DOI: 10.1002/fuce.201700034

Fuel Cells

ORIGINAL RESEARCH PAPER

Low Temperature Domestic Wastewater Treatment in a Microbial Electrolysis Cell with 1 m^2 Anodes: Towards System Scale-Up▲

S. E. Cotterill[1]*, J. Dolfing[1], C. Jones[2], T. P. Curtis[1], E. S. Heidrich[1]

[1] School of Civil Engineering and Geosciences, Newcastle University, Newcastle Upon Tyne, NE1 7RU, United Kingdom
[2] Research and Development, Northumbrian Water Ltd., Wheatlands Way, Pity Me, Durham, DH1 5FA, United Kingdom

Received March 03, 2017; accepted May 08, 2017; published online ■■■

Abstract

The potential benefits of applying microbial electrolysis cell (MEC) technology to wastewater treatment are clear and profound. Previous pilot studies have demonstrated a 'proof of concept' with domestic waste at ambient temperatures, but have not yet treated waste to required discharge standards, and have not reached energy neutrality. In addition, these reactors have been many orders of magnitude smaller than would be needed for full scale wastewater treatment plants. Scale-up affects many of the parameters that underpin performance; understanding its impact will be vital to further progress. Modifying a previously tested cassette-style design, we reduced the internal resistance, and increased the module size by a factor of 16, constructing an MEC with six 1 m^2 anodes. This created an anodic surface area to volume ratio of 34 m^2 m^{-3}. The system was operated at a hydraulic retention time of 5 hours on settled domestic wastewater for 217 days, producing more current than a scaled-down reactor, which was run in parallel. The large MEC produced 0.8 L of 93% pure H_2 d^{-1} at ambient winter temperatures (11.4 ± 2.5 °C). Chemical oxygen demand (COD) removal averaged 63.5% with an average effluent quality of 124.7 mg_{COD} L^{-1}, achieving the European Urban Wastewater Treatment Directive (1991) consent.

Keywords: Bioelectrochemical System (BES), Chemical Oxygen Demand Removal (COD), Energy Recovery, Hydrogen Production, Microbial Electrolysis Cell (MEC), Scale-up, Wastewater Treatment

1 Introduction

Conventional wastewater treatment is a series of unit processes that provide different levels of treatment: preliminary, primary, and secondary. Secondary treatment, which aims to remove biodegradable organic matter, suspended solids and nutrients, often uses aerated processes (such as activated sludge) to achieve a high effluent quality [1]. However, aerating wastewater accounts for 50% of the energy use in wastewater treatment [2]. This is costly for two reasons; (i) the process of aeration uses 0.3 kWh m^{-3} of wastewater treated [3] and (ii) failing to recover the chemical energy stored in the wastewater (estimated at 2.1 kWh m^{-3}) is wasteful [4].

Microbial electrolysis cells (MECs) have been promoted as an emerging technology that could change the energy balance of wastewater treatment [5,6]. In an MEC, electrochemically active bacteria form a biofilm on an electrode, consuming the organic material in the wastewater, donating electrons to the anode and liberating protons in the process. The electrons travel in a circuit producing an electrical current, which can be used to produce electricity, or (with the protons released and an added potential) products at the cathode, such as hydrogen gas [7].

The potential of MEC technology is clear, but the practical relevance is less explored: few studies publish results using

▲ *Paper presented at the 3rd Meeting of the International Society for Microbial Electrochemistry and Technology (EU-ISMET 2016); held 26–28 September 2016 in Rome, Italy; organized by www.is-met.org.*

[*] *Corresponding author, sarah.cotterill@newcastle.ac.uk*

realistic conditions. Zhang et al., estimated only 2% of bioelectrochemical systems (BES), an overarching term used for MECs and microbial fuel cells (MFCs), have reactors larger than 1 L [8]; Escapa et al., indicated that only 16% of MEC studies (using real wastewaters to produce hydrogen) are carried out at scales more than 10 L [6]; and most systems that have been operated more than a year are <1 L in size and fed synthetic substrates [9–12]. Whilst the value of small scale, controlled research is not questioned, it does not inform us about the challenges of operating MECs under realistic conditions at realistic scales.

In theory, modular electrode assemblies could be used to fill different sized tanks, increasing the scale of treatment, without increasing the scale of the MEC unit. Fornero et al., [13] suggested that the use of multiple electrode assemblies meant it was only necessary to scale from mL to L. Many scale-up attempts have reached the litre scale: all of which use multiple electrode assemblies within a single vessel [6, 14–16]. Fornero et al., present a scheme where 1,667 modular L-scale cells are connected in series and parallel to run a small 100,000 L d^{-1} treatment plant with a volume of 33.3 m^3 [13]. If these L-scale modules were 0.3 m deep [15] this would require a tank surface area of 111 m^2. The practicalities of building and maintaining a site with many thousands of individual modules would be onerous; the costs may be prohibitive [13]; and the land requirements would be huge. Though the use of multiple electrodes reduces the extent of scale-up required [13], we believe scaling-up the electrode assemblies to the meter scale (and possibly beyond), to retrofit into the existing tanks, may provide a more realistic path to implementation.

The electrochemical design challenges of scaling-up BES, including methanogenic competition, pH gradients, and internal resistance, have been well documented [13, 17, 18]. There is still a need to understand the effect of a large surface area on the development of a productive electrogenic biofilm. Two such developments, may be to model optimal biofilm thickness for commercially viable current densities; or to develop non-invasive imaging techniques to monitor and characterize the structure of the biofilm during its development [19].

The results from a pilot scale MEC arising from a research collaboration between an academic and an industrial partner (Northumbrian Water Ltd) are described in this paper, following on from a pilot which functioned as a "proof of concept" [15, 16]. The pilot trial outlined in this paper was installed on a domestic sewage treatment works (STW) in the north east of England. The aims of the study were to improve reactor design and performance, and to assess the effect of scale-up. Two pilot MEC reactors were operated in parallel: one reactor was comparable in size to the one used in previous studies [15, 16], and thus served as a "control" (referred to as small MEC); the second was 16 times larger, with modules measuring 1 m^2 (referred to as large MEC). Both MECs were fed settled wastewater (average influent COD of 340 mg L^{-1}) at ambient UK temperatures, with no addition of acetate or buffers.

