ENERGY RECOVERY FROM A HIGH STRENGTH WASTE STREAM USING PILOT-SCALE BIOELECTROCHEMICAL SYSTEMS

DANIEL DAVID LEICESTER

Thesis submitted for the degree of
Doctor of Philosophy

School of Engineering
Newcastle University
Newcastle upon Tyne
United Kingdom

July 2020

Acknowledgements

First and foremost I would like to thank Dr Elizabeth Heidrich. I can't express how great a supervisor she has been, providing me with unwavering support, guidance and advice from day 1. I would also like to thank Dr Jaime Amazega for his excellent supervision, helping with the bigger picture and constantly keeping me motivated. I couldn't have asked for a better supervisory team.

I would like to thank all my colleagues and co-workers, who have provided advice, support and tea breaks when needed. In particular to Harry, Amy and Jess, who have been with me since the start, and Lindsey for being the best desk buddy ever. I would like to thank all my friends who I met through NUCC, and my flatmates Aaron and Andrew for keeping me sane during lockdown.

I would like to thank all the lab technicians that have helped me over the years, as well as a number of post-docs and PhD students who have selflessly given guidance when needed. In particular Andy Zealand for persuading me to do the PhD in the first place, and Dan Aiken for specialist advice throughout. I would also like to thank Carl Samuels and Carolina Ospina, for not only keeping me company during the long hours of lab work, but always being there to bounce ideas off. I would also like to thank Amy and Fergus, who have taken countless hours out of their time teaching me how to code.

I would like to thank EPSRC for the doctoral training grant, and the school of Engineering for the additional financial support. I would also like to thank all those at STREAM for the workshop opportunities, in particular to Justine who enabled all of it to happen.

I would like to thank all those at Northumbrian Water Ltd who made my pilot-scale work possible. In particular Andrew Moore and Matt Pickersgill for sorting the access, Tony Rutherford and Kevin Stobbs for help with the reactor set-up, and finally Martin Robinson, for all the time he took to help me when things went wrong.

Finally I would like to thank my mum and dad for their love and support throughout the whole process, my sister Anna for countless advice and proof reading, and my thanks to Bridget for her never ending encouragement.

Abstract

Bioelectrochemical systems (BESs) have the potential to produce energy from wastewater. However, they are far from ready to be applied into industry. This study attempts to reduce the gap between pilot-scale and commercial application.

First, we present a systematic review on the published semi-pilot and pilot-scale MECs, and benchmark their performance against existing wastewater treatment. We find that factors which are perceived to be problematic, such as low conductivities and temperatures, have been overcome by BESs at pilot-scale, and that these systems have met the regulatory requirements for discharge standards. We identify reactor depth and volumetric treatment rates (VTRs) as the areas that require further research. It was hypothesised that the use of high strength return sludge liquor (RSL), rather than raw domestic wastewater, may boost BES performance. At a laboratory scale, it was seen that MFCs fed RSL performed as well as the pure substrate when comparing wastewater treatment performance, and COD saturation was reached with respect to VTRs. The use of RSL with a high soluble COD appears to reduce the effect of the slow breakdown of complex substrates in wastewater.

Building on this success, a pilot-scale microbial electrolysis cell (MEC) was then operated in continuous flow for 6 months. The reactor was fed RSL, and successful optimisation of the hydraulic retention time (HRT) resulted in the highest VTR achieved by a pilot-scale MEC treating real wastewater. Peak HRT was 0.5-days, resulting in an average VTR of 3.82 kgCOD/m^3·day and a 55% COD removal efficiency. Using the data obtained, a direct analysis of the potential savings from the reduced loading on AS was then made. Theoretical calculation of the required tank size with the estimated costs and savings indicate that the use of an MEC as a RSL pre-treatment technique could result in an industrially viable system.

Throughout the thesis, there was a distinct variability between identical reactors when using wastewater. Variability reduces the overall performance and therefore increases costs, but more importantly, it highlights our lack of understanding of, and our inability to control and engineer these systems. Analysis in the previous chapters saw that variability was most obvious during inoculation with fresh wastewater, and appears to be caused by the total number of electrogens able to establish onto the anode. Artificially seeding the reactors enabled the creation of higher performing biofilms; however, this was only with sterile wastewater. Periods of stable current when running the pilot reactor in continuous flow give hope that modification to the design and operational conditions could reduce the impact of this variability.

Assuming that other dimensions will be overcome by the use of modular electrodes, depth remains a major challenge, and even if this is accomplished, the natural variability from using wastewater may prevent this technology from ever being implemented into industry. However, if these issues can be solved, the switch to a more sustainable wastewater treatment method would be both economically and environmentally beneficial.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

Water is fundamental to the global economy and environment. The planet is facing an unprecedented combination of water security and resilience challenges, and the need for innovative technology is clear. With the development of the Sustainable Development Goals in 2015 [14], there has been an increased push for global change. One key focus is to ensure access of water and sanitation for all, with an aim to achieve this by 2030 [15]. It was reported that 2.4 billion people lack access to basic sanitation services, and nearly 1000 children die each day from preventable water and sanitation-related diarrhoeal diseases [15]. Implementing safe sanitation systems is essential to protect human health, and cheaper or energy positive wastewater treatment systems could help improve the quality of life, preventing poor hygiene and sanitation related deaths. Unfortunately, change to the water industry is difficult, and many of the new innovative technologies are still far from being competitive enough to be implemented.

The most commonly used wastewater treatment process within the UK is called activated sludge (AS), where oxygen is pumped into the wastewater for aerobic bacteria. This encourages the oxidisation of organic compounds into carbon dioxide and water [16], and successfully reduces the organic content within the wastewater to match legal discharge standards [17]. However, the process of aeration is energetically expensive, and within the UK is predicted to cost 4818 kWh/m^3·year [18]. The organics within the wastewater have been reported to contain up to 16.1 kJ/gCOD [19], and there has been considerable research into harnessing this energy while simultaneously treating the wastewater. Recent change has seen the implementation of anaerobic digesters at some treatment sites, a process which treats waste AS sludge, recovering energy in the form of biogas [20]. However, these are additional processes to the energetically expensive AS, and therefore net energy positive wastewater treatment can never become a reality without large-scale industrial change. With the increasing national energy demands and an impending climate crisis [21], the UK water sector has launched its first draft Innovation Strategy, aiming to "drive transformational change through innovation, which delivers greater value for customers and the environment" [22]. Changing to a technology that doubles as a renewable energy source could turn wastewater treatment into an energy positive industry; however, bridging the gap between studies at a pilot-scale to commercial application remains a significant challenge.

When rediscovered in the early 2000s the technology of bioelectrochemical systems (BESs) seemed to offer this much needed transformative step, producing easy to use electrical energy directly from treating wastewater. Research in this field exploded, moving from 13 papers in 2000 to 11,418 in January 2020 (based on Scopus search term on Bioelectrochemical Systems OR Microbial Fuel Cells, which was an early and frequently used term for a type of BES). Yet despite this high amount of research, to the author's best knowledge, there are as yet no commercial or even large-scale operational BES units in existence worldwide. As a point of reference, Annamox, a biological process in the nitrogen cycle, was discovered in 1999; the first full scale plant was in operation by 2002; and by 2013 there were at least 30 full-scale plants [23].

A BES utilises electrochemically active microorganisms (EAMs) capable of extracellular electron transfer, which can be found naturally in the wastewater. These microorganisms oxidise organic pollutants and can directly produce electrical current via electron transfer to a solid anaerobic electrode. Electrons flow to a counter electrode or cathode via an external circuit; protons (H^+) arrive via an ion exchange membrane, and a reduction reaction can occur (Figure 1.1). As a result, wastewater treatment is achieved due to the oxidisation of pollutants, and simultaneously, energy is recovered due to the current generation from the flow of electrons [24].

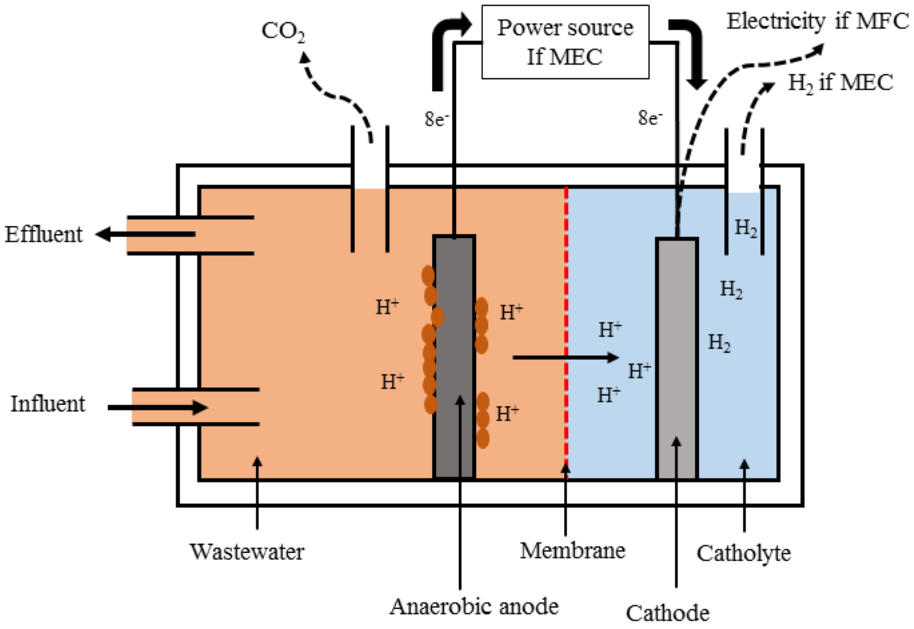


Figure 1.1: A simple bioelectrochemical system, representing the difference between a microbial fuel cell and a microbial electrolysis cell.

In relation to energy recovery from the treatment of wastewater, there are two main types of BESs (Figure 1.1): a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC). In an MFC, the cathode reduction reaction uses oxygen; water is produced as a waste product, and energy is harvested in the current that flows in the circuit. An MEC operates under completely anaerobic conditions, with the protons being reduced at the cathode to produce hydrogen gas (H_2) or other chemicals such as caustic soda. As the conversion of organic material to hydrogen is an endothermic reaction, energy needs to be supplied to the system for the reaction to proceed. For acetate to hydrogen this is 0.14V; however, the added potential also needs to overcome the systems over-potentials, which for small systems is >0.2V [25, 26] and larger systems can be closer to 1V [3, 5]. As additional energy is added by applying this potential, achieving net energy neutral treatment in an MEC requires the energy recovered in the hydrogen to be higher than this input.

Although this research focusses primarily on wastewater treatment, microbial electrochemistry can be used in other technologies [27]. The generation of electrical current can not only produce hydrogen at the cathode, but also can be used for the production of other value added chemicals (such as caustic soda or methane production) [28, 29], the remediation of organic contaminants (microbial remediation cells (MRSs)), to synthesis organic compounds (microbial electrosynthesis (MESs)) [30] or used for the desalination of saline solutions (microbial desalination cells (MDCs)) [31].

The majority of BES research is at laboratory scale, while a number of pilot-scale BESs have been attempted with varying degrees of success. However, there are still no commercially viable BESs, and scale-up of this technology is problematic. The development of large and pilot-scale systems to harness energy and value-added chemicals is widely regarded as one of the greatest research challenges in this field. There are several reasons for this: i) they are expensive, ii) they are difficult to engineer, iii) and the data that can be derived from them is often limited, rarely in duplicate and disproportionate to the time commitment. Attempts at scale-up have discovered difficulties from the effects of increased hydrostatic pressure [32], increased ohmic losses and changes to the thermodynamic and kinetic properties [33] of the biological and electrochemical processes. The current material costs may also limit larger designs from being built [34].

This project has originated in part from a previous EngD's thesis [35]. Here the student developed energy and nutrient mass balances for wastewater treatment systems, with an aim to identify areas where value recovery would be possible. It was shown that return sludge liquor (RSL), which is the liquid fraction from the dewatering of anaerobic digestion sludge (Figure 1.2), is a resource laden waste stream with currently no economical desirable methods of resource recovery. It is present at most treatment plants that use anaerobic digestion, which due to the current increased push for renewable energy alternatives is becoming much more widespread [36]. The waste stream is typically returned back to the start of the treatment works as an internal loop, and due to the much higher COD content when compared to raw wastewater, significantly increases the cost of wastewater treatment due to the added loading on AS. It was therefore hypothesised that this waste stream would be an excellent location for a BES. The high organic content could boost energy recovery and treatment rates, and focus can be on optimising this technology to be as cost effective as possible as there are no requirements to reach the exact legal discharge standards. The use of a BES at this point within the wastewater treatment process would result in a low risk entry point for pilot-scale technology.

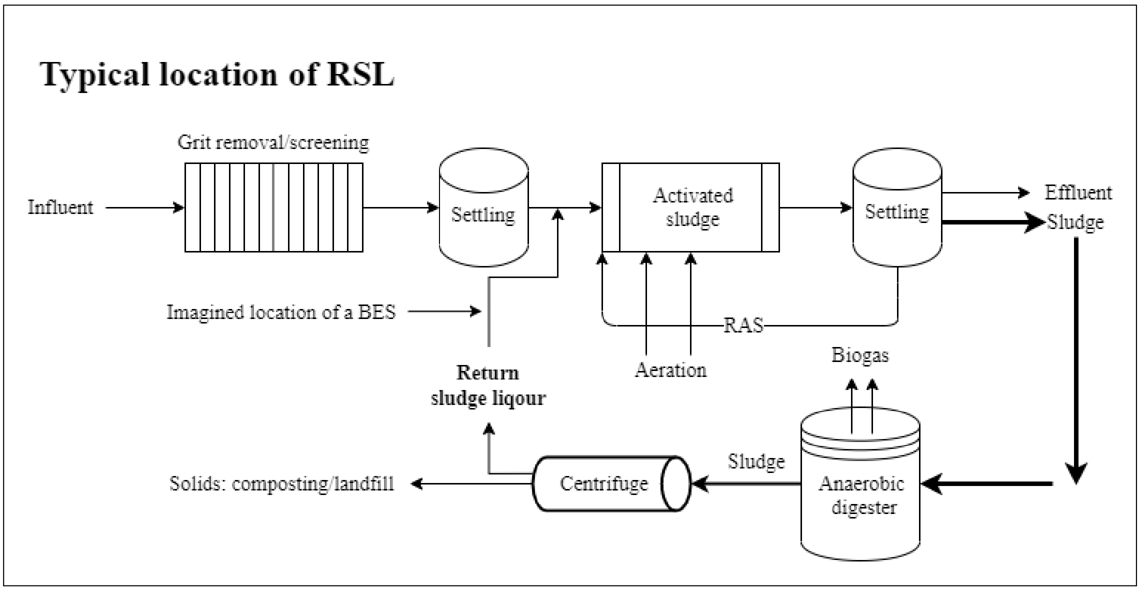


Figure 1.2: A simple flow diagram to show the location of RSL at a wastewater treatment site, using both AS and AD technology and the imagined location of a BES.

Northumbrian Water Ltd (NWL), a water company that sponsored the previous EngD's work [37], supported the hypothesis that the combination of a BES and the RSL waste stream could boost the performance of this technology. They therefore offered access to their treatment sites containing this RSL. Based on the previous success of Newcastle University pilot-scale BESs [2, 3, 4], a similar style reactor was built by a local engineering company. The experimental design planned to optimise the technology, rather than simply demonstrate it. Therefore, modifications were made to the BES to enable greater flexibility when conducting experiments.

Two sites near Newcastle University operate anaerobic digesters and have RSL. Bran Sands Wastewater Treatment Site (NWL) appeared to be an ideal location for the pilot-scale BES. This site contained other pilot-scale wastewater treatment technologies, enabling the access of using RSL wastewater pre- and post-ammonia removal. Additionally, due to the set-up, the reactor could be placed close to the RSL sample point, enabling easy access to fresh wastewater. This led to the experimental work outlined in Chapter 3 and Chapter 4 initially using Bran Sands (BS) RSL, and the pilot-reactor was set up and inoculated with BS RSL in January 2019. As similar configured reactors resulted in long start-up times [2, 4], and the wastewater used was more complex, it was expected that start-up would be similar if not greater. Therefore, it was only after 5 months of operating the pilot-reactor using BS RSL that further experimental work showed that BS RSL might be toxic to BESs.

The reactor was removed, dismantled and cleaned. All experimental work then changed to using RSL from Howdon Wastewater Treatment Works (NWL), and the pilot-reactor was then re-built and set-up at this treatment site. The RSL could be pumped directly from the sample point into an IBC, and then from the IBC into the reactor, resulting in a similar set-up to the one at Bran Sands. This wastewater was not toxic, and was used successfully throughout the thesis. The RSL at both Bran Sands and Howdon are viewed as very similar waste streams, and therefore these issues were not predicted. However, the site at Bran Sands treats both domestic and industrial wastewater, resulting in a greater number of heavy metals being present in the waste stream. This highlights the importance of the variable nature of the wastewater, increasing the difficulty in innovating in this sector. This variability is a significant issue with bioelectrochemical technology, as it already contains a great deal of uncertainty.

