have an overpotential of 0.6 V, meaning at the start of the experiment only 0.5 V of the 1.1 V added would be available. This overpotential is likely to have increased over the course of the experiment with a build-up of inactive biomass on the anode, and fouling of the membrane and wire connections, (as was observed on the decommissioning of the reactor). To avoid the need for ever-increasing input voltages and diminishing returns, a reactor design with a lower overpotential, and which allows for a program of removing and cleaning components will be critical.

3.3. Coulombic efficiency

Coulombic efficiency (the amount of hydrogen collected compared to that theoretically possible based on the current passing through the cell) was on average 41.2% with no observable trend with either temperature or time. This shows that only 40% of the

hydrogen that was theoretically produced based on the current was captured. As well as the electrical losses described above, substantial losses in hydrogen gas recovery are likely. The plastic components of the reactor such as tubing, connectors and even the gas bags are known to be permeable to hydrogen. Losses are likely to increase with time as the materials deteriorate; some parts, such as gas bags were replaced periodically others could not. Alternatives to plastic such as copper piping and bubble counters cannot survive in this corrosive environment (Heidrich et al., 2013). Dealing with the difficulties of containing hydrogen gas will be one of the design challenges for the future.

3.4. Hydrogen production

The concentration of the hydrogen gas produced in the chemical cathodic side of the reactor was consistently around 98–99% pure H₂. There was however a reduction in the total volume of hydrogen produced throughout the period (Table 1), the first half

of the year producing an average of 0.8 L/day, the second half of the year producing 0.4 L/day. There was also a reduction in the average current passing through the cell (around 35 mA at the start of the year to 25 mA at the end), and therefore energy recovery does not decline. The average H₂ production rate was 0.007 L/L reactor volume/day. Methane was not detectable in the cathode gas, and was on average only 0.8% in the anode gas.

Cusick et al. (2011) reports a higher gas production rate of 0.09 L/L/day of which 33% (± 22) was hydrogen when their reactor was operated between 15 and 22 °C in its initial phase, however analysis showed that a proportion of this was likely to be due to fermentation of the sugar rich substrate. When the temperature was artificially increased to 31 °C, this biogas increased to 0.19 L/L/day, most of which was methane with no recorded hydrogen. Gil-Carrera et al. (2013) report a maximum hydrogen production of 19.2 mL/L/day in a 2 L pilot reactor, which is also higher than the amount recorded in this study. Both studies were of membraneless systems which are known to reduce overpotential and increase performance (Call and Logan, 2008), however such designs produce a mixed biogas.

3.5. COD removal

The COD removal was highly variable; problems with the pumps caused the inflow to stop on many occasions, and at other times, sludge from the base of the channel was pumped in. The recorded values of input COD ranged between 147 and 1976 mg/L. Where the anomalous values likely to be caused by these problems are removed, as seen in brackets in Table 1, the COD removal is more consistently above 30%, sometimes reaching over 60%. The removal of soluble COD was on average 33%.

The COD removal rates did not fulfil UK discharge standards of 125 mg/L COD, or 75% removal. Removal was low and highly erratic, largely caused by the positioning of the reactor within the treatment facility and by the pumping problems encountered because of this. Wastewater was taken from the grit lanes through a concealed gap 10 m below the surface with a rising and falling flow. On many occasions wastewater from the bottom of these lanes was being pumped that was high in solids and potentially biologically indigestible particles. Also on many occasions pumping ceased to work, thus there was no flow through the reactor. With the anomalous COD values taken out average COD removal was 44%; however this is still too low for effective treatment. This problem could be improved by having longer retention times, increased electrode surface area per volume of reactor, and changing the position of the reactor within the flow of the wastewater treatment plant siting it after the primary settling tanks.

The COD removal of this reactor was considerably lower than other attempts at larger scale systems. Brown et al. (2014) used a technical 16 L scale reactor continuously fed filtered primary settled wastewater, and treatment plant effluent spiked with acetate, achieved removal rates of 60%. Cusick et al. (2011) also achieved 62% COD removal using winery wastewater supplemented with acetate. Both these studies were completed at temperatures over 25 °C, methanogenesis is reported in both studies as a potential loss of COD. However greater anode area to reactor volume and more digestible wastewater components are likely to improve COD removal. Using a series of 2 2 L reactors Gil-Carrera et al. (2013) was able to reduce COD by 87%, the second reactor in the series had a higher CE possibly suggesting that pre-fermented wastewater has more electrogenically available organics. All of these studies with greater COD removal also used recirculation pumps, which also will be a consideration in future design although the energetic cost of this must be balanced.

In most wastewater treatment plants there are a series of treatment steps to reach the final effluent standards, and it is likely that

Table 2

Average characteristics of the reactor influent and effluent wastewater over the duration of the experiment.