2 Experimental

2.1 MEC Electrode Assembly

Both MECs were based on a cassette-style design previously described by Heidrich [15, 16] with several modifications to reduce internal resistance. The cathode was redesigned to include a flat sheet of 316 stainless steel mesh, with a tab which would protrude from the module to provide a direct electrical connection (Patterson Ryan Wireworkers Ltd. UK). As in previous designs, 20 g of stainless steel wire wool was used as the cathode in the small MEC, (scaled-up proportionally to 320 g in the large MEC) (Merlin, UK), which was woven throughout the steel mesh (Figure 1a). The cathode chamber, with 9 mm thick PVC frame, was sealed on both sides by a non-selective battery separator (Entek, UK) and filled with 0.1M NaCl (Figure 1b). A 0.1M NaCl solution was chosen instead of a buffered phosphate-based salt, due to the increased conductivity of the former, which has been shown to give rise to a higher rate of hydrogen production [20], and the economic and environmental unsuitability of the use of phosphate-based salts in large-scale wastewater treatment [21]. The catholyte was periodically replaced during operation, at monthly intervals. The Rhinohide separator, (as used by Heidrich [15, 16]), was made of ultra-high molecular weight polyethylene (UHMWPE) and is routinely used in lithium-ion batteries. A tab design was also used for the stainless steel current collector at the anodes (Patterson Ryan Wirewor-

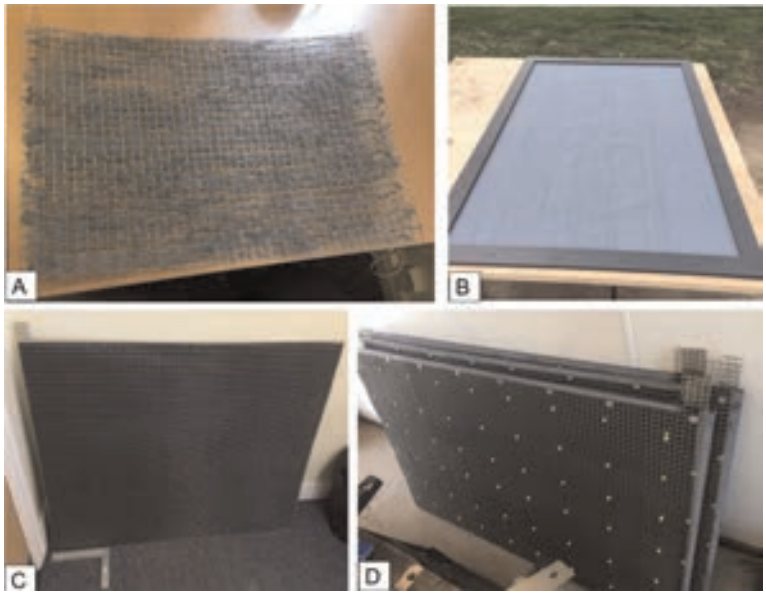

Fig. 1 Structural components of each MEC module: A) a stainless steel wire wool interwoven into the steel mesh cathode, B) the PVC frame and membrane, C) a graphite felt anode with stainless steel current collector and D) the three assembled MEC modules, showing the mesh tab connections and cable ties.

kers Ltd. UK) (Figure 1c), reducing the number of wire connections needed. The anode material was graphite felt (SGL, Germany). The current collectors were secured tightly to both sides of the anode with cable ties (Figure 1d).

The material cost for the large MEC was £1,308, of which the cathode and membrane represented only 9% of the total capital outlay. The anode (including current collector) (69%), electrical connections and power supply (11%) and PVC frame (11%) accounted for the remaining costs of the MEC. The modules were spaced 10 mm apart, on alternate sides of the MEC to create a serpentine flow path through the tank (Figure 2). Recirculation pipework (Figure 2) was built in, midway between these two points to increase mixing and minimize mass transfer limitations.

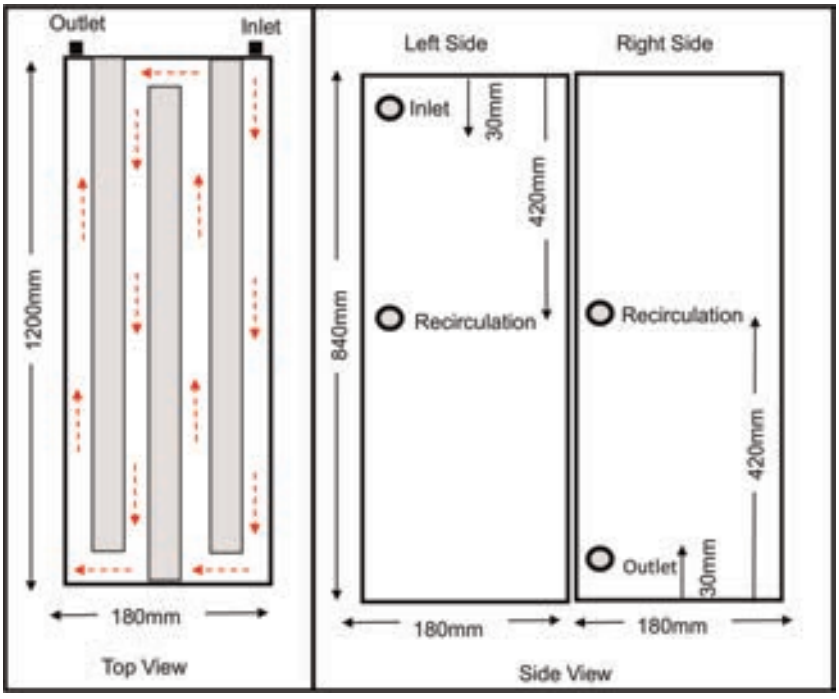

Fig. 2 Top and side view schematic of the large MEC. Top view shows the three modules, the inlet and outlet. The dashed red arrows highlight the flow of the influent through the reactor. Side views of the reactor showing the location of inlet, outlet and recirculation pipes.