This thesis first reviews the current published pilot-scale attempts to determine the major limitations with this technology. Following this, the use of RSL from Howdon Wastewater Treatment Works (NWL) is used to determine if resource recovery and cost savings are possible with BESs, first at a laboratory scale and then at pilot-scale. Finally, investigation into a major limitation preventing BESs from industrial application concludes this research.

1.1 Aims and objectives

The aim of this research was to improve the wastewater treatment and energy recovery capabilities of BESs, closing the gap between pilot-scale and industry.

To fulfil this aim, the following objectives were:

1. To identify the current limitations that are preventing pilot-scale MECs from competing with current treatment processes.
2. To determine if RSL liquor can be used successfully in BESs at a laboratory scale.
3. To run a pilot-scale BES on a wastewater treatment site with RSL, maximising performance by optimising operational conditions.
4. To investigate any limitations still preventing BESs from industrial application.

Chapter 2

Is bioelectrochemical energy production from wastewater a reality? Identifying and standardising the progress made in scaling up microbial electrolysis cells

This chapter has been published as Leicester, D.D.; Amezaga, J.M.; Heidrich, E.S. Is bioelectrochemical energy production from wastewater a reality? Identifying and standardising the progress made in scaling up Microbial Electrolysis Cells. Renewable and Sustainable Energy Reviews, 2020, Volume 133, 110279.

2.1 Introduction

Increasing demands for energy and water are the biggest issues to threaten both human health and the ecosystems we depend on [38], with fossil fuel consumption increasing by 1300 times in the last 200 years [39]. With a continuing global population rise, the world's energy requirements are predicted to increase by 28% by 2040 [40], and water demand is predicted to increase by 30% by 2050 [41]. There is a pressing need to drastically change this situation; the solutions will need to be widespread and far-reaching. All aspects of the way humans interact with the planet on which they reside will need to be considered, and improvements to, or radical reduction of their energetic costs made. One such area that has the potential for radical change is the treatment of domestic wastewater. Current methods of wastewater treatment, developed in the 1900s [42], are energy intensive, accounting for as much as 3% of electricity consumption in developed economies [43]. Furthermore, 80% of the world's wastewater goes untreated into the receiving waters [44]. This scenario is neither sustainable nor affordable for the world's growing population in a time of increasing energy costs. However, human waste contains energy locked up within its organic molecules [21]. Harnessing some of this energy whilst also treating the waste would transform this part of the human environment interaction.

Bioelectrochemical systems (BESs) are a highly complex technology, combining electrochemistry with microbiology and wastewater engineering. The strides forward in understanding these systems, and the microbes that facilitate them, have been significant and broad-reaching. These have been reviewed extensively in the literature for different reactor architectures [26, 45, 46]; anode and cathode materials [47, 48, 49]; substrates used such as synthetic and real wastewater [50, 51]; pre-treatment methods [52]; and the operational conditions of BESs [45, 53]. In addition scale-up, and the prospects for use with wastewater treatment, have also been covered in several reviews [10, 27, 51, 54, 55, 56]. Comparing each of the pilot studies published in the literature to each other is important, especially as performance criteria are often measured and reported differently. However, to understand their readiness for application, we must compare their performance to the treatment technologies that already exist. This analysis addresses this, simplifying and standardising the operational parameters of each pilot-scale reactor onto a single diagram, in order to visually illustrate the performance gap between BESs and the status quo.

In developed countries, activated sludge (AS) is the most commonly used process for wastewater treatment by volume treated [16]. It utilises the bacteria present in the wastewater, supplying oxygen via aeration to encourage the oxidation of organic compounds into carbon dioxide. The large amount of energy available to the bacteria in this aerobic digestion leads to rapid growth, which in turn removes the organic matter at a high rate. Once the organics have been removed the bacteria die and sink, producing sludge. This technology has remained largely unchanged in the past 100 years, and although highly effective at meeting discharge standards, aeration is energetically expensive, accounting for around 50% of the total treatment costs [57].

The energetic treatment cost for AS is typically between 2.52 – 7.2 kJ/gCOD [58]; this energy is spent on aerating and cannot be recovered. The energy content of domestic wastewater is estimated to be 16.1 kJ/gCOD [21]. This energy stored in the organic carbon present in the wastewater is either released as CO_2, or becomes part of the bacterial cells that form the sludge. Recent developments at wastewater treatment plants have seen an increase in the resource recovery from this sludge through the use of anaerobic digestion (AD) [59, 60]. However this only recovers the proportion of energy in the wastewater that is driven into this sludge. The typical yields in AS are about 0.4 g-COD-cells/gCOD substrate [61], though this can be lower where systems are run with low loading rates. This means that of the 100% COD entering the AS tank, a maximum of 40% becomes new biomass or sludge, and 60% is 'burnt' with oxygen producing CO_2 and is therefore lost. The biomass or sludge generated in AS enters an AD reactor, where yields are around a tenth of aerobic yields [61, 62]. Therefore, of the initial 100% COD, 60% is lost as CO_2 in the AS process, 1.6% ends up as AD sludge, and 38.4% is available for energy production i.e. methane, which must be combusted to yield energy. So although AD is a highly efficient process, it recovers energy after the energetically expensive AS process has taken place. The costs of AS are two fold: firstly the input of energy for aeration, and secondly the loss of approximately 60% of the wastewater's energy as CO_2. Thus replacement of the AS with an anaerobic process would lead to substantial energy savings and potential energy gains.

Lower energy wastewater treatment options exist and are effective. Trickling filters flow wastewater through a porous media, and aerobic conditions are created by using a large area and allowing natural ambient levels of oxygen in the air to access the bacterial biofilm. They are a simple low-cost wastewater treatment technology, with high removal efficiencies of up to 90% of the influent BOD [63], and low energy cost of 0.15 – 0.4 kWh/m^3 [64]. However, they require a larger land surface area than AS, typically 10 times higher due to a much longer hydraulic retention time (HRT) (24 hours [65]) and smaller depth (0.9 – 2.5m deep [66]), and so are often unsuitable for areas with a high density urban population [67, 68]. Rittman and McCarty [69] report the surface area treatment rates in a trickling filter are at around 0.033 kgBOD/m^2·day. Comparatively, using a volumetric treatment rate for AS of 0.6 kgBOD/m^3, and assuming a 3m deep tank, the equivalent for AS is around 1.8 kgBOD/m^2·day. Clearly there will be some site and waste stream specific issues, but there is a substantial difference in the space requirements. Additionally, poor removal of nitrogen and phosphorous often make AS a more desirable option [70]. Wastewater stabilisation ponds are also able to effectively reduce the pollutants and toxins within wastewater. These anaerobic ponds are also low cost, with removal efficiencies of up to 85% BOD [71, 72]. However, due to depth of 2.5m and a retention time of >1 day, land requirements are again high [72, 73].

Classical AD recovers energy from wastewater by using microorganisms in the absence of oxygen to digest biodegradable matter, recovering energy in the form of biogas (methane) [74]. Developed in 1895 in Exeter [75], this is a relatively simple and low cost method of wastewater treatment with energy recovery, and treatment rates can be as high as 65% with loading rates of 1.0 – 3.0 kgVS/m^3·day [76]. However, HRTs are between 5 - 40 days. AD is widely used for industrial wastewater treatment, sludge treatment and for wastewater treatment in warmer climates such as South America [77]. Unfortunately, this microbial process is impeded at low temperatures and with dilute wastewaters. Research is developing and is identifying bacterial communities which are able to successfully treat raw wastewaters at temperatures lower than 13°C [77, 78, 79]. However, currently AD is not used for low strength domestic wastewater in the UK and other temperate climates.

BESs are an alternative anaerobic wastewater treatment technology which may overcome some of these obstacles. There is continuing debate over which is the best BES configuration to use for energy neutral wastewater treatment. An MFC is technically a simpler bioreactor, it requires no additional energy input, and it produces energy directly in the form of electrical current, which although limited in amount, is readily usable to supplement energy requirements for lighting, pumping, or UV disinfection. Pilot scale MFCs have been successfully deployed in field sites for the treatment of urine in source separated toilets [80] and in the laboratory for combined wastewaters [81], and faeces [82]. The multiple MFC cell stack design is of particular importance, and the feasibility of this technology has been demonstrated in the UK [80] and Ghana [83] for decentralised treatment of the urine component for wastewater.

The development of low cost MFC air cathodes has been critical to this success; however, it is also the barrier to larger scale application. Air cathodes require exposure to the air, meaning the space requirements are likely to be large. The stacked MFC design used by Ieropoulos et al. [80] at the University of West England involves boxes that are 70×30×16cm, with a volumetric capacity of 25L, each containing 36 individual MFCs. The residence time of these boxes was at least 14 days. This gives a hydraulic loading capacity of 0.008 $m^3/m^2\cdot$day. The hydraulic loading of trickling filters is 4 -10 $m^3/m^2\cdot$day for medium rate systems [84], as these are typically 1.25 - 2.5m deep. Though the MFCs treat urine only, and therefore are not directly comparable with wastewater treatment, this means over 500 units would need to be stacked on top of each other to reach the space requirements of a trickling filter, which makes them too large for centralised urban wastewater treatment. Assuming a 4cm gap to allow air circulation, this would be a 100m tall tower. It seems unlikely that stacked MFCs would work for centralised treatment, even without considering the complexity of wiring, tubing and monitoring that this large number of small scale MFCs would entail. Scaling up MFCs will require larger area cathodes [32]. An air cathode size of 0.62m^2 has been reached, but the water pressure at a depth of just 0.85m is problematic [32].

An MEC overcomes this limitation as oxygen is not required at the cathode. Instead, a small potential is added to drive different anaerobic reduction reactions. This is usually the production of hydrogen, though the production of caustic soda is also a possibility. The gravimetric energy density of hydrogen (120 MJ/kg) makes it a highly efficient energy carrier compared to methane (50 MJ/kg), gasoline (44 MJ/kg) and ethanol (26.8 MJ/kg). It is seen as a clean, sustainable and renewable fuel, producing zero carbon emissions [45], although there are some disadvantages. Hydrogen gas molecules are small, therefore it is often difficult to capture, and there will be added costs to the purification and safe transport of such a volatile fuel. However, if MECs can produce this fuel from wastewater whilst also treating it, this could be a more economically viable prospect than producing just electricity in an MFC [55, 85].

If BESs are to achieve their "great potential to become an alternative to conventional wastewater treatment" [55] they would need to replace the AS process, currently the most prevalent wastewater treatment method (by volume treated). It has been reported that the cost of aeration can range from between 50% - 90% of the total electricity used by a treatment plant [86], of which all would be removed when operating a BES. Pumping costs are estimated to be around 7% [86], although this is very site specific, and many sites are designed so the wastewater flows downhill. As MECs are still a long way from developing a commercially viable system it is difficult to estimate the pumping costs. In a scenario of much smaller stacked units, wastewater would need to be pumped in multiple directions, and pumping costs could be high. If they were retrofitted into the existing AS lanes, then pumping costs for a BES will essentially be the same, though could be offset to some degree if energy was produced. However in both cases the pumping of the return activated sludge (RAS) will be removed (estimated as 2% of the total energy costs [86]), further reducing the energy consumed (see Figure 2.1). Additionally MECs produce less sludge than the AS process [87], so this pumping cost, and in some cases the cost of transporting this sludge to a treatment facility could be reduced.

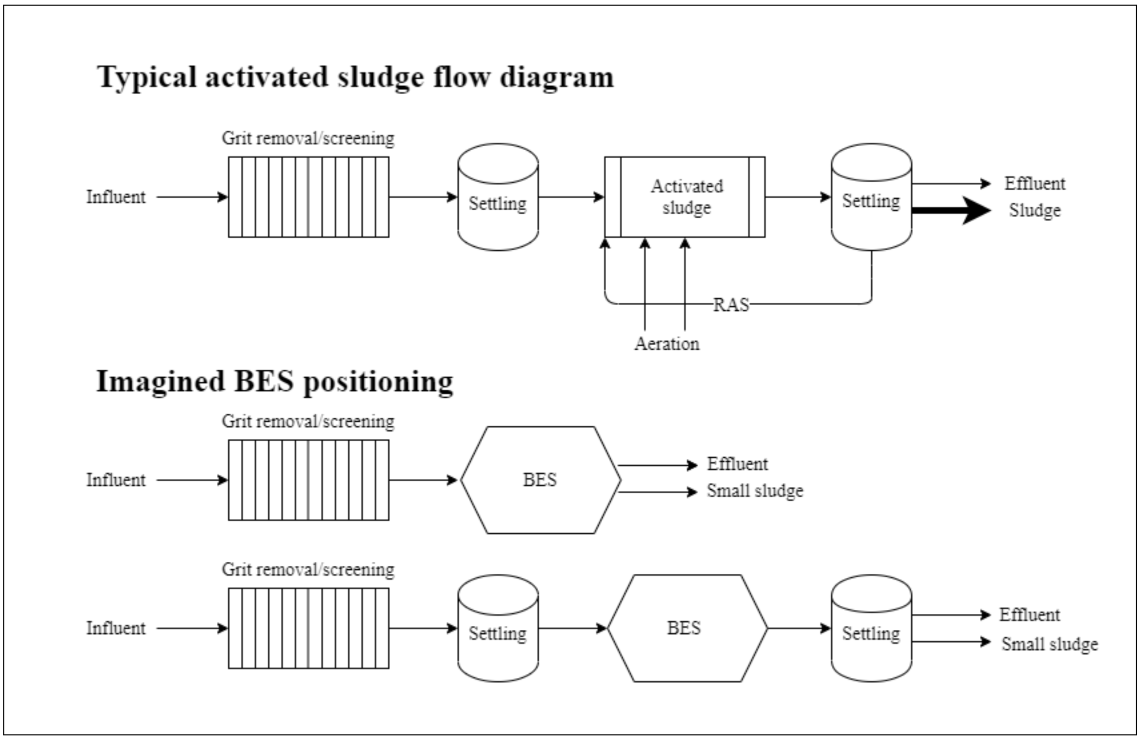


Figure 2.1: A flow diagram for a typical AS wastewater treatment plant, and the imagined positioning of a BES.

This chapter reviews the published work of pilot-scale MECs, and makes an assessment of how close they are to practical implementation via the replacement of AS. It is acknowledged for industrial application that a thorough cost-benefit analysis and life-cycle assessment would also be needed, but this is beyond the scope of this review, and covered in detail elsewhere [34, 58, 68, 85]. The methodology chosen to review the different pilot-scale MEC reactors involves a direct comparison with the operational parameters of typical AS plants. The first section of the review identifies what these parameters are, derived mainly from text books and legal standards, and the second half compares the pilot-scale MEC studies available in the literature to these parameters. Conclusions are then drawn as to which parameters we need to focus research effort into in order to take this technology from a laboratory curiosity into an industrial reality.

2.2 Operational parameters

Wastewater treatment is a compliance based industry, and its aim is to discharge 'safe' water. Safety is determined by regulatory standards. In the EU, effluent quality needs to match EU (1991) discharge standards [17] (Table 2.1). Globally there are slight differences in these guidelines. For example, in the USA COD must be <120 mg/L [88] and in China this is <60 mg/L[89]. Its infrastructure is typically built on 25 - 50 year cycles. New technologies would need to robustly demonstrate that they meet regulatory compliance, and would ideally fit into existing infrastructure to allow for a less costly transition.

Table 2.1: EU 1991 Wastewater treatment discharge standards.

Parameters	**Discharge standards**	**Units**
BOD	25	mg/L
COD	125	mg/L
TSS	35	mg/L
Total Nitrogen	15	mg/L
Total Phosphate	2	mg/L

To determine the theoretical "ideal" conditions that the MECs would be required to meet, this Chapter has used activated sludge (AS) as a benchmark. As a replacement technology MECs should: be able to treat the same types and concentrations of wastewater (complexity and conductivity); be able to treat them at realistic temperatures; be of a size similar to existing infrastructure (reactor size/depth); be able to cope with the volume and strength of wastewater at current treatment plants (organic loading rate); be able to treat the wastewater to the desired discharge standards (effluent quality and volumetric treatment rate); and be able do this using less energy (energetic treatment balance). The values for these parameters are found in Table 2.2.

Table 2.2: Summary of the parameters used for the Ideal System with their key references.

Parameter	**Values**	**Units**	**Key references**
Wastewater complexity	≥ 90	no.	Huang et al. [90]
Substrate conductivity	≤ 1.25	mS/cm	Henze et al. [91]
Organic loading rate	≥ 1.67	kgCOD/m^3·day	Logan et al. [61]
Depth	≥ 3	m	Eckenfelder et al. [92]
Volumetric treatment rate	≥ 1.25	kgCOD/m^3·day	Logan et al. [61] ; EU (1991) [17]
COD removal	≥ 75	%	EU [17]
Energetic treatment cost	≤ 2	kJ/gCOD	Pant et al [58]; Li et al. [93]
Temperature	≤ 10	°C	Ali ([94]

2.2.1 Wastewater complexity

In order to replace or compete with AS, MECs must be able to function with the complex nature of real wastewater. By this we mean firstly that the mixture itself is complex as there are lots of components within it which will vary with space and time. Secondly, these components in the mixture will themselves be complex, for example long chain organics that require multiple stages to break down. It will also contain trace metal, chemical or pharmaceutical pollutants that may be impossible to break down, or else they may be toxic to microorganisms. The issue of long chain complex organics is of particular importance, as failure to break these down would lead to poor COD removal rates and therefore non-compliance, as many of the other complex pollutants are not currently regulated for. Thirdly the microbiology of wastewater is highly complex, with 10^{15} – 10^{18} individual bacteria in an AS tank [95]. Assuming the most abundant species represents about 10% of the population (NT/NMax =10), this gives an estimated diversity of around 10^4 to 10^5 [96]. Some of this complexity will be helpful, providing the food chain for complex organics; however, some may be detrimental or competitive [97].