	Influent	Effluent
pH	7.0 (± 0.2)	6.7 (± 0.2)
Conductivity (ms/cm)	1.8 (± 0.4)	1.6 (± 0.4)
DO (mg/L)	0.0 (± 0.0)	0.0 (± 0.0)
Nitrate (mg/L)	4.4 (± 3.1)	4.22 (± 3.9)
Sulphate (mg/L)	145.2 (± 67.2)	89.4 (± 37.6)
Chloride (mg/L)	196.4 (± 120.2)	177.7 (± 131.7)
Phosphate (mg/L)	7.3 (± 3.5)	8.7 (± 5.4)
Acetic acid (mg/L)	28.6 (± 38.8)	10.5 (± 14.3)
Propionic acid (mg/L)	12.0 (± 28.0)	0.1 (± 0.5)

MEC technology would be within this series. Raw wastewater has a higher COD than settled sewage, which means potentially more energy available for conversion to hydrogen. However it has been shown that around 50% of the COD in domestic sewage is suspended solids which at low temperatures is difficult to anaerobically digest (van Lier et al., 2001). MEC technology may be better placed after the settling tanks, where although the COD would be lower with less potential energy, it would be more accessible. This would directly replace the energy intensive process of activated sludge, and leave the low energy primary settling tanks producing sludge, which is typically anaerobically digested recovering energy at this stage.

Only a small fraction (1.6%) of hydrogen theoretically available in the substrate carbohydrate was captured. Methane was not detected in either the cathode gas or anode gas, and although a small amount of oxygen could have diffused into the headspace and the surface of the wastewater, the system was essentially anaerobic. Sulphate removal accounted for 3–4% of the COD removed, and there was no substantial removal of other anions as seen in Table 2.

The substrate energy efficiency of 1.6% shows that over 98% of the total energy estimated to be within the wastewater (Heidrich et al., 2011) is not converted to hydrogen. Although not all of this estimated energy is accessible, and the erratic COD measurements distort this calculation, the value is low and indicates COD is being lost. The likely cause of apparent COD loss was a substantial build-up of sludge in the bottom of the cell as was observed during decommissioning. This can be easily re-engineered, or a pre-fermentation step added. However the lack of effective conversion of the energy in the substrate into hydrogen is a major issue. If energy positive treatment is to be achieved then a substantial portion of this wastewater energy needs to be converted.

3.6. Varying performance within the reactor

Each of the six cells within the MEC unit had an individual gas bag attached, it was observed that the production of gas across these different cells was highly variable as recorded in Table 3. Gas production was highest in the middle cells, 2 and 3, these also had the highest power density and higher average energy recovery. The sequencing data showed that there was quite a large difference in the community composition of each different anode, as seen in Fig. 3. When the biofilms on the anodes were sequenced at the end of the study, these cells 2 and 3 had very low proportions of *Geobacter* sp., the only known exoelectrogenic organism to be found in the sequencing. Cell 3 contained a large proportion (35.7%) of *Desulfomicrobium* sp., however Cell 2 did not have elevated levels of these bacteria as compared to the other cells.

The biofilms observed during decommissioning were heterogeneous, both within each anode and across all of the anodes. The biofilm appeared to be fixed within the material matrix of the carbon felt rather than just on the surface.

Table 3Average performance values over the year for each individual cell^a within the MEC unit with the % *Geobacter* found using Ion Torrent sequencing.

	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
Hydrogen production (mL per day)	38.5	175.6	153.7	–	96.4	95.5
Power density (mW/m ²)	0.62	0.71	0.88	–	0.65	0.49
Energy recovery (%)	18.1	77.2	60.7	–	44.4	50.6
% <i>Geobacter</i>	12.4	2.2	0.3	–	28.1	18.5

^a Cell 4 consistently failed to produced gas for the first few months, and therefore was disconnected, thus no values are recorded for this cell.