2.2 MEC Reactor Scales

To assess the effect of scale-up on reactor performance, a small MEC, comparable in electrode surface area to the previous pilot trials [15, 16] was built and run alongside a large MEC. The small MEC had a similar total anodic surface area, but the tank volume was 6 times smaller, and the electrodes were 16 times smaller (Table 1). Though different in size, the components of the electrode assemblies were identical in the small and large MECs. In the large MEC, each anode was increased from 0.06 m^2 to 1 m^2 (dimensions 0.3 × 0.2 m *vs.* 0.8 × 1.2 m). Each cassette-style module contained two anodes, with a total anodic surface area of 6 m^2, an anodic volume of 175 L and a surface area-to-volume ratio of 34 m^2 m^{-3}. Each of the large MEC's modules had a cathode measuring 0.8 m^2. The large MEC had a total cathodic volume of 7.2 L, leading to a cathode specific area of 13 m^2 m^{-3}: a four-fold increase on a previous trial [15, 16]. Insufficient cathodic specific area (with reactor scale-up) has been shown to negatively affect volumetric power densities in MFCs [22].

2.3 Operating Conditions

Both MEC reactors were installed in an unheated shipping container on a domestic STW in the north east of England. The site has a population equivalent of 24,581, a dry weather flow of 8,356 m^3 d^{-1} (97 L s^{-1}) and a full flow to treatment consent of 194 L s^{-1}. A small submersible pump drew settled wastewater into a header tank from a distribution chamber (used to distribute wastewater to the trickling filters) following primary settlement and dosing of sodium hydroxide. Sodium hydroxide was dosed on site to raise pH (to between pH 7 and 8.5) prior to the trickling filters. Adjustment of pH was required after ferric sulfate dosing for phosphorous removal. We do not believe dosing sodium hydroxide had a material effect on performance, as the measured pH (8.0 ± 0.3) and conductivity (812 ± 116 µS cm^{-1}) were routinely within the boundaries of un-dosed wastewater. A peristaltic pump (Watson Marlow 520S, UK) was used to pump the wastewater into each of the MECs at a hydraulic retention time (HRT) of 5 h (304 min): a pumping rate of 98 mL min^{-1} in the small MEC and 575 mL min^{-1} in the large MEC. Wastewater left both MECs by gravity. Reactors were operated in continuous flow mode and a voltage of 0.9 V was applied from PSM 2/2A variable DC power supplies (Caltek Industrial Ltd., Hong Kong). No acetate or synthetic substrates were used to supplement the wastewater. The MECs were operated for 217 days from October 2015 until May 2016.

Table 1 Dimensions of MEC reactors, including anode, cathode and tank sizes.

Scale	Anode / m^2	Number of modules	Total anodic area / m^2	Cathode / m^2	Tank volume / L	Anodic surface area to volume ratio / m^2 m^{-3}	Cathodic specific area / m^2 m^{-3}
Small MEC	0.06	8	0.96	0.05	30	32	13
Large MEC	1.00	3	6.00	0.80	175	34	13

2.4 Sample Collection and Analysis

The voltage from each module was recorded every 15 minutes across a 1 Ω resistor (RS Components, UK) using 4-differential input (ADC-20) and 8-differential input (ADC-24) data loggers (Pico Technology, UK). This data was used to calculate the current produced by each module. Conductivity, pH and temperature measurements were taken from the front and back of each MEC twice weekly using a Hach HQ40D multi portable meter with a PHC10105 pH gel probe and CDC40105 conductivity probe (Hach Lange, UK).

Gas was captured from the cathode into Tygon F-4040 tubing (VWR, UK) connected to a Tedlar gas bag (Sigma Aldrich, UK). The volume of gas produced was measured using a 100 mL borosilicate glass syringe (SGE Analytical, Australia) twice weekly. Gas composition was analyzed using gas chromatography (GC) with a thermal conductivity detector (GC-TCD) (oven temperature: 40 °C for 1.5 min, ramping up from 110 °C by 30 °C min^{-1} and held at 200 °C for 1.5 min; detector temperature: block = 220 °C, transfer = 190 °C) with argon as the carrier gas (flow rate 10 mL min^{-1}) (Thermo Scientific, USA). A five-point calibration was carried out prior to the analysis, with a 99.999% hydrogen standard (Calgaz, USA). Later, these measurements were duplicated using a HPR40 membrane inlet mass spectrometer (Hiden Analytical Ltd, UK), confirming the results from the GC-TCD.

Liquid samples of influent and effluent were taken three times per week. Total chemical oxygen demand (tCOD) was measured in duplicate with Hach LCK314 (range 40–150 mg L^{-1}) and LCK 514 (range 100–2,000 mg L^{-1}) COD cuvette test kits with a LT200 laboratory analysis dry thermostat and a DR3900 spectrophotometer (Hach Lange, UK). Volatile fatty acids were measured twice weekly on site using LCK365 kits, and confirmed in the laboratory using a Dionex ion chromatograph (IC). Sulfate (LCK153), sulfide (LCK653), nitrite (LCK341), nitrate (LCK339) and ammonium (LCK305) were measured weekly on site using Hach cuvette kits. These results were supported by anion analysis using a Dionex IC. Samples were also sent to Northumbrian Water's United Kingdom Accreditation Service (UKAS) accredited laboratories. Two 1 L samples were taken once a week for the following analyzes: total suspended solids (TSS), ammonium, nitrate, nitrite, biological oxygen demand (BOD_5), chemical oxygen demand (COD), soluble BOD, soluble COD and alkalinity. These results are reported separately (Table 3).