The complex organics which act as a bacterial food source in domestic wastewater include proteins, fats and carbohydrates. These organic compounds are typically digested in a food chain, with different groups of organisms being responsible for different stages of this chain. Velasquez-Orta et al. [98] suggests that the pathway to break down these complex organic compounds within a BES is similar to AD, with hydrolysis followed by fermentation. Hydrolysis breaks complex organic molecules into simple molecules, and then these are broken down into volatile fatty acids (VFAs) via fermentation. The final step however, is not completed by methanogens but by electrochemically active microorganisms which convert acetate to CO_2 and H^+, and also transfer an electron to the electrode. In AD some of these stages are known bottlenecks. Hydrolysis (when bacteria attempt to break down complex polymers into simple sugars, VFAs and amino acids) is often described as the rate limiting step [99], and subsequently research involving AD often performs pre-treatment techniques in order to boost this step [100, 101, 102]. Therefore, within an MEC, breakdown may be slow, or not even possible, depending on the components of the wastewater. In small scale MFCs at lab temperatures Velasquez-Orta et al. [98] determined the combined rates of hydrolysis and fermentation was 0.0024 h^{-1}, whereas the rates for both fermentation alone and acetate consumption were an order of magnitude faster. This was 0.018 h^{-1} and 0.017 h^{-1} respectively, in small scale MFCs at laboratory temperatures.

The use of synthetic wastewater cannot replicate or model this complex reality. The difference in running a system with real wastewater, compared to that of synthetic wastewater, is well observed [103]. In laboratory scale MFCs, Zhang et al. [104] reported coulombic efficiencies of 90% in acetate fed reactors, compared to 22% in identical domestic wastewater fed reactors. At pilot-scale, Baeza et al. [5] observed higher removal efficiencies but lower hydrogen production when using glucose (36.8% COD removal, 0.028 $m^3H_2/m^3\cdot day$)

and glycerol (26.3% COD removal, 0.015 $m^3H_2/m^3\cdot$day) as compared to domestic wastewater (6% COD removal, 0.031 $m^3H_2/m^3\cdot$day) using the same reactor. The reason for this higher production was not determined.

In order to make a comparison of different wastewaters and artificial substrates, our method requires an actual number to be assigned for complexity. For the purpose of this study we have chosen to use complexity as the number of organic compounds present in the substrate. It is acknowledged that this does not account for complexity in terms of variability over time and space, and may not account for the recalcitrant nature of some individual components or the microbiology. The number of different components within the given wastewater could be used for this; however, detailed knowledge of the composition of wastewater is quite limited [105]. Huang et al. [90] determined with GC/MS analysis that there were at least 90 organic compounds within wastewater. Eriksson et al. [106] found 900 compounds in grey water, ranging from trace hydrocarbon pollutants such as oil and grease, to heavy metals such as manganese or zinc. Real wastewaters from an industrial process such as food and drink production may contain fewer and more simple compounds. Colin et al. [107] found that ethanol, fructose and sucrose represented more than 90% of winery effluent. Synthetic substrates typically contain between 1 - 10 different compounds [5, 12, 13, 108]. The use of domestic wastewater with an MEC is therefore graded as 90 using the number found by Huang et al. [90], which is the maximum, or ideal value, for other substrates to be set against. For simpler wastewaters the number of typical components are used as determined either in the paper, or in other literature. For synthetic wastewaters we use the number of components likely to contribute as a food source.

2.2.2 Substrate conductivity

Conductivity is the ability for an electrolyte solution to conduct electricity, and is measured by the concentration of charged ions which are free to move in a liquid. Wastewater conductivity is relatively low 0.7 - 1.8 mS/cm. The limited number of ions which are free to flow increases resistance and electrolyte Ohmic losses, reducing the current density and lowering the electricity harvested [33]. In artificial wastewaters, conductivity can be boosted by the addition of buffers, such as a phosphate buffer, typically in the range of 7.5 – 20 mS/cm [26, 109]. An increased performance of 0.13 to 0.82 $m^3H_2/m^3\cdot$day was observed by Verea et al. [110] when the conductivity of the synthetic wastewater was doubled from 7.5 mS/cm to 15 mS/cm.

Systems using real wastewaters could be dosed with the same buffers to artificially increase the conductivity and boost performance. However, the cost for a large continuous flow reactor to be routinely dosed with chemicals may be too high to consider for application to industry [58]. The ideal system should therefore work with the typical conductivity of wastewater. The reported values for these in the literature are: 1.80 mS/cm [2]; 1.25 mS/cm [5]; and 0.8 mS/cm [4, 6] for domestic wastewater, and 0.7 mS/cm for winery wastewater [1]. Similarly, Henze et al. [91] reports for high strength wastewater a value of 1.20 mS/cm. A value of 1.25 mS/cm has been selected in this study to represent the 'Ideal System'. If a reactor uses a substrate with a lower conductivity than 1.25 mS/cm, it shows that it is successfully coping with greater Ohmic losses, while a higher conductivity indicates the reactor is operating in an unrealistic and advantageous scenario.

2.2.3 Organic loading rate

The organic loading rate (OLR) is the rate at which the organic content of the wastewater is supplied to the system. It is a critical measurement in the design of a wastewater treatment plant. It takes into account both the volume and strength of the wastewater that is treated, and is calculated based on the flow rate and size of reactor (or HRT) and the COD concentration of the wastewater. It enables the size of the tank required to be determined as follows:

$$Ks = \frac{S}{HRT \times 1000}$$

where S is the COD concentration (mg/L), HRT is hydraulic retention time (days), and Ks is the OLR (kgCOD/m^3·day). Using an average COD of 500 mg/L for medium strength wastewater [111], and an average HRT of 7.2 hours (0.3 days) [112], an 'Ideal' OLR based on AS has been determined at 1.67 kgCOD/m^3·day. This aligns with the typical loading rate for AS described by Logan et al. [61] of 0.2 - 2 kgCOD/m^3·day, or slightly above that described by Rittman's et al. [69] of 0.5 - 1 kgCOD/m^3·day.

The ideal system needs to have a similar or higher OLR, to the treatment process it is replacing to allow it to sit on the same land footprint. A lower OLR would possibly require the purchase of land and building of new infrastructure, which would add a large cost to any proposed change and may be prohibitive. In many of the papers reviewed, the organic loading rate was not given and sometimes the influent COD of the substrate was not given either. In these cases an estimate of the influent COD has been made using the average effluent and COD removal rates. Calculation of the loading rate was possible when a HRT was reported (see Appendix A).

2.2.4 Reactor depth

With a retention time of 6 - 8 hours, the size of the AS tank is governed by the population it serves. As these can range from small towns to large cities, the volume will vary hugely. To limit the need for large scale modifications to infrastructure, new systems should be compatible with the AS tanks currently in place. One of the biggest challenges in advancing MECs is the scale–up of designs. Problems include lower power densities, hydrogen production decline, cost increase, and manufacturing [5, 10, 11, 13]. Currently the biggest reactor operated was 1000L, with several others between 20 and 200L; yet the majority are less than 1L. Most of the large-scale reactors have been modular in design, with multiple electrode pairs run together within the same tank. At pilot-scale the number of these units has ranged from 3 - 24 [1], yet this number could be increased to fill the volume of a larger tank. Size of reactor, in terms of volume, is therefore not such a useful comparison.

Although AS tanks come in a wide range of sizes they all have approximately the same depth, typically between 3 - 6m [92, 113]. The depth controls the aeration efficiency, which can range from 0.5 - 1.5 kg O2/kWh when using a surface aerator [114]. They are designed to be deep enough to maximise oxygen contact with the bacteria, yet not so deep as to increase the head pressure and reduce the efficiency of the blower [86, 115]. The depth of the tank is therefore taken in this case to represent the value of the ideal size the system needs to attain. Assuming retrofitting of existing infrastructure, and that each electrode should reach from the bottom of the tank to the surface, an ideal value of 3m is used. Depth will be critical to many of the issues relating to scaling MECs. Manufacturing electrodes with dimensions of 3m or over will be difficult, and factors such as resistance, water pressure, turbulence and sludge accumulation will also become more significant at this scale, and may be difficult to predict based on smaller systems.

2.2.5 Volumetric treatment rate

The volumetric treatment rate is a measure of the ability for the systems to successfully reduce the COD of the substrate to the required concentrations, taking into account reactor volume, flow rate, substrate strength, removal rate and effluent standards. It gives a clearer comparison between reactors that utilise different operating conditions. A replacement system would not only need to cope with the organic loading, as previously discussed, but also achieve national discharge standards of <125 mg/L or >75% removal [17]. Therefore, taking the calculated loading rate of 1.67 kgCOD/m^3·day, a volumetric treatment rate of 1.25 kgCOD/m^3·day has been determined using the 75% removal requirement [17]. The VTR can also be calculated as follows:

$$Ks = \frac{CODr}{HRT \times 1000}$$

where CODr is the COD removed (mg/L), HRT is the hydraulic retention time (days), and VTR is the volumetric treatment rate (kgCOD/m^3·day).

2.2.6 Energetic treatment balance

The AS process uses energy to power the air blowers that aerate the tanks. The amount of energy can be calculated per gram of COD removed, giving the energetic treatment cost. This value will be highly variable based on the individual equipment and the aeration regime used at different wastewater treatment plants. Therefore, finding values in the literature is difficult. Pant et al. [58] determined that the energy consumption for AS lies in the range of 0.7 – 2 kWh/kgCOD removed, which converts to 2.52 – 7.2 kJ/gCOD removed, and Li et al. [93] reports 1.08 – 2.1 kJ/gCOD. We chose the value of 2 kJ/gCOD as an ambitious but realistic target.

For each pilot study reported, the energetic treatment balance is calculated by first determining the energy costs, and then the energy gains from the hydrogen produced using the following calculations.

Energy cost:

$$kJ/gCOD = \frac{(V \times A) \times 86.4}{gCOD/day}$$

Energy recovery:

$$kJ/gCOD = \frac{\frac{LH_2}{L \cdot day^{-1}} \times Volume(L) \times \%H_2 \times \frac{H_2kg}{m^3} \times \frac{H_2MJ}{kg}}{gCOD \cdot day^{-1}}$$

Where gas has been recovered, the energy content of this has been calculated and compared to that of grams of COD removal. Volumetric densities used for methane and hydrogen are 0.656 kg/m^3 and 0.0898 kg/m^3 respectively, with energy densities (higher heating values) of 55.6 MJ/kg CH_4 and 142 MJ/kg H_2 [116]. The energy balance is therefore the difference between the cost and recovery (kJ) to remove 1 gram of COD.

The energetic treatment balance is an important parameter as it takes into account the OLR, the efficiency of COD removal, power input, and energy recovered as a usable product. In theory MECs should be energy positive. Successful reactors will offset this cost by energy recovery from gas production, i.e. they should produce energy per gram of COD removed. The pilot studies reviewed may not achieve this; however, they may still have a lower energetic cost than AS.

It is noted within these costs that pumping the wastewater is not considered. It is likely that this may be similar between AS and an MEC, if a desired treatment capacity is similar. It is also acknowledged that the energy recovery from the gas may be an overestimation; for example if the gas needed separation, purification or pressurisation, then this would add cost.

2.2.7 Temperature

The temperature at which biological treatment is run is a critical parameter, but will also be highly variable across the world and with seasons. Lower temperatures slow down biological activity, and in the case of anaerobic digestion have been shown to be a limiting factor [117]. In both the USA and UK winter temperatures will reach below zero; however, there is a latent heat within wastewater caused by biological activity. Average wastewater temperatures recorded in the UK are in the range of 10 - 25°C [94]. The value of 10°C is used as the ideal as this is the most challenging temperature these systems might face.

2.3 Research method

Rose diagrams are used to standardise and visualise a series of metrics about the performance of a system which may be of differing scales and dimensions. They are commonly used in the computer game industry, for example to plot the speed, manoeuvrability, acceleration, and cost of a racing car. They can also be a powerful data visualisation method in research [118]. The multiple parameters are presented in a circular plot where the centre value is the 'worst' and the outer circle the 'best' (these values could be high or low). The proportion of the petal filled in gives an indication of how good the unit is in that parameter; with all petals filled in they give a quick impression of the total performance across the multiple parameters. In this study, they are used to show the performance of each of the pilot studies, by indicating how far they are away from the operational parameters they need to meet in order to replace AS (Figure 2.2).

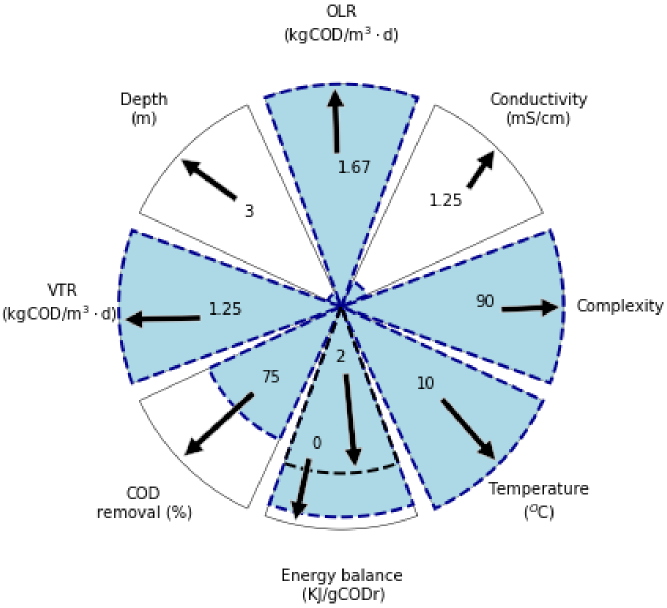


Figure 2.2: An example rose diagram to represent the actual values for each component.

For each pilot study used, the values for each of these parameters are taken directly from the paper, or calculated or estimated based on the information contained within them (Figure 2.3, 2.4). In some cases the values have been re-calculated and are different from those directly in the paper, in order to ensure the method for calculation is comparable across the studies (see Appendix A). The values for each of the studies are then shown as a shaded section of the rose petal. Where data is entirely absent from the paper and cannot be reasonably estimated or calculated, the entire petal is removed. In a perfect situation all petals of the rose would be fully shaded. Each rose petal is scaled using the parameters in Table 2.2. In most cases the inside of the petal is zero, representing the very worst level of operation. For some of the parameters (conductivity, temperature and energy balance) a smaller number is desirable, and in this case the petals are scaled in the opposite direction, again with the centre representing the worst level of operation. In the case of the energetic balance, as described in Section 2.2.6, two ideal values are plotted. The outer boundary of the petal is the point at which the MEC is energy neutral, as would be desirable, but the inner dashed line is the energetic cost of AS. A shaded petal above this line but below the outer limit is an MEC that has better energy costs than AS, but is not yet energy neutral. Full details of the scaling are given in Appendix A.

The term 'pilot-scale' is used differently among different studies, as is the word 'scalable'. Pilot-scale, or scalable reactors should be of a design which has the potential to be scaled; to use real wastewater; to be operated outside the laboratory; and to be continuously flowing. Wang et al. [119] analysed the language used in BES research and found that of all the articles with 'scalable or pilot-scale BES' in the title, only 13.8% meet the above criteria. San–Martin et al. [87] and Sugnaux et al. [120] also review work on pilot-scale BESs, and discount all pilot studies which operate solely in batch mode. For our review we have selected to use only those studies which use continuous flow, are at a scale over 0.01m in height and run over a greater length of time than 1 month. This gives us twelve pilot studies in total.

2.4 Description of the rose diagrams

2.4.1 Wastewater complexity

Nine out of the twelve studies have a complexity value as good as that needed to replace AS. Additionally, all of these studies use real domestic wastewater, therefore the other complexities, (not simply the number of constituent parts as discussed in Section 2.2.1) will have been overcome. It is clear that although there may be reduced performance with wastewater, which has been well documented in laboratory studies [121], this is not preventing pilot-scale MEC reactors from working. The rose diagrams show that the MEC pilots which operate with real wastewater do not have generally lower performance in the other sections than those operated with synthetic substrates or simpler wastewaters.

The pilot study by Cusick et al. [1] was also run on real wastewater from a winery. It is important as industrial wastewaters such as this might be an easier entry point for commercialisation of MEC technology. Analysis of the constituent parts of this wastewater was not given, but Mosse et al. [122] lists an average of 26 different organic compounds in 10 different winery wastewaters, and therefore this value is used. This pilot study demonstrates the applicability of MECs for industrial wastewaters as well as domestic or municipal wastewaters, as has been reviewed elsewhere [121].