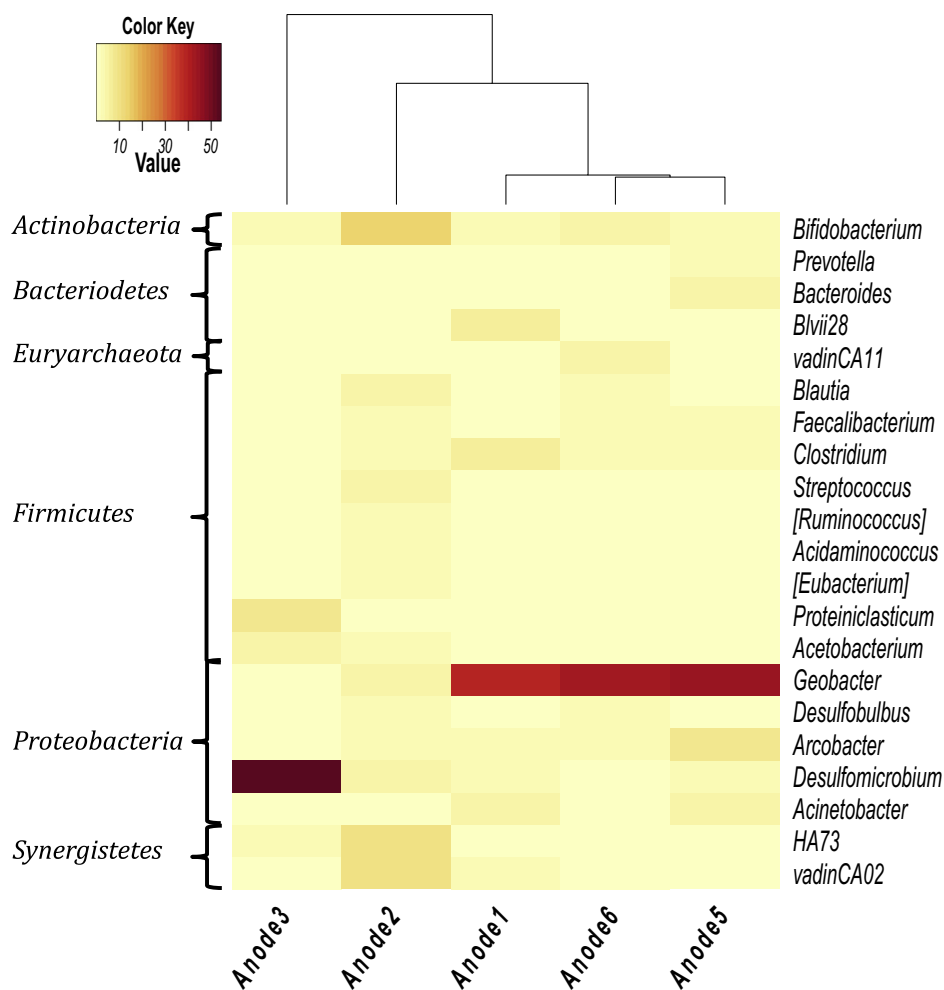


Fig. 3. Heatmap representing all OTUs present at a relative abundance of 2% or more in at least one of the samples. The colour scale ranges from 0% to 50% relative abundance, and the taxonomy is shown at the phylum level (left column) and at the lowest determined level, i.e. order or genus (right column). Ordering of the anodes is based on hierarchical clustering, shown in the dendrogram at the top of the diagram.

The gas volume data collected from each cell confirms that reactor positioning, and overpotentials are likely to be the main issues affecting performance. Cells 2 and 3 in the series, which would not be hit by the initial extremely dirty flow, but still be fed with high COD performed far better than cell 1, and better than 5 and 6. Although all cells were ostensibly identical, cells 2 and 3 seem to have less overpotential and resistance and therefore produce higher power densities. If all cells within the reactor had similar performance to cells 2 and 3 the hydrogen recovery rate would be 0.09 m³/m³ reactor and the energy recovery would be around 70%.

The individual gas data demonstrates that subtle changes in reactor configuration and flow are likely to have large impacts on

cell performance and potentially the microbial community. It is observed surprisingly that the higher performing cells have low levels of *Geobacter* spp., contrary to many laboratory studies where high levels of *Geobacter* increases performance, this is especially the case when just acetate is used (Kiely et al., 2011), although mixed cultures out-perform monocultures (Call et al., 2009b).

Spearman's rank rho correlations were calculated for the 44 most abundant taxa and: hydrogen production, power and energy recovery. There was no significant correlation with *Geobacter* for any of the criteria. However significant rho values were observed for 5, of the 44 most abundant taxa for hydrogen production, 6 for power density and 5 for energy recovery, of these 1,1 and 4 respectively were positive. The taxa with a positive correlation

were: Carnobacteriaceae; Bifidobacteriaceae *Bifi*; Clostridiaceae *Proteiniclasticum*; Dethiosulfovibrionaceae; Clostridiales; and Dethiosulfovibrionaceae HA73, a full table of this data is in [Supplementary material](#). These fermentative and hydrolytic bacteria seem to have a more positive impact on reactor performance rather than the identified electrogens such as *Geobacter*. It has been previously reported that it is these hydrolytic processes which are rate limiting within microbial fuel cells (Velasquez-Orta et al., 2011).

Due to the destructive nature of the sampling, microbial data could be collected only at the end of the study period and thus provides insufficient evidence to draw firm conclusions about the effect of microbial composition on performance. However it may be that the presence but not dominance of *Geobacter* is needed for increased performance, with hydrolytic and fermentative bacteria being more important. Understanding the factors that lead to and produce a highly functional anode community is clearly an area for future work we anticipate that new electrogens and new electrogenic mechanisms will be discovered.

The overall performance of this reactor was in many respects less than that seen in optimised laboratory conditions or than what we might hope for in full-scale applications. However, innovation theory (Thomke, 1998) and industrial practice outside the water sector (Brown, 2008) suggest that multiple iterations of imperfect prototypes accelerate the rate of innovation faster than striving for perfection in a single one off design. Thus we anticipate that further and rapid but imperfect iterations will bring us to application more quickly than an attempt to achieve perfection in a single “one off” design.

4. Conclusions

This study has tested the robustness and applicability MEC technology for domestic wastewater treatment to a far greater extent than previous studies. Valuable insight has been gained into the areas of design that need to be improved, and where greater understanding is needed. The challenge in scaling this technology remains great. The signs are promising in that the major hurdles are in design and engineering rather than problems with the biology. We believe the outcome of this research gives grounds for cautious optimism that MEC technology has the potential to become an energy neutral wastewater treatment option of the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2014.09.083>.

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