Calculations were carried out to determine the efficiency of the reactor based on the electrical and substrate energy supplied. These included: coulombic efficiency (CE), which is the ratio of coulombs recovered as current, divided by the coulombs available in the substrate (i.e., in COD removed); cathodic efficiency (CCE), calculated as the amount of hydrogen gas captured relative to the theoretical value derived from the current; electrical energy recovery (ηE), calculated as energy out vs electrical energy in, where the moles of hydrogen produced (multiplied by the standard higher heating value of hydrogen, 285.83 kJ mol^{-1}) were converted to electricity (kWh) with a unit of conversion of 3,600 kJ kWh^{-1}; substrate efficiency (SE), calculated as the amount of hydrogen produced relative to the theoretical amount possible from the COD removed; and total energy efficiency (EE), which is the energy recovered from the combination of input energies (electrical and substrate). The equations used have been described previously [14]. All statistical tests were carried out using IBM SPSS statistics 23 (IBM Corp. NY, USA)

2.5 16S DNA Sequencing

Liquid samples of the catholyte were taken from the MEC cathode chambers during operation to understand problems observed with performance. The cathodic tCOD and VFA concentration was measured (as described in Section 2.4), which indicated the presence of biological material, so DNA sequencing was performed. We did not take anodic samples for DNA sequencing during operation, as this process would be destructive and interrupt the ongoing activity of the MEC. To minimize disruption to the catholyte, whilst obtaining enough sample for analysis, the samples from each cathode chamber were pooled, to create one sample for the cathode of the small MEC and one sample for the cathode of the large MEC. For each sample, 16 mL of catholyte was spun down in a centrifuge to obtain a pellet. The supernatant was discarded and the pellet was re-suspended to create a measured volume of 500 µL. Due to the high salt content of the catholyte solution, a freeze-thaw process was applied to the samples instead of using a commercial DNA extraction kit. Freeze-thaw protocols have been suggested as a method to extract and purify DNA quickly and efficiently, without the need for chemicals [23–25]. The samples were stored in a –80 °C freezer for three minutes, adjusted to room temperature, and incubated in a 95 °C heated block for two minutes. This procedure was repeated five times, to fracture the cells and make their DNA accessible. Cell fracturing occurs as the cells swell and break due to the formation of ice crystals during the freezing process. Samples were analyzed using a Qubit fluorometer (Thermo Fisher Scientific, USA) to assess the quantity of DNA. Samples were visualised under UV on a 2.5% weight by volume agarose gel. DNA extracts were pooled and sent for PCR and Illumina MiSEQ 16S DNA sequencing at LGC Genomics (Berlin, Germany).

3 Results and Discussion

3.1 Start-up

Both MECs were operated at a HRT of 5 hours; 4.8 times shorter than the previous trial [15, 16]. Fornero et al., suggested shortening the HRT in BES would be necessary to make them competitive with activated sludge and high-rate anaerobic digestion systems [13]. After 90 days, the large MEC began to produce gas. Five days later, the small MEC was also producing gas. The longer start-up period (than the 64 days observed previously [15, 16]), was likely due to lower temperatures and the use of lower strength wastewater. This trial

started in autumn-winter, with wastewater temperatures averaging 9.9 ± 1.4 °C. In comparison, the MEC in the previous trial was started in spring-summer with wastewater temperatures averaging 16.6 ±1.2 °C. A switch to primary treated wastewater led to an average COD of 347 mg L^{-1} with a conductivity of 812 µS cm^{-1}. This is slightly lower than the raw wastewater (COD 450 mg L^{-1} and conductivity =1.8 mS cm^{-1}) used in the previous trial [15, 16]. During start-up, current production in the large MEC increased from 0.18 A (0.03 A m^{-2}) at day 10 to 0.45 A (0.075 A m^{-2}) at day 70.

The time for MEC acclimation did not appear to be affected by scale-up: an important factor for the implementation of MEC. Cold BES systems have been observed to acclimatise substantially faster at laboratory scale [26]. However, this may be due to the inoculation regime, which will affect competition for resources, rather than scale directly. A direct comparison of acclimatisation methods at difference scales (at ambient temperatures) has not yet been made.

3.2 Effect of Scale

There was negligible difference in the volumetric gas production (0.004 and 0.003 L of hydrogen per litre of reactor volume per day respectively) and level of COD removal (62.5 and 63.5% respectively) of the small and large MECs, respectively (Table 2). However, the average hydrogen production, relative to the anode surface area, was four times larger in the small MEC (2 L m^{-2} of anode) than in the large MEC (0.5 L m^{-2} of anode) (Table 2). If this observation reflects a linear decrease in hydrogen gas captured with scale, then a 30% further increase in scale (to 1.3 m^2) could, theoretically, result in a module where no gas is captured. This would support Fornero's prediction that it is only necessary to scale from the mL to L scale [13].

The current density was an order of magnitude larger in the large MEC (Table 2). This may have been a true effect of scale; though the apparent difference may also have been introduced by the high margin of error on the small MEC. The standard deviation on the current observed in the small MEC was 2.08 times the value recorded (Table 2). Pico ADC 20/24 data loggers are operational between 0 and 45 °C, but accuracy can only be guaranteed between 20 and 30 °C. Air temperature was below this for most of the operational period reported. Although subject to considerable error, current density was comparable with the previous trial [15, 16], and increased with time. A maximum of 0.79 A m^{-2} was achieved in the large MEC in April: three times larger than the average current density calculated across operation, including periods when the biofilm was still maturing. Cathodic specific area was four-times greater in this study (13 m^2 m^{-3}) than in the previous trial (3 m^2 m^{-3}, [15, 16]), which may have supported an increased current density. An increase in power density has been observed in MFC with increased cathodic specific area [22].

As scale-up did not appear to have a negative effect on MEC performance, the rest of the paper describes the results obtained from the large MEC alone. Data relating to gas yield and current production are provided individually, for each of the three modules, where data were recorded and analyzed in isolation. This was not possible for wastewater treatment data, as the three modules were hydraulically connected. Therefore, wastewater treatment data reflect the influent and effluent of the large MEC in its entirety.

Table 2 Effect of scale on MEC performance. Data are reported both as raw values (current, COD removal and H_2 production) and subsequently adjusted relative to scale (current density, daily removal of COD and volumetric yield of hydrogen).