Only two of the pilot studies use synthetic wastewater [12, 13] and in both cases acetate is

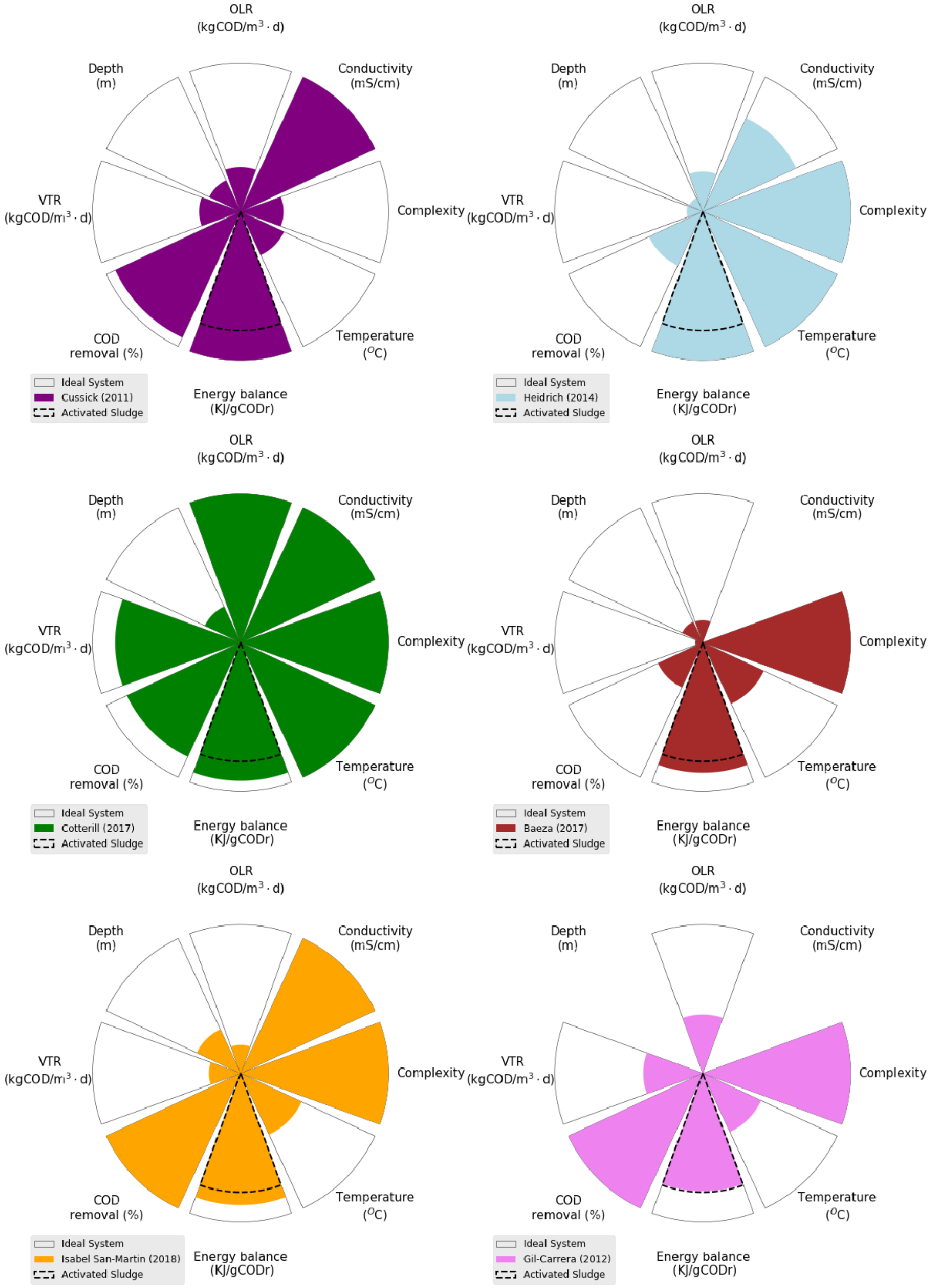


Figure 2.3: Rose diagrams to compare the operational performance of each pilot-scale MEC against the parameters for activated sludge as indicated by the outer edge of the rose petals, in the case of energy balance the dotted line represents the level for activated sludge, the outer petal is an energy neutral system, as would be the aim with an MEC.

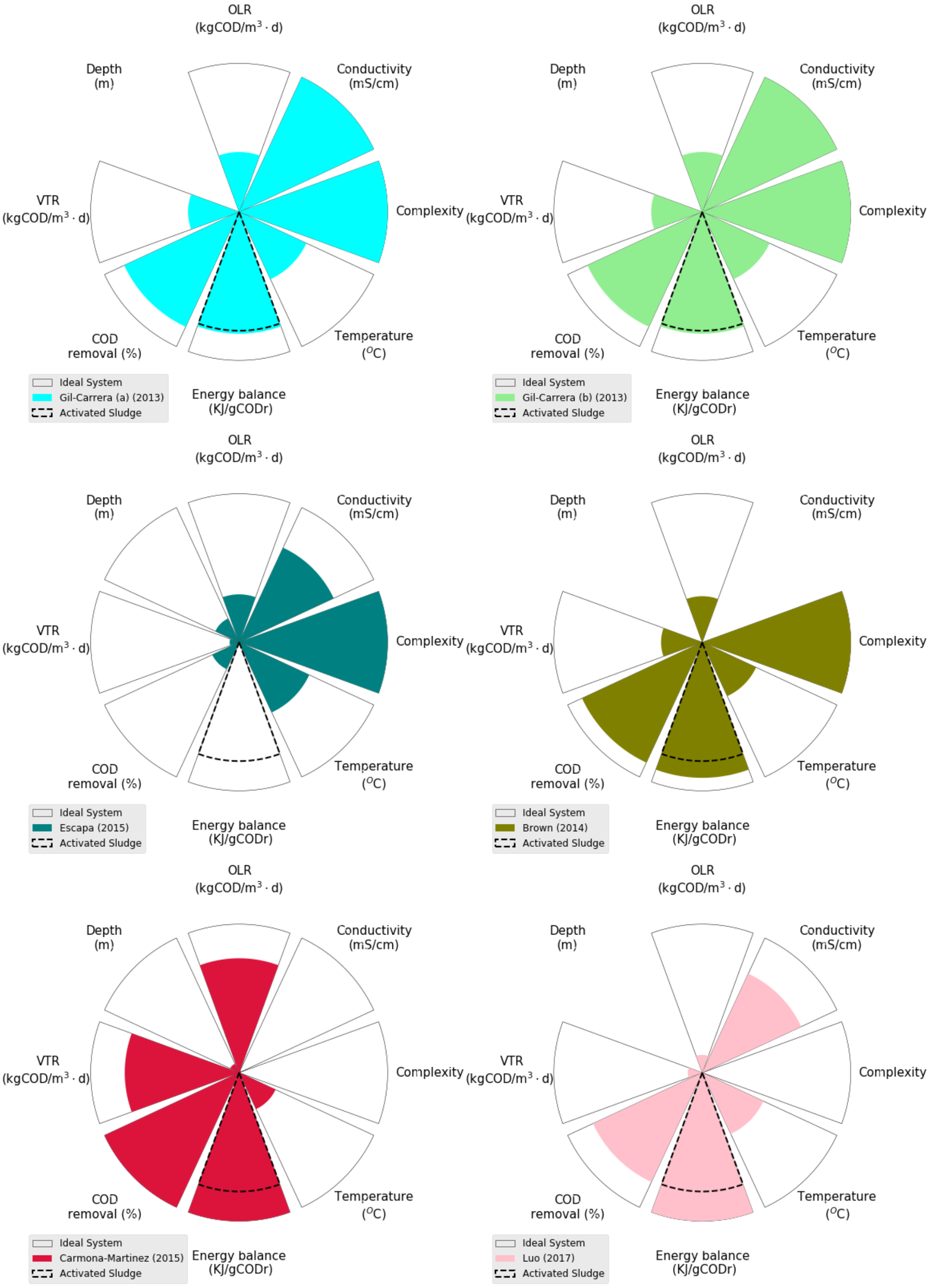


Figure 2.4: Rose diagrams to compare the operational performance of each pilot-scale MEC against the parameters for activated sludge as indicated by the outer edge of the rose petals, in the case of energy balance the dotted line represents the level for activated sludge, the outer petal is an energy neutral system, as would be the aim with an MEC.

Table 2.3: A table of operational performances from each pilot-scale MEC reviewed. These have either been taken directly from the papers, or calculated based on reported values. Justification and calculations of each parameter can be found in Appendix A.

Paper	**Date published**	**Complexity**	**Conductivity (mS/cm)**	**OLR (kgCOD/m^3·day)**	**Depth (m)**	**VTR (kgCOD/m^3·day)**	**COD removal (%)**	**Energetic cost (kJ/gCOD)**	**Energy recovered (kJ/gCOD)**	**Energy balance (kJ/gCOD)**	**Temperature (°C)**
Cusick	2011 [1]	26	0.70	0.50	0.7	0.35	70	1.64	14.34	-12.70	31.0
Heidrich	2013/14 [2, 3]	90	1.80	0.45	0.3	0.14	30	1.37	1.37	-0.005	8.5
Cotterill	2017 [4]	90	0.81	1.67	0.8	1.06	64	0.73	0.04	0.69	9.9
Baeza	2017 [5]	90	n/a	0.25	0.46	0.06	25	7.84	6.60	1.24	22.0
San-Martín	2018 [6]	90	0.80	0.32	0.98	0.27	84	1.13	0	1.13	22.0
Gil-Carrera (a)	2013 [7]	90	n/a	0.66	n/a	0.50	76	3.24	1.16	2.08	23.0
Gil-Carrera (b)	2013 [8]	90	0.61	0.67	n/a	0.43	64	3.14	1.34	1.80	20.0
Gil-Carrera (c)	2013 [9]	90	0.61	0.67	n/a	0.43	64	3.14	1.34	1.80	20.0
Escapa	2015 [10]	90	1.78	0.54	0.53	0.08	15	25.20	0	25.20	19.2
Brown	2014 [11]	90	n/a	0.52	n/a	0.35	67	0.87	0	0.87	25.0
Carmona-Martinez	2015 [12]	1	90	1.28	0.2	0.96	75	0.78	2.65	-1.87	37.0
Luo	2017 [13]	1	1.70	0.20	n/a	0.12	61	0.55	9.51	-8.95	22.0

used as the sole carbon source. In the case of Carmona-Martínez et al. [12] this coincides with good performance in terms of energy recovery, OLRs and VTRs; however, the use of saline conditions may have been the cause for this. The performance of the other reactors which were fed synthetic wastewater was similar to studies using real wastewaters. The use of synthetic substrates is sometimes justified on the basis that it allows the reactor to function better, and therefore enables greater understanding and optimisation, which can be the case at a laboratory scale. However, this review suggests that the use of real wastewaters is not the limiting factor in pilot systems.

Applications of BESs for wastewater treatment will rely on their ability to treat complex mixtures of organic compounds. Even relatively simple wastewaters which are high in sugar, such as winery waste, have different and complex components [107] that require multiple stages of digestion by different organisms. It is clear from the low conversion efficiencies in the pilot studies examined, that these complex waste organics are not all transferred to current. Understanding and optimising the metabolic pathways in BESs will be vital for their successful application. Increasing the efficiency of which waste organics are converted into products will increase both treatment and resource recovery.

2.4.2 Conductivity

The use of real, non-supplemented wastewaters in most of the studies means that the conductivity was of a level found in real wastewater, though there is inherent variability in wastewater from different places and of different concentrations. In the case of the pilot reactor by Heidrich et al. [2], wastewater conductivity was higher than the ideal wastewater value at 1.80 mS/cm. This is possibly due to the use of raw wastewater, whereas other studies used effluent from primary settlement [4, 5, 6, 9, 11]. Escapa et al. [10] also used primary effluent but this had a conductivity similar to the raw wastewater, showing the inherent differences in wastewater taken from different areas. In the study by Heidrich et al. [2], the energy recovery was high, reaching the energy neutral level, which may suggest this higher conductivity inferred an advantage by reducing resistance loss. However, this was also achieved by Cusick et al. [1] who had a conductivity lower than 1.25 mS/cm.

Only four of the pilot studies reviewed had a conductivity higher than that which would be needed in our ideal system. Carmona-Martínez et al. [12] used saline water. This conductivity was in an order of magnitude higher than those other studies, being in the range of 90 mS/cm, but this did not produce better performance in other areas such as energy recovery. Luo et al. [13] used a synthetic medium with 1.70 mS/cm conductivity, and did have high energy recovery. However, this was lower than the real wastewater used by Heidrich et al. [2] and Escapa et al. [10]. In this case, the use of acetate may have been of greater significance.

In the study by Cusick et al. [1] there was an addition of a phosphate buffer, and average conductivity was 1.80 mS/cm. During boiler water dilution this was 0.70 mS/cm.

Interestingly, it was reported that a decrease in conductivity had little to no effect on the reactor performance. Similarly, Tartakovsky et al. [123] noticed only a minor impact on MEC performance when conductivity was dropped from 15 to 9 mS/cm. However, Verea et al. [110], observed that an increase of 7.5 mS/cm to 15 mS/cm resulted in hydrogen production rates of 0.13 to 0.82 m^3H_2/m^3day respectively in an acetate fed reactor.

Application of this technology cannot rely on the artificial supplementation of conductivity, as this would be too costly to achieve with high volumes of liquid. It is possible that this technology could be targeted only at wastewaters from certain industry, which already have high conductivity or salinity; however this would be a very small market. From the studies reported here it is seen that lower conductivities do not automatically mean lower performance, and that MECs can function with the increased resistance caused by the conductivities experienced in real wastewaters. Advances in material science and reactor design to overcome these resistances, may be a better solution to the low ionic conductivity. Supplementing conductivity with a buffer is not likely to be a cost effective or practical solution for large-scale treatment, and nor does it seem necessary.

2.4.3 Organic loading rate

The organic loading rate gives the value of the amount of organic substrate that the reactor receives per day, and is a balance between the influent COD and the HRT. In nearly all the pilot reactors, the OLR is considerably lower than that of the ideal system. This means that reactor infrastructure, were this to be built to scale, would need to be considerably larger than that currently used for AS. This is likely to be very costly, and may not be feasible, especially in urban settings. It therefore represents a critical area for improvement, and should be a focus for future pilot studies.

The ideal OLR is based on a COD of 500 mg/L, which is higher than many of the influent CODs used in the pilot studies. This means even in those with a HRT approximating that of AS (i.e. 8 hours) the OLR was the same or lower. The only pilot study that achieved the necessary OLR was Cotterill et al. [4]. Here, even though the influent COD was lower than 500 mg/L, the fast HRT of 5 hours resulted in a OLR of 1.67 $kgCOD/m^3\cdot$day. Although decreasing the residence time of the substrate has been shown to decrease COD removal [8], Cotterill et al. [4] still achieved average effluent to match discharge standards at this speed. Gil-Carrera et al. [8] also had a fast HRT of 4 hours, but only achieved a OLR of and 0.67 $kgCOD/m^3\cdot$day, due to the very low strength wastewater (COD of 112 mg/L).

In the pilot studies with a low OLR, the low rate was often due to a longer HRT than is typical in an AS treatment plant. With the high strength wastewater used in the Cusick et al. [1], a reduction from 1 day to an 18-hour retention time would see it fit with the ideal OLR. Baeza et al. [5], Heidrich et al. [2] and San-Martin et al. [6] used similar strength wastewater to the average value, and so would need to reduce the HRTs down to 8, 5.8 and 5.8 hours respectively. However, the rest of the studies [6, 9, 10, 11, 13] used a wastewater or synthetic equivalent with values much lower than average wastewater. These would have to operate at a retention time of less than 5 hours, and in cases such as Gil-Carrera et al. [8], as low as 1.5 hours. This may well impact the diffusion of substrate into the biofilm layer and reduce performance, and it could also cause biofilm detachment. Simply reducing the retention time will most likely reduce the removal rates and worsen effluent quality (as shown by Gil-Carrera et al. [9]), although the study by Cotterill et al. [4] shows that HRT can be faster than in AS. Most reactors in the study were only run at one single HRT. Therefore, optimising the HRT would be a valuable step forward in understanding how this technology fits with existing infrastructure, and where it might best be applied.

2.4.4 Reactor depth

Cusick et al. [1] used the biggest reactor to date [87], with 24 modules containing 6 electrode pairs each. The electrodes in this reactor were 0.7m high, which is one fifth of the minimum 3m needed. In this study, performance was compared to small-scale laboratory reactors (2.5cm high), which were run in the same conditions, and it was found that the estimated possible current density was 44% less. The authors attributed this to changes to the reactor design, specifically the use of stainless steel mesh compared to platinum carbon cloth cathodes. However, greater resistance and the relatively slow start up were also mentioned. If the reactor increased to 3m of height, it is expected that further loss in performance would occur. If this was at the same rate seen between the laboratory and pilot scales, this would cause failure.

Heidrich et al. [2] adopted a different design, and reported the size of the electrodes as 0.2m wide by 0.3m high, 10 times smaller than would be needed to fit into AS tanks. The electrodes for Cotterill et al. [4] and Baeza et al. [5] were based on the Heidrich et al. [2] design, but were 0.8m and 0.46m high respectively. Cotterill et al. [4] operated $1m^2$ anodes, in the dimensions of 1.2m by 0.8m high, which makes them the second tallest electrodes to date. In this study a smaller scale MEC in-situ was run alongside the larger one so that this scale-up can be directly compared. Increasing the size of the electrodes by 16 times did not have a detrimental impact on current density or other performance metrics, though hydrogen production was reduced. However, microbial contamination of the cathode chamber was the likely cause of this, rather than scale. The study did also highlight the unwieldy and structurally weak design of the flat plate anodes at this size, suggesting that further scaling of this design may not be possible.

The largest electrodes used in a pilot study to date was in the study by San-Martín et al. [6], which used 0.98m deep by 0.48m wide anodes. Although modular, these had a different design than previous reactors in which the cathode chamber was equally as large as the anodic, and the wastewater was recirculated through both chambers to facilitate denitrification and the subsequent conversion of nitrite to molecular nitrogen. These could still be placed within an AS tank, although modification to the design might present some difficulty in order to keep each chamber sealed. As these are the largest electrodes, they are the closest to fitting the ideal scenario. When operated as an MFC, the author comments that power densities were much lower than similar smaller laboratory scale designs (0.06 mW/m^2 from $0.47m^2$ anodes compared to 3.6 W/m^2 from $0.005m^2$ anodes [124]).

The rest of the reactors were not modular, and so are not compatible with an AS tank. Carmona-Martínez et al. [12] had a 20cm high anode, but at 1cm thick, the scale-up to 3m would be structurally difficult. The remaining reactors used anodes and designs too small to be considered able to scale-up to 3m [7, 8, 11, 13].

Depth may be a critical factor in the application of BESs. Existing wastewater treatment technologies, even those with a large land footprint such as treatment ponds and trickling filters, operate at depths much greater than has been tested with these pilot systems. Stacking multiple using on top of each other could be a solution, however, this may be difficult to implement, and is likely to add to both building and pumping costs. Pilot-scale demonstration of this technology over large depths will be vital in understanding if this technology is suitable for applications beyond those which are very small and localised. Research at this scale is needed, as in addition to the practical issues of building structurally sound electrodes to fit this size, there may be important effects on the microbiology, thermodynamics, hydraulics and electrochemistry caused by the pressure difference at depth.

2.4.5 Volumetric treatment rate

The volumetric treatment rate is a similar parameter to OLR, and is necessary in determining if the MEC could fit into existing infrastructure. It is arguably more important than OLR as it is the actual amount of wastewater that can be treated per unit area. Simply increasing the HRT could result in optimal OLRs as discussed above, but if the substrate or wastewater is then moving too quickly through the reactor for the substrate to diffuse into the biofilm, it will not treat it. Due to the modular designs of the electrodes for some reactors, more could be added into the tank, reducing the anode working volume but increasing anodic surface area. This would reduce the retention time while increasing the loading rate and increasing the ratio of the wastewater which comes into contact with the anodes and the biofilm. Increased anode surface area has been shown to enable greater COD removal, and subsequently increased reactor performance [125]. Studies to optimise HRT must be based on the VTR. If a critical HRT can be found, which achieves similar removal efficiency (percentage of COD removed) but at a faster rate, this will boost the VTR. Recio-Garrido et al. [126] used a combined bioelectrochemical–electrical model to investigate HRT on removal efficiency, removal rate and power production. It was found that a retention time of 10.5 hours was the optimum for COD removal rates, and any higher resulted in the same effluent quality but lower treatment rates.

The highest VTR is in Cotterill et al.'s [4] reactor. This reactor was run on relatively low strength wastewater, but the authors did note that COD removal efficiency was higher when the strength of influent COD was higher. It is possible therefore that if this reactor was run with higher strength wastewater, the target VTR may have been reached. The treatment rate reported by Carmona-Martínez et al. [12], when the reactor was fed 0.64 g/L of acetate, is also high (0.96 kgCOD/m^3·day). This reactor was also subject to increased loading rates up 6.4 g/L of acetate. Although these values are not used in the rose diagrams, as they are unrealistic in comparison to the concentration of acetate found in wastewater, it shows that a VTR of 7.04 kgCOD/m^3·day could be achieved in this reactor.

All the other reactors had very low VTRs, and the worst performing reactors in terms of pollutant removal were Baeza et al. [5] and Escapa et al. [10] with removal rates of 0.06 and 0.08 kgCOD/m^3·day respectively. Baeza et al. [5] altered the HRT following the unsuccessful pollutant removal. It was found that a 10-day HRT was needed to achieve 72% COD removal. With an OLR of 0.05 kgCOD/m^3·day, this would result in a VTR of 0.04 kgCOD/m^3·day, 34.7 times lower than needed.

Volumetric treatment rate is arguably the most important parameter for understanding the applicability of this technology in the wastewater treatment industry. It encompasses the removal rates of the organic pollutants, flow rates and reactor size. The pilot studies examined show a range in VTRs of between 0.06 – 1.06 kgCOD/m^3·day, the worst performing reactor would need to be over 17 times larger than the best performing reactor to treat the same amount of wastewater. Furthermore, all the VTRs achieved by the pilot studies are lower than AS, so more space would be required. This has a significant impact on their applicability into existing treatment work, and would also add to the material costs of the reactors. Understanding and defining the optimal rate at which substrate can be transferred out of the wastewater flow and into the BES biofilm will be vital in taking this technology forward. With this value the correct size of reactor can be designed for the given flow rate, and the costs accurately estimated.

2.4.6 Effluent quality

Volumetric treatment rate and effluent quality are highly connected, the solutions for the reactors need to benefit both. Running the reactors at different retention times and measuring COD removal would then help indicate at what point the system ran at peak removal rates. Once this has been found, improving the efficiency of COD removal at this loading rate would help the reactor produce an effluent quality closer to the ideal model of <125 mgCOD/L, or a 75% removal rate, while also fitting with the VTR of an ideal system. As the organic compounds in the wastewater have to be funnelled through the anode as current, the anode could be the component that needs optimisation. There have been a number of anode pre-treatment techniques used in order to make the system more efficient, including: heat treatment, chemical treatment and high temperature ammonia gas treatment [127, 128], although these have been used in the laboratory, not at pilot scale.

Escapa et al. [10], Baeza et al. [5] and Heidrich et al. [2] had the lowest COD removal, with average values of 15%, 25% and 30% respectively. As these reactors operated longer HRTs than desired, but still did not remove enough pollutants, a greater anode to volume ratio to improve the loading rate may also benefit the treatment rate. Increased surface area of the anode has been reported to increase both current densities and subsequent organic degradation when using real wastewater [129].

Those reactors which achieved discharge standards [4, 6, 7] all used lower than average strength wastewater. Gil-Carrera et al. [8] achieved discharge standards, but was run using a wastewater already below 125 mg/L COD. Cotterill et al. [4] suggested that the low influent COD was detrimental to the start-up and hydrogen production of the reactor. Lower strength wastewater has been reported to limit current densities and treatment rates [104]. Targeting an expensive technology such as an MEC at a wastewater stream that needs minimal treatment is unlikely to be viable. Importantly, these studies do show that MECs can continue to work down to low COD levels, and that it would be possible with the correct volume of treatment space to reduce COD from a loading of 500 mg/L to below 125 mg/L. Reactors which achieved relatively high removal efficiencies, but not high enough removal rates to achieve discharge standards, could be improved by optimising HRT, anode surface area and reactor size.

To be successful for wastewater treatment applications, BESs must not only demonstrate that they are able to meet discharge standards, but that they can also do this reliably and consistently. Variability in the levels of performance in these systems to date [3, 4] could be a significant problem. Initial applications of these technologies may be better suited to places within the treatment works where full treatment in not required, i.e. where they would be a pre-treatment rather than a finishing step. They might also be better placed where the organic load is high, and therefore the costs of building these reactors can be mitigated by the reduction in energy cost of high COD removal via aerobic digestion.

Currently in the UK and Europe, effluent quality is primarily focused on removal of the organic load (through BOD, COD and total suspended solids), and levels of Nitrogen and Phosphate. It is possible in the future that these regulations will be extended to other pollutants such as metals and pharmaceuticals [43]. Investigating the ability of BESs to remove these other pollutants and harvest them as resource could offer a significant advantage of this technology over those currently available.

2.4.7 Energetic treatment balance

Most of the reactors had an energy treatment cost that is lower than AS, and some reached an energy neutral and even positive state. The most cost efficient reactor to date in terms of energy recovery was the reactor fed winery wastewater by Cusick et al [1]. This had an energetic treatment cost of 1.64 kJ/gCOD removed, less than typical AS costs of $\leq$ 2 kJ/gCOD removed, and the energy recovered was 14.34 kJ/gCOD removed. This gives a net energy positive energetic treatment balance of +12 kJ/gCOD removed, although this seems very high given the energy value of 16.1 kJ/gCOD for wastewater, or indeed the value for glucose of 14.6 kJ/gCOD [21]. The methane content produced was much higher than the calculated stoichiometric conversion of current to methane, and it is suggested that current was produced from COD removal, while anaerobic digestion occurred separately. This would most likely be due to the installation of the thermostat to heat the wastewater to 31°C. The energetic cost of heating was not considered in the balance. However, heating a litre of water by 10°C requires 41.9 kJ, equivalent to the energy it would contain with a COD of 2600 mg/L. Carmona-Martínez et al. [12] and Luo et al. [13] also achieved a positive energy balance with +1.87 and +8.95 kJ/gCOD removed respectively. However, in both these cases synthetic wastewater was used, with the substrate being acetate.

In order to compare Heidrich et al. [2] with other papers, it was necessary to recalculate the energy balance by only using one face of the anode. This recalculation shows this reactor was also very marginally energy positive, gaining +0.005 kJ/gCOD removed. Cotterill et al. [4] used a similar but larger reactor design and had similar energy costs to power the systems. However, due to the poor hydrogen capture this reactor was not energy positive, and required a total net energy input of 0.69 kJ/gCOD removed. It should be noted that this is still less than AS. Gil-Carrera et al. [7], Gil-Carrera et al. [8] and Brown et al. [11] also all achieved energy balances less than AS, with 2.08, 1.8 and 0.874 kJ/gCOD respectively. In the studies by both Gil-Carrera et al. [7] and Gil-Carrera et al. [8] the energy cost was higher than AS, but successful hydrogen recovery counterbalanced this. Baeza et al. [5] had one of the highest energy costs, 7.84 kJ/gCOD, due to the 1.5V potential difference used. However, due to the high hydrogen production, and low COD removed, the energy balance was still better than the AS comparison, with a value of 1.24 kJ/gCOD removed. The pilot project by Isabel San-Martín et al. [6] also performed less well with respect to this parameter. The gas produced comprised of a mix of methane, CO_2 and hydrogen but it was in such small quantities, that virtually no energy was recovered from the system. In the study by Escapa et al. [10], the calculated treatment cost of 25.2 kJ/gCOD is the largest of the discussed reactors (see Appendix A). Part of the problem of addressing the energy balance in MECs is the consistency with which terms are calculated and reported [119]. However, it has been shown that even in these first pilot studies, where build quality and conditions may not be optimal, energy neutrality can be achieved.

Actual application of BESs may however require a greater incentive than energy neutrality. Even with advancements in finding low cost materials, these reactors will be far more costly to build than the existing technologies [34]. The ability for BESs to harness reducing equivalents from wastewater, and then convert these into products is the technology's greatest asset. By manipulation of the cathode reaction it is possible to produce different products that may have higher value than the energy value alone [93]. This means that they have the potential to produce different things at different times, responding to industrial need, market prices and incentive schemes. The energy balance remains an important performance parameter, and future research should seek to improve upon this, in particular by reducing the energy costs: lower overpotentials on the anode would mean less energy input [46]. This should be a goal of design modifications. Future research should also target the efficient bioproduction of different products at the cathode which may bring greater cost benefits. Defining a way in which to incorporate this into the energy balance, for example by using the energy offset by typical industrial production, will also be important.

2.4.8 Temperature

The temperatures at which the reactors are run is critical if they are going to operate under ambient conditions in temperate climates, as heating raw wastewater on an industrial scale will never be viable. Unfortunately the majority of pilot studies reviewed were either set up in a laboratory, were artificially heated, or both, operating at over 22°C. Only 2 have shown that MEC wastewater treatment can operate all year round outside of the laboratory [3, 4]. Heidrich et al. [2] reports minimum and maximum wastewater temperatures of 8.5 – 27.0°C for the influent, highlighting the large temperature range MECs will have to cope with if placed on a wastewater treatment site. Cotterill et al. [4] also operated a reactor at ambient temperatures, with the average temperature during start-up 9.9°C. It was discussed that this low temperature could have been the cause of the longer than expected start up. Despite laboratory work showing a correlation between performance and temperature [130], seasonal variations in both of these studies did not map significantly onto changes in performance. This is likely to be due to other factors masking the relationship, as all biological activity is affected by temperatures. Fully understanding this relationship will be important in predicting reactor behaviour and performance, and it is possible they may have to run at an energetic and financial loss in the winter and make this up in the summer.

Low temperatures are described as the Achilles heel in advancing anaerobic digestion technology [117], and so a major advantage of MECs over AD is their ability to operate at low temperatures. Gaining a full understanding of this disparity, despite the similar food chains involved could be vital in advancing not only BESs, but also low temperature AD. Initially application of this technology is likely to be in places, or on waste streams, where there is a high energy load i.e. a high concentration of organic matter, but where anaerobic digestion is not possible. This may be on small-scale operations where heating is not viable, or on the treating AD centrate which is too liquid to be fed into the AD process. It is clear that to gain this information needed, to both advance the fundamental science of these systems, and for their applications, testing, validation and experimentation should be done in environmentally relevant conditions. In order to develop a full understanding of the limits of BESs, reactors deemed to be pilot-scale, which are aiming to advance the technology towards application, should be operated at representative temperatures for the UK, Europe and North America.

2.5 Practical and policy implications of this study

Currently, MEC technology sits around the technology readiness level (TRL) 5: there has been validation of the technology in a relevant environment [131]. Future research should therefore seek to optimise the performance, rather than just demonstrate it. Based on the analysis of the different pilot studies, this chapter has highlighted two key areas required for BES to become a competitive wastewater treatment technology. Firstly, achieving the optimal volumetric treatment rates will be vital in understanding the applicability of this technology. Previous literature shows that increasing the organic loading rate can boost this volumetric treatment [132]. This can be achieved either by increasing the strength of the wastewater or by increasing its flow rate [133]. Each of these modifications will increase the rate at which the organics within the wastewater are supplied into the reactor, which in turn should boost the rate of organic removal. Only by fully understanding and optimising this mass transfer can the correct size of reactor be designed for the given flow rate and the costs accurately estimated.

Secondly, size also remains a significant problem. The largest MEC to date was 1000L, with a hydraulic retention time of 1 day [1]. This is far from the size required at wastewater treatment operations. Many BESs have therefore been designed with modular electrodes, multiple units that can be placed in any existing tank. However, these existing tanks are deep, and this depth is necessary to achieve treatment on the small land footprint in urban areas. Designing an electrode that can span to the depth of 3m will need to overcome the effect that hydrostatic pressure has on both the biofilm formation, the performance and the structural integrity. It will also need to cope with the changes in the thermodynamic and kinetic properties of the biological and electrochemical processes. Further research investigating these new reactor designs that retain the same land foot print of existing assets is needed.

A further issue demonstrated in this research, and which has been highlighted previously [55, 134], is the standardisation of the design, methods and the reporting of BESs. The data we present in this study seeks to compare the performance of each pilot-scale study and the parameters used in the wastewater treatment industry. However, comparable information was often difficult to find within the research papers, and in some cases was absent or had to be calculated from other values given. If policy makers and industry promote this technology, relevant data must be presented in a clear and systematic way. The commercial implementation of these systems will depend not only on their ability to meet with current and future wastewater treatment regulations, but will also need to consider policy decisions, energy targets, and carbon trade schemes [135]. If BES technologies are to reach their full potential, researchers must not only provide the data to show this is possible, but also present it so that it can be accessed by those which will promote or implement these.

The barriers to bring this technology further from TRL 5 are not only technical but also related to policy incentives for innovation. The strict regulatory constraints on performance and the economics of water utilities do not favour investments in technologies that cannot fulfil both criteria straight away. However, the imperative of defining zero-carbon pathways should benefit technologies able to reach this goal. BES technologies fit in this category. Accordingly, there is strong argument for R&D policies able to invest in deblocking the technical constraints of BES technologies, and for utility regulatory frameworks that encourage explorative applications in real settings, in order to bring them to practice in the future.

2.6 Conclusion

The journey towards zero carbon will require far-reaching solutions across all aspects of the human/ environment interaction. Wastewater treatment would seem like an easy win in this regard, as there is more energy contained within the wastewater than is currently being used to treat it. However, the locked-in infrastructure and strict environmental regulation to protect receiving waters means change is difficult to implement and taking risks are avoided. The benefits of MECs are clear, as they are simultaneously treating wastewater while recovering the energy harnessed from the organics as valuable products. Their ability to do this with dilute wastewaters and at low temperatures sets them apart from classical anaerobic digestion. However, the technology is still far from being commercialised. By standardising the data across all of these studies and benchmarking them against industry standards, we are able to identify the operational parameters that MECs are repeatedly able to attain, and importantly clearly identify those where further research is needed. MECs have been shown to cope well with the conditions that might theoretically stop their performance, such as low temperatures, low conductivities and complex real wastewaters. None of these factors appear to be the Achilles heel of MEC operation, and therefore supplementing or amending them is not necessary. There is however, a significant performance gap with MECs and AS in terms of the volumetric treatment rate and the reactor depth. Solving these issues will require both improved reactor design and increased fundamental understanding of the metabolic pathways of waste organic digestion. Critically though, in four out of the twelve pilot studies examined, the MECs were energy neutral or even positive, demonstrating that energy recovery from wastewater treatment using this technology is possible.