	Small MEC	Large MEC
Size of electrode / m^2	0.06	1.0
Current / mA	2.24 ± 4.66	294 ± 185
Current density / A m^{-2}	0.04	0.29
COD removal / %	62.5	63.5
Daily ΔCOD / kg_{COD} d^{-1}	0.03	0.18
Average H_2 production / mL d^{-1}	124	521
Average H_2 production relative to scale / L m^{-2}–electrode d^{-1}	2.07	0.52
Volumetric H_2 yield / L–H_2 L–MEC^{-1} d^{-1}	0.004	0.003

3.3 Wastewater Treatment

In the gas production phase (Jan-May), the average effluent COD was 120.6 mg L^{-1}, achieving the European consent of < 125 mg L^{-1}. During the 217 days of operation, the average measured COD of the effluent from the large MEC (125 ± 50 mg L^{-1}) was significantly lower [t (434) =15.496, p = 0.000] than the influent (340 ± 200 mg L^{-1}), equating to a removal efficiency of 63.5%. A Spearman's correlation analysis was run to determine the relationship between the strength of the influent COD and the percentage of COD removed. There was a strong, positive correlation between the two variables, which was statistically significant (r_s (109) = 0.693, p = 0.000). Therefore, COD removal efficiency was higher, when the strength of the influent COD coming in to the reactor was higher. Observations in laboratory BES have shown that when total influent COD drops below 200 mg L^{-1} (or sCOD below 100mg L^{-1}), current production declines drastically [27]. Influent tCOD was below 200 mg L^{-1} in 49 of the 218 spot samples in this trial (22.5%). This may be a limiting factor: there may not have consistently been sufficient COD to drive the MEC process.

Gil-Carrera et al., found that COD removal improved with increasing OLR, in a continuous flow tubular MEC fed primary settled domestic wastewater [28]. These authors suggest that exoelectrogenic activity is limited at low COD concentrations, reducing COD removal and hydrogen production [28]. Furthermore, a Pearson's correlation of data presented by Escapa et al., [29] in an MEC fed synthetic wastewater, shows a significant positive relationship (r (6) = 0.917, p = 0.010) between influent COD and percentage of COD removed, provided a voltage is applied (i.e., excluding V_{app} = 0).

Removal of biological oxygen demand (BOD) and total suspended solids (TSS) was 65.7% and 74.2%, respectively; yet, with average effluent concentrations of 35 mg BOD L^{-1} and 42 mg TSS L^{-1}, neither parameter achieved the Urban Wastewater Treatment Directive (UWWTD) consent through this process alone (Table 3). This suggests a final polishing step is necessary, to achieve a satisfactory effluent standard to discharge to the environment. Effluent VFA concentration, of which acetic acid comprised on average 20%, was 44 ± 18 mg L^{-1}. This was 46% lower than in the influent (80 ± 42 mg L^{-1}).

The ratio of sulfate to COD provides information about COD removal in the large MEC. The ratio of COD to sulfate in the influent (which was 2.4), and the large MEC's pH (8.0 ± 0.3) would favor sulfate-reducing bacteria (SRB) to out-compete methanogens [30, 31]. There was an average of 7.8 g d^{-1} of sulfate removed from the reactor. An independent samples t-test showed that the amount of sulfate in the large MEC's effluent (105 ± 16 mg L^{-1}) was significantly lower ($t(40) = 3.722$, $P = 0.001$) than in the influent (141 ± 41 mg L^{-1}). Removing 1 mol of sulfate (98 g) requires 64 g of COD. The maximum COD that could be consumed by the removal of 35.9 mg L^{-1} of sulfate is 23.4 mg L^{-1}, accounting for 11% of the total COD removal. However, other metabolic pathways may be involved, reducing this value.

The ratio of BOD to COD in the influent (0.38) (Table 3) was slightly below the typical values observed in primary settled waste (0.4–0.6) [1]. The ratio of BOD to COD in the large MEC's effluent (0.33) (Table 3) was slightly higher than typically observed in final effluent (0.1–0.33) [1]. The soluble COD (45 mg L^{-1}) and soluble BOD (15 mg L^{-1}) in the large MEC's effluent was very low (Table 3), suggesting that availability of organic material for the electrochemically-active bacteria could be limiting current and gas production.

3.4 MEC Performance

The first gas measurements recorded a hydrogen purity of 60 ± 18.5%. After the residual gases were flushed out, hydrogen purity increased to 77.5 ± 18% and then stabilized at 92.8 ± 7% for 4 months. The volume of hydrogen produced increased each month between January and April, but dropped by 18% in May (Table 4). This may be attributed to a 22% decrease in average influent COD from 352 ± 145 mg L^{-1} in April, to 274 ± 120 mg L^{-1} in May (Table 4). The maximum gas yield obtained, 0.86 L d^{-1}, is equivalent to 0.005 L-H_2 L-anode^{-1} d^{-1}. This is considerably lower than typical values obtained in laboratory studies with acetate (0.12 m^3 H_2 m^{-3} d^{-1}, [32]) or real wastes (0.061 m^3 H_2 m^{-3} d^{-1}, [33]). However, these laboratory values were obtained with a considerably higher influent COD at 1298 mg L^{-1} [33].

Table 4 Average yield (in mL) of hydrogen gas per MEC module per day during the gas production phase of the large MEC.

	January	February	March	April	May
Module 1 / mL d^{-1}	156	162	590	813	652
Module 2 / mL d^{-1}	27	30	12	13	30
Module 3 / mL d^{-1}	0	45	20	31	24
Total MEC / mL d^{-1}	183	237	622	857	706
Average temperature / °C	8.6 ± 0.5	9.7 ± 1.3	10.6 ±1.1	12.6 ± 1.2	15.6 ± 1.4
Average influent COD / mg L^{-1}	328 ± 198	413 ± 156	267 ± 150	352 ± 145	274 ± 120

Table 3 Analysis of total suspended solids (TSS), Biological Oxygen Demand (BOD5), Chemical Oxygen Demand (COD), soluble BOD, soluble COD, alkalinity, ammonia, nitrate, and nitrite in the influent and effluent of the large MEC, carried out by Northumbrian Water's UKAS accredited Scientific Services. Removal efficiency was calculated, following a paired t-test to determine a significant difference between influent and effluent. The urban wastewater treatment directive requirements (UWWTD, 1991) are listed alongside, including variations for population equivalent (PE) i.e. the pollution load produced during 24 hours by one person.

Analysis	Influent / mg L^{-1}	Effluent / mg L^{-1}	Percentage removal / %	UWWTD Consent / mg L^{-1}	Minimum removal / %
TSS	161 ± 50	42 ±12	74.2	35 (>10,000 PE) 60 (2,000–10,000 PE)	90 (>10,000 PE) 70 (2,000–10,000 PE)
BOD_5	101 ± 33	35 ± 16	65.7	25	70–90 (>10,000 PE) 40 (2,000–10,000 PE)
COD	261 ± 85	103 ± 38	60.6	125	75
sBOD	18 ± 10	15 ± 11	No difference (p = 0.19)	–	–
sCOD	57 ± 24	45 ± 23	No difference (p = 0.08)	–	–
Alkalinity	183 ± 39	143 ± 24	21.9	–	–
Ammonia	15 ± 4	13 ± 2.5	18.8	Total N 15 (10,000–100,000 PE) 10 (100,000 + PE)	Total N 70–80
Nitrate	1.3 ± 0.9	1.1 ± 0.9	No difference (p = 0.49)		
Nitrite	1.8 ± 2.3	0.9 ± 0.8	No difference (p = 0.08)		

A Spearman's rho was used to determine the relationship between the volume of hydrogen gas produced and the temperature of the wastewater. There was a positive correlation which was statistically significant (r (24) = 0.567, p = 0.004), suggesting that more hydrogen gas is produced when the temperature of the wastewater is higher, as observed in April and May (Figure 3). However, a partial correlation was run to control for the month in which the sample was taken. The partial correlation was not significant, when controlling for which month the sample was taken (r (21) = –0.226, p=0.300). This implies the increase in hydrogen obtained at warmer wastewater temperatures may be an artefact of a winter start-up, as the MEC reached maturity during the spring when temperatures happen to increase.

The electrical energy efficiencies were particularly low during the start-up phase (October 2015 to January 2016), but showed an increase throughout operation (Figure 4). A maximum coulombic efficiency of 27.7% was achieved, 6 months into the period of operation. Cathodic coulombic efficiency remained below 10% throughout, implying significant hydrogen losses. This will have affected the electrical recovery throughout operation, which remained below 3.5% (Figure 4). Diffusion of hydrogen gas (a very small molecule) is frequently a problem in bioreactors.

3.4 Module Variability

Current production (mean current = 294 ± 185 mA) was highly variable throughout the operation, and between the three identical electrode assemblies within the large MEC. The average current produced throughout operation was 207 ± 49 mA for module 1; 392 ± 252 mA for module 2; and 192 ± 43 mA for module 3. A Pearson's correlation showed there was not a significant relationship between the current produced in each of the MEC's modules and the hydrogen gas obtained (r (15) = –0.079, p = 0.780). Module 1 produced more gas (475 ± 299 mL d^{-1}) than module 2 (22 ± 9 mL d^{-1}) or 3 (24 ± 17 mL d^{-1}) (Table 4). However, module 2 produced more current (327 ± 219 mA) than module 1 (204 ± 37 mA) or 3 (189 ± 33 mA).

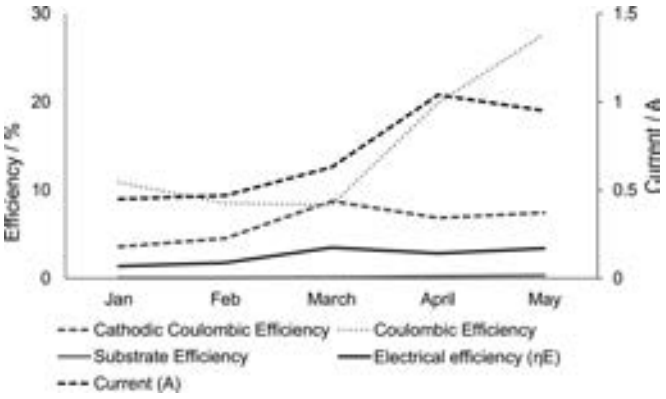

Fig. 4 Average reactor efficiencies in the large MEC per month during the period of hydrogen production (day 90–200). Most of the efficiencies show an increasing trend during this time. Substrate efficiency remains very low throughout.

The yield of gas collected from module 1 was 20 times larger than that collected from module 2 and 3 (Table 4). If the latter two modules had produced this amount of hydrogen, the average yield would have been between 1.8–2.4 L d^{-1} between March and May. This would treble the electrical energy recovery, and increase cathodic coulombic efficiency (CCE) to 21%. The low CCE, and the high variability in gas collected from each module, is likely to be caused by activity at the cathode, rather than the anode, such as hydrogen-scavenging. The catholyte sampling method, designed to minimize disruption to the cathode during operation, precluded the ability to differentiate between the communities of individual modules within a reactor. It is likely that hydrogen loss (to acetogenic bacteria and diffusion through materials) was common across all modules, even though the yield was substantially larger in module 1.

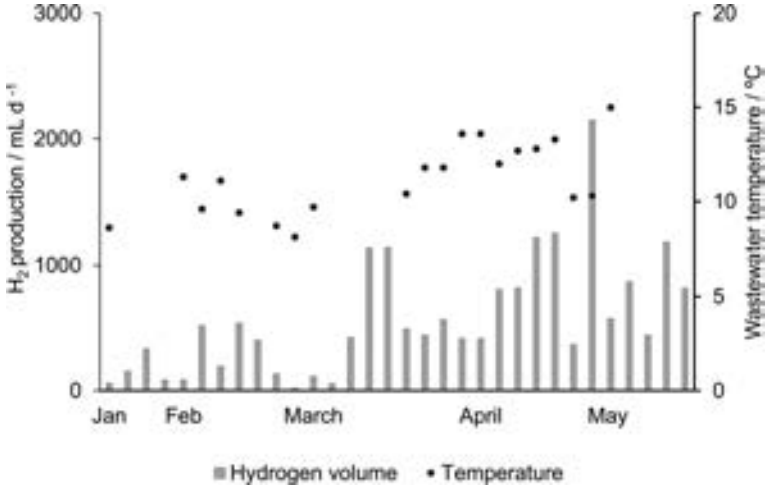

Fig. 3 Volume of hydrogen gas produced by the large MEC, in millilitres per day, per month, relative to the temperature of the wastewater at the time of the gas measurement.