Chapter 3

Investigating the COD saturation point in bioelectrochemical systems

3.1 Introduction

The results found in Chapter 2 showed that achieving a volumetric treatment rate (VTR) to equal activated sludge (AS) remains one of the major challenges in scaling up microbial electrolysis cells (MECs). Optimal VTRs would enable the design of correct sized reactors for real flow rates, while also providing accurate predictions on cost savings. In the review of pilot-scale MECs, if each study is taken as one single entity, none met all the criteria required to replace AS. For example, although San-Martin et al. [6] achieved the highest COD removal (84%) when using real wastewater, the organic loading rate (OLR), reactor depth, VTR and temperature all fell short of an ideal system. However, all performance parameters other than VTR and depth were met by a minimum of one reactor. Pilot-scale MEC VTRs have ranged from 0.06 kgCOD/m^3·day [5] to 1.06 kgCOD/m^3·day [4], and for pilot-scale microbial fuel cells (MFCs) these range from 0.53 kgCOD/m^3·day[136] to 0.92 kgCOD/m^3·day [137]. Compared to the average VTR for AS, which is estimated at 1.25 kgCOD/m^3·day (Chapter 2, Section 2.2.5), it is clear that improvement is still needed. VTRs were therefore identified in Chapter 2 as one of the key areas for further research to advance MEC technology.

The majority of current MEC wastewater treatment focusses on the chemical oxygen demand (COD) removal efficiency. Specifically, this indicates how effective the reactors are at treating the wastewater to legal discharge standards ($\leq$125 mg/L [17]). Although this is still important, the volumetric rate at which the reactor achieves this removal is equally significant. Wastewater flowrates at treatment sites are unchangeable. Therefore, reactors need to achieve discharge standards while using the same or faster hydraulic retention time (HRT) as current technologies; otherwise, a much larger land footprint would be needed. For areas with large space, current technologies other than AS are already used. However, in densely populated areas these are not an option.

In a biological system, bacteria will use the organics available to survive. They grow until the available organics are used up, and then decay or cease to metabolise. In a batch-fed BES, this end of growth is represented by reaching a peak current, and the greater the number of electrogens within a BES, the greater the maximum electron uptake rate available. However, maximum VTRs when using a mixed culture of bacteria is not solely dependent on the number of electrogens, but more importantly will be affected by the total number of bacteria within the system, and their ability to oxidise the different organics. Therefore, when operated in batch mode, the relationship between total organics and the theoretical maximum VTR can be described by both the Monod equation and the Michaelis-Menten equation. The Monod equation describes the relationship between the concentration of the limiting substrate and growth of microorganisms [138]. Bacterial growth increases logarithmically with increased substrates, with a plateau occurring due to substrate saturation. The equation is used to model bioremediation in AS plants [139]. The Michaelis-Menten equation describes a similar relationship, however, the relationship here is between the concentration of the limiting substrate and the bacterial reaction rates [138].

Therefore, for a BES with a mixed culture, the theoretical limitations for VTRs will include factors that affect both growth and reaction rates. This includes the available biological space, the lack of required nutrients to enable enzyme activity, and the amount of useable organics available. The biological space is governed by both the size of the anode and the total volume of substrate within the reactor. Maximising the anodic surface area to increase anode-to-substrate volume ratios has been a proven way to increase COD removal [129]. The lack of the desired nutrients can also limit bacterial growth and enzyme activity [140], preventing the breakdown of the substrate and limiting the number of electrons available for electron transfer. Due to this, studies that use a simple substrate such as acetate often supplement their reactors with trace vitamins and minerals to prevent this occurrence [141, 142]. Finally, the amount of organics available for the bacteria within the systems is typically reported as the OLR, which is measured by the total amount of COD supplied to the reactor per volume per day. This can be influenced by either the strength of the substrate, or the rate at which the substrate is supplied into the reactor. COD removal was shown to increase with increased OLRs in a twin tubular semi-pilot MEC [8].

Previous pilot-scale studies have often used low strength wastewaters [4, 6, 7, 8, 9, 11, 13]. There is evidence that this leads to high coulombic efficiencies (CEs) [143] and makes reaching discharge standards easier [17]. However, CE is a measure of how efficient the transfer of electrons is to the anode, not the efficiency of wastewater treatment. Therefore, to compete with AS, there needs to be a focus on VTRs before CEs. Cotterill et al. [4] reported that VTRs decreased as influent COD of the wastewater dropped below 200 mg/L, while a study by Zhang et al. [104] showed that VTRs decreased over time as COD was removed in air-cathode MFCs. At a laboratory scale when using acetate as the substrate, Lee et al. [144] successfully achieved high VTRs of 27 - 49 kgCOD/m^3·day when an MEC was subject to high COD loading rates of 32 - 133 kgCOD/m^3·day. Additionally, Kim et al. [132] reported that high strength swine wastewaters achieved higher VTRs when compared to domestic wastewater. Although an increase in the substrate concentration will boost VTRs, it can be hypothesised that in BESs this will only increase until a certain point. Once the bacterial growth exceeds the biological space available, removal rates will be capped [140]. To the author's knowledge, no study reports how increasing the COD of the substrate results in a maximum VTR in BESs. Understanding this limit will enable determination of the correct strength substrate, while aiding reactor optimisation for high removal rates.

The use of return sludge liquor (RSL), rather than raw domestic wastewater as a substrate for bioelectrochemical systems (BESs) may therefore improve performance. This wastewater is high strength, and is the effluent from the dewatering of sludge at a wastewater treatment plant, which is simply returned to the start of the treatment process. RSL was used briefly by San-Martin et al. [6] with a 150L pilot-scale MEC. However, the majority of data was collected when using raw wastewater and the reactor was only run briefly (four days) with pure RSL (referred to as centrate in the paper, meaning it is the effluent from a centrifuge). When fed with this centrate, the reactor performance appeared to decrease in comparison to the raw domestic wastewater and the authors suggested this could be due to the lower biodegradability of the centrate. Using a high strength domestic wastewater will increase the complexity and the overall number of organics present when compared to raw domestic wastewater. However, some RSL has a high soluble COD content [35], making the organics more readily available, and so has the potential to boost wastewater treatment efficiency.

As discussed in Chapter 2 (Section 2.2.1), BESs must be able to function with substrates that are as complex as real wastewater. Often a synthetic substrate is used to replicate real wastewater in order to improve performance, as electrogenic bacteria can use the acetate directly. Great progress has been made using synthetic substrates, including the discovery of cheaper materials [145], novel reactor configurations [45] and improved operational conditions [146]. However, the use of synthetic substrates results in an unrealistically high level of performance, which has been well observed [50, 103, 147, 148]. When using a simple substrate, the electrogenic bacteria in the biofilm can access this substrate directly, transferring electrons to the anode. When a more complex structure is used, complex carbohydrates need to be broken down by hydrolytic bacteria, before fermentative bacteria convert these products into simple compounds. Only then can electrogenic bacteria access the organics. What therefore seems like a more efficient process cannot actually be replicated by using raw wastewater. Additionally, wastewater typically contains some competitive electron acceptors, which outcompete the electrogenic bacteria and reduces the CE [148]. Despite this, all but two of the reviewed pilot-scale MECs in Chapter 2 were successfully run with real wastewater, and the use of a synthetic substrate did not appear to be beneficial in terms of COD removal and total charge produced. In one case the switch to real wastewater from glucose actually enhanced hydrogen recovery [5]. There is clearly conflicting evidence that the use of a synthetic substrate will improve performance, and no two wastewaters are comparable. Further understanding of the exact metabolic pathways is required.

Velasquez–Orta et al. [98] suggests that the metabolic pathways of anaerobic digestion (AD) are similar in BESs fed real wastewater. However, some of these stages within AD are known to result in bottlenecks and significantly slow the process. Several studies have shown hydrolysis to be the rate-limiting step in the conversion of organic wastes to methane [149, 150, 151], and it is suspected that in BESs this is the same. Using six 200ml single chambered glass MFCs fed acetate, glucose and starch in duplicates, the calculated combined hydrolysis and fermentation rates were seven times slower than just fermentation [98]. In contrast to this, acetate accumulation was noticed in an electrically assisted anaerobic digester fed activated sludge from a municipal wastewater treatment site, which indicated that aceto-clastic methanogens were the rate-limiting factor [152] rather than hydrolytic and fermenting organisms. If hydrolysis is limiting, then increasing the number of electrogens in the system is not as important as maximising the other bacteria within the biofilm or skipping this step. If electrogenesis is the rate-limiting step instead of hydrolysis, acetate uptake and VTRs could improve with an increased number of electrogenic bacteria.

The use of RSL in BESs could increase VTRs and charge produced due to the higher concentration of organics. In addition, the soluble nature could result in skipping the potential rate-limiting steps found during the breakdown of complex wastes, mimicking the performance seen when using a simple substrate. The aim of this experiment is therefore to determine how successful using RSL with BESs would be, while investigating if a COD saturation point exists. This study uses 25 air-cathode MFCs to test the following hypotheses.

1. Identically set up reactors can be replicated with a small margin of error.
2. Using a higher strength wastewater will be beneficial, as total charge produced will correlate positively with influent COD.
3. At a certain influent COD concentration, there will be a saturation point in terms of VTRs.
4. The use of acetate compared to RSL at identical COD concentrations will increase the performance of the MFCs with respect to charge produced and organic removal.
5. In a batch-fed system, the electrogenic step is rate limiting.
6. The high soluble COD content of RSL will aid VTRs and COD removal efficiency.

3.2 Materials and methods

3.2.1 Reactor configuration

In Chapter 2, it was discussed that MECs have a greater potential to be scaled up compared to MFCs. Comparatively, at a smaller scale, MFCs are often used due to the simplicity in manufacturing, especially when using a single chamber air-cathode design. The ease of measuring energy recovery in the form of the charge produced, compared to hydrogen gas capture and analysis, is a major benefit. The experiment demanded an MFC design that enabled high replicability, high throughput and the ease to analyse the substrates simply and quickly. Therefore, the 25 identical air-cathode MFCs were cylindrical chambers with three sample ports, sealed at the opposite end with a plastic bung and each had a 60ml working volume. The anode was 40mm diameter, 3ml width carbon felt (SGL Carbon, Wiesbaden, Germany) connected to 0.6mm^2 stainless steel wire (Clarke © Tools, Chronos Ltd, Dunstable, UK), fed out of a sample port through a rubber bung. The air cathode was 0.2 mg/cm^2 20% platinum on Vulcan carbon cloth electrode (Fuel Cell Store ©, Texas, USA), platinum side facing the air. A 300Ω resistor connected the anode wire and the carbon cloth cathode, secured by crocodile clips. All wire connections were soldered to maintain high connection. The carbon cloth cathode was 5cm x 5cm, with an extra 1cm x 2cm section cut at the top and folded at 90 degrees to allow crocodile clips to connect. This was glued to a 7cm x 7cm rubber square with a 40mm diameter circle cut out, and glued onto the end of the tube using Gorilla Epoxy Resin (Gorilla glue Ltd, Chorley, UK) (See Figure 3.1).

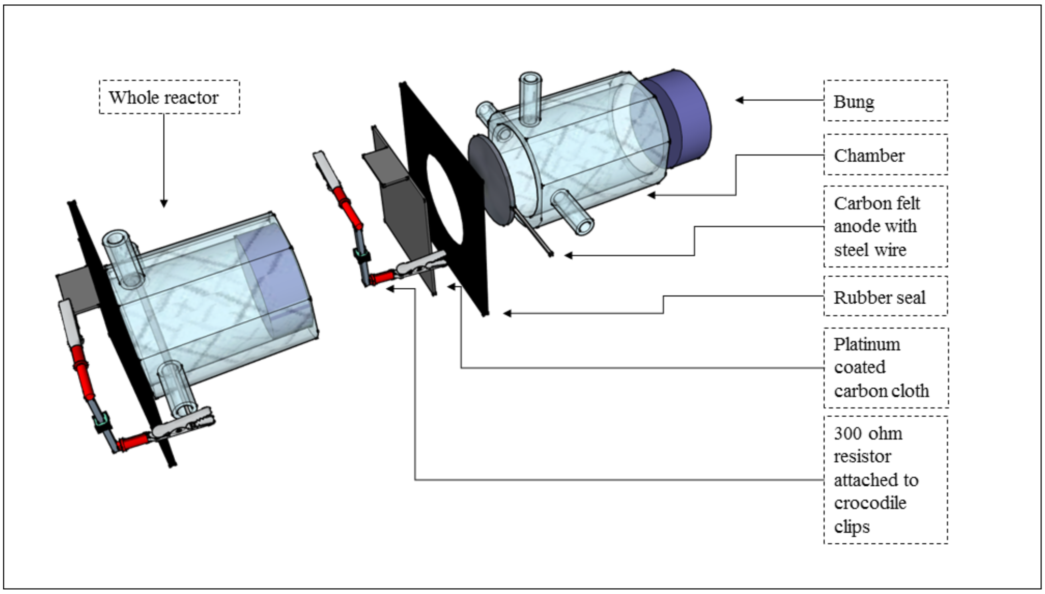


Figure 3.1: Air-cathode MFC design (left) with the individual components in an exploded view (right).

3.2.2 Multiple runs

All reactors were subject to the same experimental conditions, with the temperature set at 22°C, in order to determine if identically set up reactors can be replicated with a small margin for error (hypothesis 1). All reactors were inoculated with 50% raw wastewater 50% acetate mix and left until current production ended. Raw wastewater was collected from Birtley Wastewater Treatment Works (NWL). The synthetic components consisted of a phosphate buffer, acetate, vitamin solution (ATCC™, Teddington, UK) and trace mineral solution (ATCC™, Teddington, UK). This resulted in a COD of 431 mg/L, with a conductivity of 1.7 mS/cm. The mixture was homogenised and then nitrogen gas (80%) was bubbled through for 30 minutes. Reactors were then filled with the inoculant and sealed with rubber bungs.

Following inoculation, reactors were emptied and filled with a 100% synthetic mix to try to minimise the variation from the wastewater. Reactors were left until all had stopped producing current. This was repeated two more times to try to get equal total coulombs across all reactors. COD of the substrate was reduced for each run to show the effect of lower strength influents and the need for large replicates. However, COD remained consistent between all reactors for each run.

Following three standardising runs, reactors with total coulombs outside the interquartile range were removed. This left 20 reactors, enabling five COD concentrations for both acetate and RSL in duplicates (hypothesis 2, 3, 4). RSL was collected from Howdon Wastewater Treatment Site (NWL), and the average components can be seen in Table 3.1. Fresh RSL was collected with a COD of 2643 mg/L. Concentrations of 101 mg/L, 507 mg/L, 1269 mg/L, 2114 mg/L and 2643 mg/L were made with both the RSL and acetate substrate. Each COD concentration was randomly assigned to a reactor. Once they had all stopped producing a current, VTRs, total coulombs, peak current, coulombic efficiency (CE) and COD removal were compared across the different substrates and their concentrations.

Table 3.1: Full wastewater components measured of return sludge liquor (RSL) taken prior to COD dilutions.

COD mg/L	**sCOD** mg/L	**Phosphate** mg/L	**Sulphate** mg/L	**Ammonia** mg/L	**Nitrate** mg/L	**Nitrite** mg/L	**Acetate** mg/L	**Butyric** mg/L	**Formic** mg/L
4535	1772	56.8	180.6	298.3	8.9	0.8	274.0	49.2	n/a

Following this, 12 working reactors were randomly chosen and had their anode sliced in half to help determine if the eletrogenic step is rate limiting step (hypothesis 5). Half of these reactors were then fed three acetate concentrations in duplicates and the other half fed the equivalent RSL concentrations. The concentrations of COD were 1269 mg/L, 2114 mg/ and 2643 mg/L. The higher CODs were chosen due to the negative and seemingly random effect low COD in the influent had on reactor performance in the previous runs. Results from both experiments were then compared to determine if the high soluble COD content of RSL will aid VTRs and COD removal efficiency (hypothesis 6). The components and COD of the reactors for all runs can be seen in Table 3.2.

Table 3.2: The substrate and COD used for each run.

Run	**Type**	**Substrate**	**Number of reactors**	**COD (mg/L)**
1	Inoculation	Wastewater–acetate mix	25	431
2	Standardising	Sterilised acetate mix	25	269.5
3	Standardising	Sterilised acetate mix	25	211
4	Standardising	Sterilised acetate mix	25	182
5	COD dilution	RSL or acetate	20	101/ 507/ 1269/ 2114/ 2643
6	Halved anode	RSL or acetate	12	1269/ 2115/ 2643

3.2.3 Analytical methods

Following the end of each run, all reactors were sampled, and COD removal was measured using Merck COD cuvette tests (25 - 1500 mg/L) in duplicate according to standard methods. Voltage was measured using Pico 6 software across a 300Ω resistor, with ADC-20 and ADC-24 PicoLogers for continuous measurement.