3.5 Hydrogen Scavenging Bacteria

The applied voltage used in this trial should permit the MEC to be energy-producing [15]. During March and May, the MEC produced on average 0.73 L of hydrogen gas per day. To meet the energy requirements of the power supply, and 'break-even', the MEC would need to produce on average 12 L per day: 16 times more than the average achieved in this pilot trial. If the yield obtained from modules 2 and 3 had matched module 1, then total yield would be 2.45 L d^{-1}: a volume 4.9 times lower than required to meet the energy requirements of the power supply.

The cathodic efficiency remained below 10%, meaning at least 90% of the current generated was not captured as hydrogen. Although there will have been losses caused by leakage of hydrogen gas, sequencing data suggest that hydrogen scavenging bacteria also played an important role. Homoaceto-

genic bacteria may have populated the cathode chamber, which was not sterilised at start-up or during operation, after passing through the Rhinohide separator.

Analysis of the pooled cathode samples indicated that several genera of sulfate- and sulfur-reducing deltaproteobacteria were present, totaling approximately 5% of the sampled community. These included *Desulfomicrobium* and *Desulfovibrio*, genera which are unable to oxidize acetate and use hydrogen as an electron donor for anaerobic respiration; and *Sulfurospirillum*, a dissimilative sulfur reducer which uses hydrogen to reduce elemental sulfur [34]. An acetogen, *Acetobacterium*, was observed in all catholyte samples (~1% of sampled community), suggesting some of the hydrogen may have been converted to acetic acid. Hydrogen-oxidizers, such as *Acidovorax* (3–4% of each community), *Hydrogenophaga* (1–10% of sampled community) and pseudomonads such as *Pseudomonas* (6–11% of sampled community) and *Brevundimonas* (1–2% of sampled community), were also found. It is likely that hydrogen was lost to these bacteria.

The chemical composition of the catholyte was analyzed prior to sampling for DNA sequencing. At the onset of gas production, there was negligible difference between the total VFA concentration recorded in the anode (17.9 $\pm$ 0.9 mg L^{-1}) and cathode (16.4 $\pm$1.6 mg L^{-1}). However, there was a considerable increase in the amount of total VFAs, proportional to tCOD. In the cathode, VFA concentration comprised on average 42 $\pm$ 11% of the tCOD compared with 6.6 $\pm$ 0.8% of the tCOD in the anode. This may indicate hydrogen loss to acetogenic and fermentative processes in the cathode. In January, 183 mL d^{-1} of H_2 was captured from the cathode (Table 4). In theory, based on cathodic VFA concentration, a maximum of 110–130 mg of acetate (2 mmol) was produced in cathode. Per mol of acetate formed, 4 mol of H_2 are consumed: therefore, 8 mmol of H_2 (i.e. 200 mL) would be required to produce 120 mg of acetate. Thus, if acetogenic bacteria accounted for the loss of 200 mL of H_2, the yield captured in January (183 mL) would have been only 48% of the total yield. If the loss of gas was linear with time, then in April (when gas yield was highest) a percentage loss of 52% to acetogenic conversion, would equate to a loss of 936 mL H_2 d^{-1}. This may be a conservative estimate: the mixed consortia in the cathode suggests that hydrogen may be scavenged by more than one pathway.

The presence and, more importantly, activity of hydrogen-scavenging bacteria is problematic for the efficiency of an MEC system producing H_2. This problem could be solved by: eliminating the bacteria from the cathode; or removing the hydrogen from the system before it can be scavenged. The former could be achieved by modifying the pH or sterilizing the catholyte solution periodically, inhibiting the anaerobic acetogenic bacteria. Recovering hydrogen more efficiently from the system, may be more achievable than creating and maintaining a sterile environment in the cathode whilst submerged in wastewater. Recirculating the catholyte would decrease the retention time of the hydrogen in the cathode, limiting the potential for the hydrogen to be scavenged [35]. Another possibility would be to design the MEC architecture (i.e., wide, shallow modules) to decrease the distance for the H_2 to travel from the site of production to capture and therefore, reduce the likelihood of diffusion out of the module. An alternative option may be to harvest acetate, with hydrogen as the secondary product [36].

4 Conclusions

In this paper, we describe the scale-up of an MEC treating authentic domestic wastewater, without significant detrimental impact on performance. MEC technology has advanced from the litre to the m^3 scale, accompanied by a 16-fold increase in electrode size and a reduction in HRT to 5 h. This study demonstrated the MEC's capability of treating 0.9 m^3 of wastewater per day to the European standard for COD removal (<125 mg L^{-1}) at winter temperatures in the UK. Hydrogen was produced for 127 consecutive days, but it was evident from the poor cathodic efficiency (<10%) that hydrogen losses were significant. Reducing hydrogen loss is a priority for improving the efficiency of the technology. Future research efforts should address modifications to the electrode assembly required, most notably to the Rhinohide separator, which was porous (allowing permeation of bacteria into the cathode); and ridged (influencing the ability to form a seal, particularly under the pressure of recirculation).

Engineering these systems at a large scale is new territory. Even with the scale-up demonstrated in this paper, thousands of modules would be needed for full scale treatment, as suggested by Fornero. These 1 m^2 electrodes would need to be 30–50 times larger to span, and crucially reach the bottom of, a typical aeration lane. Dealing with gases (either supplying oxygen for an MFC or capturing hydrogen from an MEC) may be the limiting factor for scale-up, rather than current production or resistance. Perhaps now, scale-up beyond m^3 is not as critical as addressing how to operate a system with thousands of modules. It will be increasingly important to assess how factors such as: gas diffusion; hydrodynamics; and the distribution of organic load, influence the stability and efficiency of scaled-up reactors. There is still much to be understood: this study invites further pilot scale research to optimize configuration and energy efficiencies under realistic conditions.

Acknowledgements

The authors are grateful for the continuous support of staff at Northumbrian Water: with special thanks to Chris Hepple, Laura Wilkinson, Trevor Fenwick and Stuart Barnes. The work was funded by the Engineering and Physical Sciences Research Council and Northumbrian Water, through a STREAM IDC engineering doctorate (EP/G037094/1).

Data supporting this publication is openly available under an "Open Data Commons Open Database License". Additional metadata are available at: http://dx.doi.org/10.17634/154300-49. Please contact Newcastle Research Data Service at rdm@ncl.ac.uk for access instructions.