3.2.4 Calculations

Current density, COD removal, VTR, CE, peak current density and total coulombs were calculated as follows:

Voltage was measured using high resolution multi-channel data loggers from PicoTech [153]. This was converted to current density based on projected anode surface area. According to Ohms law, the current can be calculated as:

$$I = \frac{V}{R}$$

where I is the current (amps), V is the voltage (volts) and R is the resistance (Ohms). This was then divided by the surface area to get current density:

$$J = \frac{I}{A}$$

where A is the projected anode surface area (m^2) and J is the current density (A/m^2).

VTR has been calculated using the COD removed and the retention time of the substrate, where S is COD removed (mg/L), HRT is the retention time (days), and Ks is VTR (kgCOD/m^3·day).

$$Ks = \frac{S}{HRT \times 1000}$$

Total coulombs (C_p) was calculated by the sum of the total current. As the Pico Log software recorded the Volts over a 300Ω resistor every minute, this was multiplied by 60 to convert to seconds, and then summed.

$$C_p = \sum(I \times 60)$$

CE was calculated by:

$$E_c = \frac{C_p}{C_n} \times 100$$

where E_c is the CE, C_p is the total coulombs (current over time), and C_n is the theoretical coulombs that could be recovered from the COD removed. Theoretical coulombs were calculated based on Logan et al [61], and therefore CE was calculated by:

$$E_c = \frac{8 \times C_p}{F \times V_A n \times \Delta COD} \times 100$$

where F is Faraday's constant (96,485 C/mol of electrons), 8 is a constant used for COD [61], V_{An} is the liquid volume in the anode chamber (L), ΔCOD is the change in COD (g/L) that has occurred over the batch cycle.

3.2.5 Statistical analyses

All statistical tests were run in Python, using packages reasearchpy, scipy.stats and statsmodels.

3.3 Results

All 25 reactors were run in batch mode and inoculated with a wastewater-acetate mix (COD = 431 mg/L). Following this, the reactors were subject to three runs with a sterilised acetate mix. In the final acetate run, five reactors were out of the interquartile range when measuring the total coulombs and so were removed from the experiment. The remaining 20 reactors were then randomly assigned five COD concentrations of both acetate and RSL and were run in duplicate. Finally, half the anode was removed from 12 of the reactors and re-run with three COD dilutions of both acetate and RSL in duplicate. This was to investigate the effect that reducing the surface area had on the electrogens' capability to produce current, using both simple and complex substrates.

For all runs, reactors were filled and then left until current production ceased before analysing the COD of the effluent. The performance indicators used were as follows: total coulombs, which is a measure of the total number of electrons recovered in each MFC over the entirety of the batch mode; the VTR, which is the volumetric rate at which organics (represented by COD) are removed from the reactor; the peak current, which is the peak current observed in a single MFC; the CE, which is a measure of the efficiency of electron transfer at the anode; and COD removal efficiency, which is the percentage of organics (represented by the COD) removed at the end of the batch mode.

3.3.1 Identically set up reactors can be replicated with a small margin of error.

The first hypothesis was to determine if multiple identical reactors could be set-up and run, with equal performance between them all. This was proven incorrect. Analysis of the current generation during inoculation saw high variability between reactors. Total coulombs recovered across all the reactors averaged 132.4 A·s, with a range of 137.3 A·s. Similarly, the average peak current from all the reactors was 0.23 A/m^2, with a range of 0.36 A/m^2. The use of acetate to standardise the reactors reduced the variability in total coulombs between the reactors, with an average of 45 A·s and a range of 34.2 A·s in the final run. However, this variability did not solely disappear, and peak current remained highly variable, with an average of 0.17 A/m^2 and a range of 0.17 A/m^2 (Figure 3.2).

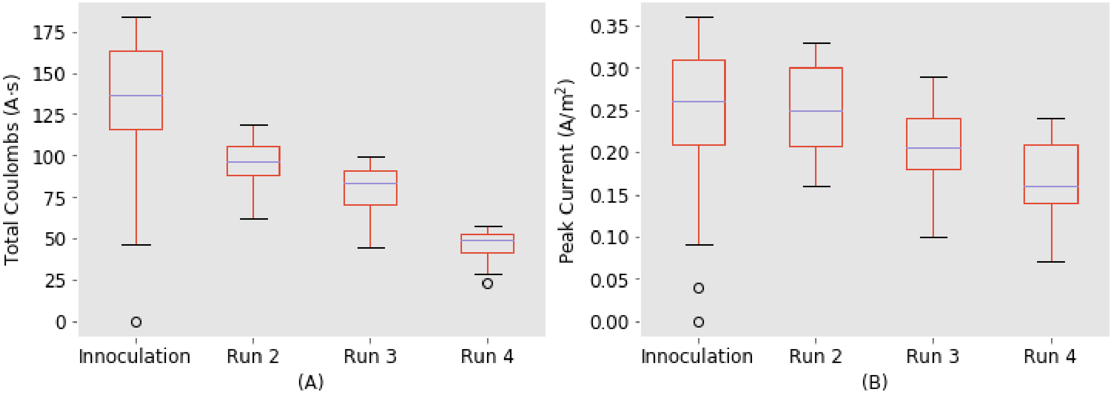
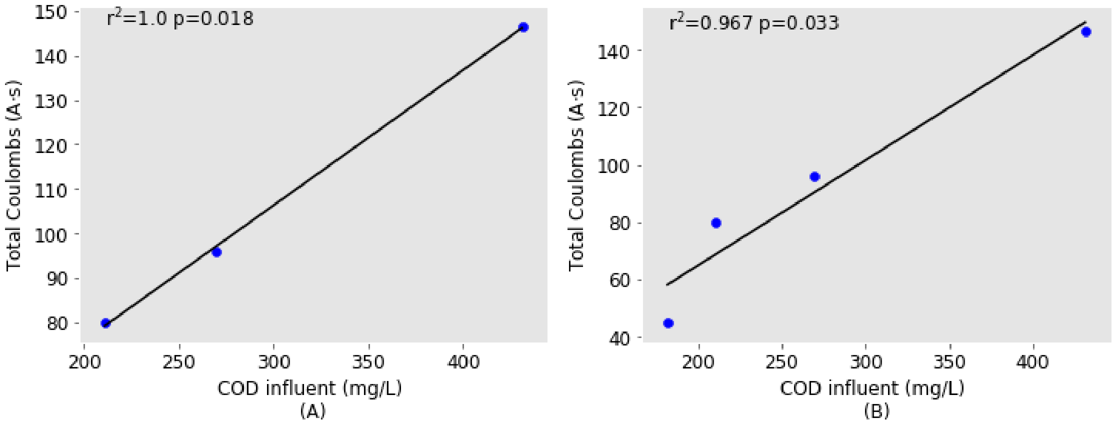


Figure 3.2: Total coulombs (A) and peak current (B) for inoculation and the following three standardising runs.

3.3.2 Using a higher strength wastewater will be beneficial, as total charge produced will correlate positively with influent COD.

The second hypothesis was to determine the relationship between influent COD and total charge produced, represented by total coulombs. With each standardising run, the influent COD of the acetate mix was reduced, which showed that the average from all the reactors follows a linear trend as influent COD increased ($r^2 = 1$). However, this trend is broken once the influent COD drops below 200 mg/L ($r^2 = 0.967$) (Figure 3.3).



(A)

(B)

Figure 3.3: A comparison between the influent COD and total coulombs recovered during the inoculation and standardising runs. Figure A (left) presents the inoculation run and the following two standardising runs, and Figure B (right) shows the same, with the additional standardising run < 200 mg/L COD.

This same trend was seen when increasing the influent COD up to 2643 mg/L (Figure 3.4). Although only two repeats were enabled for each COD concentration, total coulombs increased as influent COD increased in both acetate and RSL reactors, with r^2 values of 0.964 and 0.996 respectively. These results show the hypothesis to be correct, that in a batch-fed system total charge will increase linearly with increasing COD.

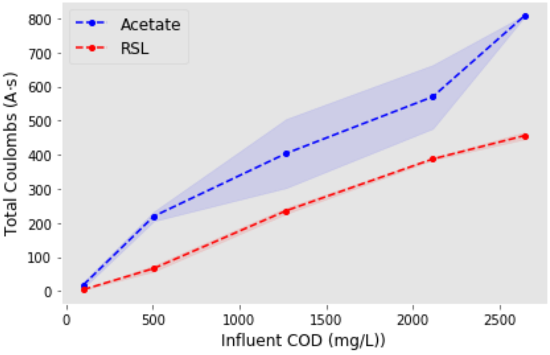


Figure 3.4: How influent COD affected total coulombs in both acetate and RSL reactors.

3.3.3 At a certain influent COD concentration, there will be a saturation point in terms of VTRs.

The third hypothesis was to see if COD saturation could be reached with respect to VTRs. VTRs were expected to plateau from an increased influent COD as there are only a fixed number of bacteria within the reactors when operated in batch. A continued increase in COD concentration will eventually exceed the bacteria's capacity to increase the rate of removal. This hypothesis was seen to be correct. VTRs of the RSL started very low and increased steadily at influent CODs of 507 and 1269 mg/L, before plateauing with values of 0.126 – 0.146 kgCOD/m^3·day. In acetate reactors, VTRs started to plateau at 0.147

kgCOD/m^3·day when the influent COD was set as 2114 mg/L (Figure 3.5).

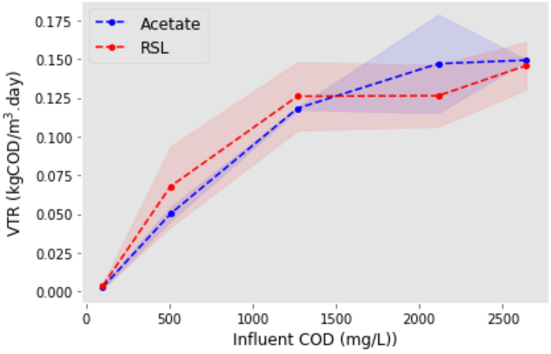


Figure 3.5: How influent COD affected VTRs in both acetate and RSL reactors.

A saturation point was also reached when comparing CE, although different values were seen between the two substrates. At influent CODs of 101 mg/L, CEs gave values of greater than 100%. This indicates that greater current is being produced than theoretically available in the removed COD, and so these values have been omitted. For the remainder of the COD concentrations, CE in RSL based reactors plateaued around ∼30%. Comparatively acetate reactors achieved a CE of 80% at an influent COD of 507 mg/L, before plateauing down to an average of 45% for the remainder of the concentrations (Figure 3.6).

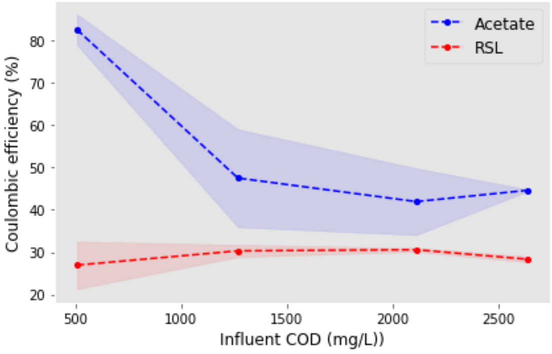


Figure 3.6: How influent COD affected CE in both acetate and RSL reactors.

3.3.4 The use of acetate compared to RSL at identical COD concentrations will increase the performance of the MFCs with respect to charge produced and organic removal.

It was hypothesised that the use of acetate would result in higher performing reactors, which was observed in both total coulombs (Figure 3.4) and CE (Figure 3.6). However, this was not the case with VTRs, peak current and COD removal efficiency. Both acetate and RSL reactors achieved similar peak VTRs of 0.149 and 0.146 kgCOD/m^3·day respectively (Figure 3.5). Each plateaued at high influent COD concentrations, with high overlap between the substrates. Similarly, there was also high overlap when comparing peak current throughout all CODs in both acetate and RSL (Figure 3.7). At the lowest COD concentrations (101 mg/L), acetate and RSL had the same average peak current of 0.17 A/m^2. Following this, acetate reactors increased to 0.34 A/m^2 at 507 mg/L, and then plateaued for the remainder of the COD concentrations, while there was a linear increase seen in RSL reactors (r^2 = 0.873) (Figure 3.7).

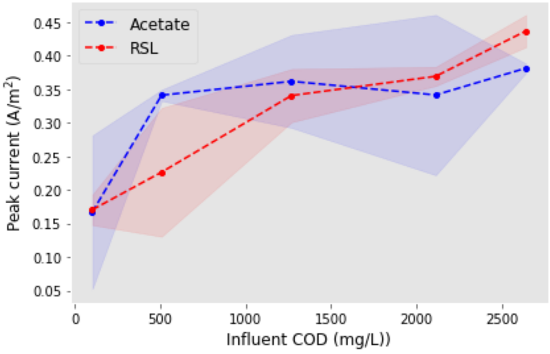


Figure 3.7: How influent COD affected peak current in both acetate and RSL reactors.

Although the percentage of COD removal with acetate reactors was slightly higher than RSL, this was much closer than predicted and both substrates plateaued at an influent COD of 1269 mg/L. For acetate reactors this was a plateau at a COD removal of ∼94%, while RSL reactors plateaued at ∼84% (Figure 3.8).

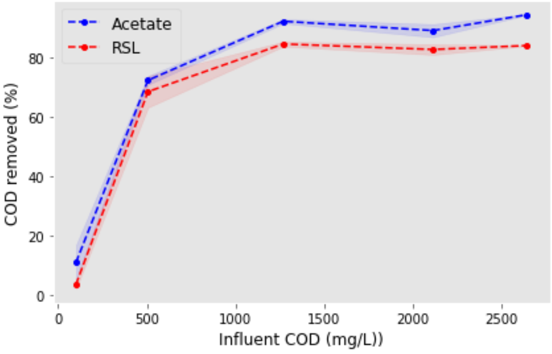


Figure 3.8: How influent COD affected COD removal efficiency in both acetate and RSL reactors.

3.3.5 In a batch-fed system the electrogenic step is rate limiting.

With a halved anode, acetate reactors remained similar in total coulombs and CE. Total coulombs still increased linearly with increasing COD (r^2 = 0.837) and CE plateaued at ∼40% (compared to ∼45%) at all three COD concentrations (Figure 3.9, A). Contrastingly, in RSL fed reactors total coulombs recovered dropped by over 50% while still increasing linearly (r^2 = 0.837) and CE plateaued at 15% rather than 30% (Figure 3.9, B).

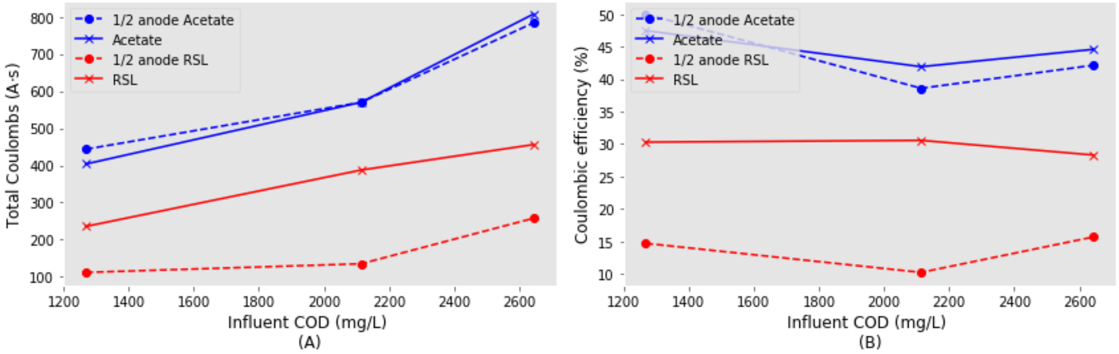


Figure 3.9: The effect of halving the anode on both acetate and RSL reactors for total coulombs (A) and CE (B).

COD removal efficiency and peak current were both higher with acetate fed reactors once half the anode was removed. COD removal efficiency plateaued at 96% instead of 92%, and peak current fluctuated between 0.5 and 0.8 A/m^2 compared to plateauing at 0.47 A/m^2 (Figure 3.10). Similarly, in RSL reactors removal efficiency remained between 84% and 86% for all influent COD concentrations, compared to 83% and 85% seen previously. Peak current increased in RSL reactors ($r^2 = 0.916$) from 0.24 to 0.46 A/m^2, compared to the previous increase ($r^2 = 0.873$) of 0.34 to 0.44 A/m^2 (Figure 3.10).