References

[1] G. Tchobanoglous, F. L. Burton, H. D. Stensel, *Wastewater Engineering: Treatment and Reuse*, McGraw Hill, Boston, **2004**, pp. 97.

[2] G. Olsson, *Water and Energy: Threats and Opportunities*, IWA Publishing, London, **2012**, pp. 313.

[3] P. L. McCarty, J. Bae, J. Kim, *Environ. Sci. Technol.* **2011**, *45*, 7100.

[4] E. S. Heidrich, T. P. Curtis, J. Dolfing, *Environ. Sci. Technol.* **2011**, *45*, 827.

[5] J. M. Foley, R. A. Rozendal, C. K. Hertle, P. A. Lant, K. Rabaey, *Environ. Sci. Technol.* **2010**, *44*, 3629.

[6] A. Escapa, R. Mateos, E. J. Martinez, J. Blanes, *Renewable Sustainable Energy Rev.* **2016**, *55*, 942.

[7] J. Ditzig, H. Liu, B. E. Logan, *Int. J. Hydrogen Energy* **2007**, *32*, 2296.

[8] F. Zhang, G. Zheng, J. Grimaud, J. Hurst, Z. He, *Environ. Sci. Technol.* **2013**, *47*, 4941.

[9] H. Liu, S. Cheng, L. Huang, B. E. Logan, *J. Power Sources* **2008**, *179*, 274.

[10] H. Moon, I. S. Chang, B. H. Kim, *Bioresour. Technol.* **2006**, *97*, 621.

[11] F. Zhang, D. Pant, B. E. Logan, *Biosens. Bioelectron.* **2011**, *30*, 49.

[12] G. Zhang, K. Wang, Q. Zhao, Y. Jiao, D. J. Lee, *Bioresour. Technol.* **2012**, *118*, 249.

[13] J. J. Fornero, M. Rosenbaum, L. T. Angenent, *Electroanalysis* **2010**, *22*, 832.

[14] R. D. Cusick, B. Bryan, D. S. Parker, M. D. Merrill, M. Mehanna, P. D. Kiely, G. Liu, B. E. Logan, *Appl. Microbiol. Biotechnol.* **2011**, *89*, 2053.

[15] E. S. Heidrich, J. Dolfing, K. Scott, S. R. Edwards, C. Jones, T. P. Curtis, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 6979.

[16] E. S. Heidrich, S. R. Edwards, J. Dolfing, S. E. Cotterill, T. P. Curtis, *Bioresour. Technol.* **2014**, *173*, 87.

[17] R. A. Rozendal, H. V. M. Hamelers, K. Rabaey, J. Keller, C. J. N. Buisman, *Trends Biotechnol.* **2008**, 26, 450.

[18] B. E. Logan, *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1665.

[19] A. P. Borole, G. Reguera, B. Ringeisen, Z. W. Wang, Y. Feng, B. H. Kim, *Energy Environ. Sci.* **2011**, *4*, 4813.

[20] J. Y. Nam, B. E. Logan, *Int. J. Hydrogen Energy* **2012**, *37*, 18622.

[21] D. Pant, A. Singh, G. Van Bogaert, Y. A. Gallego, L. Diels, K. Vanbroekhoven, *Renewable Sustainable Energy Rev.* **2011**, *15*, 1305.

[22] B. E. Logan, M. J. Wallack, K. Y. Kim, W. He, Y. Feng, P. E. Saikaly, *Environ. Sci. Technol. Lett.* **2015**, *2*, 206.

[23] T. de Baera, G. Claeys, D. Swinne, G. Verschraegen, A. Muylaert, C. Massonet, M. Vaneechoutte, *BMC Microbiol.* **2002**, *2*, 21.

[24] A. M. Borman, C. J. Linton, S. J. Miles, C. K. Campbell, E. M. Johnson, *Med. Mycol.* **2006**, *44*, 389.

[25] G. Ligouri, A. Lucariello, G. Colella, A. D. Luca, P. Marinelli, *J. Clin. Pathol.* **2007**, *60*, 1035.

[26] Personal communication between S. E. Cotterill and E. S. Heidrich, Feb. 28th, 2017 about submitted manuscript: E. S. Heidrich, J. Dolfing, M. Wade, W. T. Sloan, C. Quince, T. P. Curtis, *Bioelectrochemistry* **2017**.

[27] X. Zhang, W. He, L. Ren, J. Stager, P. J. Evans, B. E. Logan, *Bioresour. Technol.* **2015**, *176*, 23.

[28] L. Gil-Carrera, A. Escapa, B. Carracedo, A. Moran, X. Gomez, *Bioresour. Technol.* **2013**, *146*, 63.

[29] A. Escapa, A. Lobato, D. M. Garcia, A. Moran, *Environ. Prog. Sustainable Energy* **2013**, *32*, 263.

[30] A. Visser, Y. Gao, G. Lettinga, *Bioresour. Technol.* **1993**, *44*, 113.

[31] E. Choi, J. M. Rim, *Water Sci. Technol.* **1991**, *23*, 1259.

[32] Y. H. Jia, J. H. Ryu, C. H. Kim, W. K. Lee, V. T. T. Thi, H. L. Lee, R. H. Zhang, D. H. Ahn, *J. Ind. Eng. Chem.* **2012**, *18*, 715.

[33] Y. H. Jia, J. Y. Choi, J. H. Ryu, C. H. Kim, W. K. Lee, H. T. Tran, R. H. Zhang, D. H. Ahn, *Korean J. Chem. Eng.* **2010**, *27*, 1854.

[34] M. T. Madigan, J. M. Martinko, D. A. Stahl, D. P. Clark, *Brock Biology of Microorganisms*, Pearson Education Inc., Boston, **2012**, pp. 540.

[35] J. A. Baeza, A. Martínez-Miro, J. Guerrero, Y. Ruiz, A. Guisasola, *J. Power Sources* **2017**, *356*, 500.

[36] S. Gildemyn, K. Verbeeck, R. Slabbinck, S. J. Andersen, A. Prévoteau, K. Rabaey, *Environ. Sci. Technol. Letters* **2015**, *2*, 325.