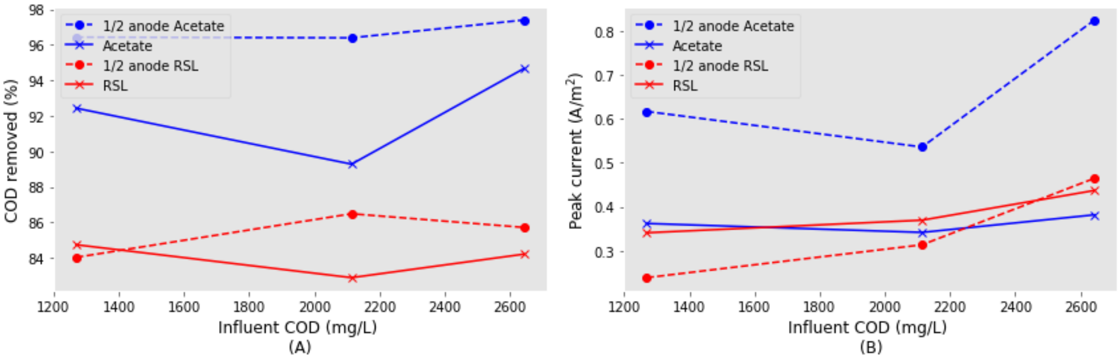


Figure 3.10: The effect of halving the anode on both acetate and RSL reactors for COD removal efficiency (A) and peak current (B).

VTRs increased slightly in both reactors with half the anode removed, and still followed very similar trends when comparing both substrates (Figure 3.11).

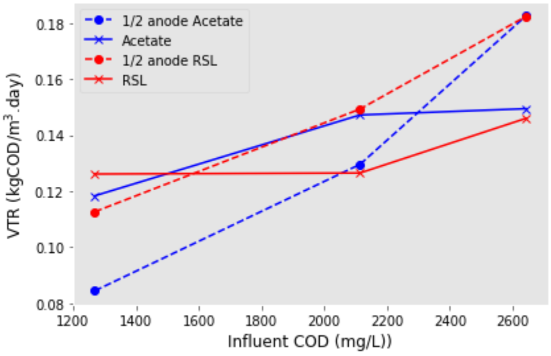


Figure 3.11: The effect halving the anode had on both acetate and RSL reactors for VTRs.

3.3.6 The high soluble COD content of RSL will aid VTRs and COD removal efficiency.

The final hypothesis was to determine if the high soluble COD content of the RSL aids wastewater treatment. Figure 3.5, Figure 3.7, Figure 3.8 and Figure 3.11 all suggest that this is the case, due to the lack of a significant difference between acetate and RSL reactors with respect to VTRs, peak current and removal efficiency.

3.4 Discussion

Bioelectrochemical systems have the potential to remove pollutants while simultaneously recovering energy from wastewater; however, this technology is still in development. Comparing the pilot-scale MECs to an ‘ideal’ reactor in Chapter 2 highlighted that the VTR has never matched that of AS. Kim et al. [132] demonstrated that the use of higher strength wastewaters achieved higher VTRs in BESs, however, this was with rice mill, mustard, cheese and swine wastewater. The composition of these wastewaters may vary hugely from domestic wastewaters and so are not comparable to the studies reviewed in Chapter 2. Raw domestic wastewater is low strength and dilute (COD values typically 300 – 700 mg/L), whereas RSL in comparison has a much higher COD content (COD values typically 2000 – 4000 mg/L). Therefore, 10 air-cathode MFCs were run with five increasing concentrations of RSL in duplicate. The aim was to determine if RSL could be used in BESs, and to investigate the effect increasing COD has on reactor performance. In order to compare the performance of RSL based reactors to a simple substrate, the equivalent COD concentrations were run using just acetate.

The hypothesis that using a simple substrate (such as acetate) would result in a higher performance was, surprisingly, shown to be false with respect to peak current and VTRs. The similar peak current and VTRs seen in both the acetate and RLS reactors suggest that the high soluble COD content of the RSL (39.1% sCOD) was easier to breakdown than other typical wastewaters. This removes upstream steps, aiding the wastewater treatment capabilities. Achieving a lower total coulombs and a lower CE in RSL reactors was as hypothesised, and is a well-observed trend in wastewater based reactors due to greater loss of electrons to alternative pathways [148]. Additionally, although lower than acetate, removing 84% of the COD and achieving 30% CE from the RSL is quite high in comparison with MFCs fed domestic [154, 155, 156, 157, 158, 159] or other high strength wastewaters [132, 160, 161]. Zhao et al. [148] reported that CE seems primarily affected by hydrolysis, therefore achieving this higher CE supports the idea that RSL is less affected by these upstream steps due to higher soluble COD. This 16% indigestible fraction which remained in the effluent is assumed to be non-biodegradable soluble and particulate COD, which cannot be broken down by conventional wastewater treatment processes and is often discharged [162]. The results from Choi et al. [162] highlighted that domestic wastewater on average can contain 20 - 30% indigestible COD, which makes the remaining 16% quite low.

As predicted, total coulombs increased linearly with increasing COD. As the reactors were batch-fed, the increased supply of organics within the chambers as influent COD increased led to a linear increase in charge production. However, during inoculation and standardising runs, the total coulombs recovered broke the linear trend as the reactors were subject to an influent COD <200 mg/L. This was also the case when reactors were subject to concentrations of 101 mg/L for both substrates. These observations of reduced reactor performance at low CODs aligns with findings by Zhang et al. [104], who reported the current production in air-cathode MFCs rapidly decreased as the COD concentration was less than ∼100 mg/L. This reduction in current production at low CODs will make achieving UK discharge standards [17] while achieving high energy recovery difficult [104].

COD saturation was reached with respect to VTRs. At higher CODs the supply of organics exceeded that which the bacteria could breakdown. This led to a maximum VTR for the MFCs, which fits the hypothesis that the reactors would follow a Monod-like trend when the organics in the wastewater increased. This saturation point is a function of both the concentration of COD and the size of the biofilm. In a batch-fed system, this is not problematic, as the wastewater will remain in contact with the biofilm until all available pollutants are removed. However, in a continuous flow system, the HRT affects the contact time between the wastewater and the biofilm. If the use of a high strength wastewater is going to be successful, the reactor needs to be designed so that the concentration of influent COD does not exceed the biofilms capacity to remove the pollutants. This will require either optimisation of the HRT or an increase in the wastewater volume to anode ratio.

The peak VTR achieved with RSL is lower than with other MFCs fed domestic wastewater [154, 156, 157, 163, 164] (<0.25 kgCOD/m^3·day), yet similar to the VTRs achieved with MFCs fed high strength rice mill (0.09 kgCOD/m^3·day) [165] and high strength mustard wastewater (0.19 kgCOD/m^3·day) [166]. However, this is comparing a variety of wastewaters in very different reactors, and multiple factors can affect VTRs. These include the inoculum used [167], the anode surface area to volume ratio [104], the temperature [159], the cathode [47, 48, 49] and the operational conditions [8]. In addition, VTRs recorded in these reactors would have been much higher during initial current production, and subsequently lowered as the COD of the substrate drops. This was also shown by Zhang et al. [104], who discovered the VTRs for MFCs fed domestic wastewater followed first order kinetics, resulting in the concentration of COD decreasing at an exponential rate with respect to time. Therefore, measuring over the entirety of the batch mode will result in much slower VTRs than reactors operated in continuous flow [11]. However, it is clear that this higher strength RSL has the capability to show increased VTRs.

The highest CE was seen at the lowest influent COD for both acetate and RSL, which matches the results from both Sleutels et al. [143] and Zhang et al. [104]. In these studies, it was reported that using lower strength wastewaters compared to high strength wastewaters achieved greater CE. It was suggested that at lower concentrations, electrogenic bacteria could out-compete other bacteria for the produced electrons. However, the low VTRs seen when using low strength wastewaters are reducing the ability for these systems to compete with AS. Zhao et al. [148] recommended that a reduced CE should be incorporated into the preliminary plans to anticipate these losses, and accepting a lower CE but high VTRs seems more logical with respect to wastewater treatment.

When the anode was halved, RSL based reactors produced 50% less charge and achieved 50% lower CE when compared to the previous whole anode reactors. However, these reactors still maintained the same COD removal at the same or higher VTRs. This suggests the loss of 50% of the electrogens from these reactors, as electrons were lost to competitive pathways rather than limited breakdown of organics. This loss of charge was not seen in acetate-based reactors when 50% of the biofilm was removed. As acetate can be readily used by the electrogens, no upstream steps were required, and the removal of the electrogenic bacteria did not limit the uptake of electrons. This agrees with previous work, demonstrating that the rate limiting step within BESs is more likely to be hydrolysis rather than electrogenesis [98].

In this study, a highly variable difference in performance was seen between the 25 identical MFCs used, and although repeating three standardising runs with acetate reduced this variability, it did not completely remove it. Due to this variability, a clear linear trend between average charge and influent COD was only seen with a large number of replicas.

3.5 Conclusion

This study successfully demonstrates the rationale behind the use of high strength waste streams with BESs in order to increase VTRs, specifically RSL from Howdon Wastewater Treatment Works (NWL). The high soluble COD in RSL aids COD removal and appears to reduce the effect of the slow breakdown of complex substrates in wastewater. Running a pilot-scale MEC with RSL should be the next step for this technology, as the increase in performance could help bridge the gap between MECs and competitively competing with AS.

Chapter 4

Optimising the hydraulic retention time in a pilot-scale microbial electrolysis cell to achieve high volumetric treatment rates using concentrated domestic wastewater

This chapter has been published as Leicester, D.D.; Amezaga, J.M.; Moore, A.; Heidrich, E.S. Optimising the Hydraulic Retention Time in a Pilot-Scale Microbial Electrolysis Cell to Achieve High Volumetric Treatment Rates Using Concentrated Domestic Wastewater. Molecules 2020, 25, 2945. [133]

4.1 Introduction

Bioelectrochemical systems (BESs) are a wastewater treatment technology that use anaerobic and electrochemically active microorganisms within the wastewater to break down pollutants and recover energy. Typically, they consist of two chambers, one containing an anode and the other a cathode. Most of the research for BESs has been at a laboratory-based scale of millilitre to litre volumes, and scaling up these reactors still remains a challenge [1, 4]. This is due to a number of reasons: pilot reactors are expensive; they are difficult to engineer; scale-up shows decreased performance; and data from a pilot-scale reactor is often limited and rarely in duplicate. Therefore, only a small number of pilot-scale BESs have been built, as reviewed by Rousseau et al. [54] and Gajda et al. [168]. All use a range of configurations, operate under different conditions and use different substrates. Standardising and comparing the results from these studies is difficult, as even the phrase ‘pilot-scale’ can be ambiguous. To call a reactor ‘pilot-scale’, Wang et al. [119] determined that it must fulfil certain criteria: the reactors must be in operation for greater than two months, be continuously flowing, be run using real wastewater, and have 0.1 to 5% of the practical flow of the wastewater facility. The majority of published pilot or semi-pilot BESs do not fit these criteria.

The most common configurations of BESs are microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) [87]. As MECs are able to recover valuable hydrogen gas rather than electricity, it has been suggested that scaling up an MEC is more economically viable than scaling up an MFC [32, 55]. MFCs produce current that is simple to use, but do so only at a very low level. They also require oxygen at the cathode, and therefore need constant aeration or the use of an air-cathode. Constant aeration requires extra energy and the largest air-cathode to date is only 0.62m^2 [32]. The hydraulic pressure exerted on air-cathodes poses not only structural difficulty, but also increases charge and diffusion transfer resistance, as well as suppression of bacteria in the biofilm [169]. An MEC has the advantage that both chambers are anaerobic with no oxygen required, meaning they may be more able to fit into the infrastructure of large-scale treatment works.

Currently, six MEC studies have been published as pilot-scale [1, 2, 4, 5, 6, 170], one described as technical-scale [11], two as scale-up [7, 10], two as semi-pilot [8, 9] and one as a mini-pilot [171]. The first and still largest to date was a 1000L MEC treating winery wastewater [1], which successfully removed 70% of the influent chemical oxygen demand (COD). Unfortunately, due to the single chamber design and heating of the reactor, methane was produced rather than hydrogen. However, it was still energy positive (+14.3 kJ/gCOD). Since then, there have been a number of ‘proof of concept’ designs, which have successfully produced hydrogen while also treating wastewater. The most successful reactors include Carlotta-Jones et al. [171] in terms of hydrogen production (0.066 m^{3}H$_2$/m^3·day), San-Martin et al. [6] in terms of COD removal efficiency (84%) and Cotterill et al. [4] in terms of COD volumetric treatment rates (VTRs) (1.06 kgCOD/m^3·day). However, none of these reactors achieved both discharge standards and net energy recovery from hydrogen capture, and VTRs were far lower than can be achieved with activated sludge (AS). The technology is still not ready for industry, and performance at a pilot-scale falls short of the performance needed to make it competitive with current technologies.

Application into the commercial sector is an enormous challenge. The water industry is typically conservative and risk adverse, being driven by the need to comply with regulation more than to innovate. BESs are unlikely to leap from academic research to full-scale water treatment. If this technology is to progress, it will be important to find places where there is a need or economic drive that cannot currently be met by other technologies. This could be in developing countries where there is little existing safe sanitation [80], or it could be treating high concentration wastes which are too liquid to feed a standard anaerobic digestion system [172]. Developing a minimal viable product that is usable and can add value could allow entry of BES technology into the water industry, enabling further commercial development and cost reduction to take place.

Most of the pilot-scale work to date has used domestic wastewater, before or after primary sedimentation [2, 4, 5, 6]. Although the goal would be to eventually replace the energetically expensive AS [55], the change required to achieve this would be a huge undertaking, and the risks of failure prohibitively high. Alternative waste streams within the treatment process could provide a safer option, and introduce this new technology into the commercial sector. A recent drive to generate renewable energy and improve the efficiency of the treatment works has seen anaerobic digestion added to many large treatment sites in the UK and elsewhere [22]. At these treatment sites, following the AS treatment process, waste sludge is dewatered and then anaerobically digested. During this process, energy is recovered in the form of biogas while the sludge is further treated. The effluent from this process is then dewatered again, with the bio-solids safely and beneficially re-cycled to land. The combined liquid fraction from these dewatering steps returns to the top of the treatment works and passes back through the AS process in an internal loop. This waste stream is one of the most energetically resource-laden sections of the wastewater treatment plant, and currently there is no economically attractive solution [35]. This return sludge liquor (RSL) is much higher strength than raw sewage, i.e. it contains more organic matter. Importantly, a high proportion of this organic matter is soluble and therefore may be more biologically available. The organic content, represented by the COD values, ranges from 1500 – 6000 mg/L (or 24.2 – 96.6 kJ/L [21]) in RSL, whereas low to medium strength raw wastewaters have COD values of 300 – 700 mg/L [111] (or 4.83 – 11.27 kJ/L).

At a large treatment plant such as Howdon Wastewater Treatment Works in the North East of England (Northumbrian Water Ltd), this RSL may have a flow rate of 100 L/s and an average COD of 2500 mg/L. The energetic treatment cost for AS is predicted to be between 1.08 and 2.1 kJ/gCOD [93], or 2.52 and 7.2 kJ/gCOD [58]. Therefore, using an ambitious but realistic energetic treatment cost of 2 kJ/gCOD, with an average energy cost (for business rates) of £0.13/kWh or £3.6 $\times 10^{-5}$/kJ [173, 174], treating this RSL in the AS process could cost the treatment plant around £1500 a day. As BESs have shown increased performance when treating a substrate with a high COD content [12, 132], this waste stream could be an ideal location for a pilot-scale BES, where it may be possible to get economically viable treatment rates. In addition, as the RSL simply returns to the top of the treatment process rather than being discharged, there is no demand to meet effluent standards. Any reduction in COD and energy recovery will be beneficial from the reduced loading on the AS process.

BES have large capital costs [34], therefore it is important that the reactors perform to their highest potential. One way to achieve this could be to optimise operational conditions, such as the hydraulic retention time (HRT), which would then have consequential effects on performance parameters, such as the VTRs. The HRT of these systems describes the length of time the wastewater remains in the reactor. It is a function of the volume of the tank and the flow rate, which determines the organic loading rate (OLR), and the contact time between the wastewater and the biofilm. Faster flow (or lower HRT) increases the OLR as more food is delivered into the tank. Increasing the OLRs by using high strength wastewaters has been shown to improve the performance of BESs in terms of the VTRs [132]. As the HRT will affect both the OLR and the subsequent pollutant removal, as well as electron transfer and hydrogen/electricity recovery, the HRT is a critical operational parameter for MECs [175]. If the HRT is not optimised, pollutant diffusion may be limited, and the reactors could underperform. Furthermore, tanks may be built to the wrong size, adding to their cost.

Despite the importance of optimising the HRT, this has not been done in most of the previous pilot-scale studies. Many pilot reactors use different architectures, however, three have been based on the cassette design outlined by Heidrich et al. [2]. This is where wastewater flows around rectangular cassette style electrodes with two external anodes and an internal cathode (Figure 4.1). In these studies, the reactors were run using different HRTs and supplied different voltages. Cotterill et al. [4] used a HRT of 5 hours and inputted 0.9V; Heidrich et al. [2] used a HRT of 1 day and inputted 1.2V, and Baeza et al. [5] ended up using a 2-day HRT with 1.5V. As these were proof of concept reactors, conditions to maximise the reactor performance were not optimised by Heidrich et al. [2] or Cotterill et al. [4], with no explanation as to why these conditions were chosen. Baeza et al. [5] switched from a 1-day to a 2-day HRT to improve COD removal, successfully increasing it from 6% to 25%. At the end of the experiment, they determined that a 10-day retention time would be needed to reach the desired 75% COD removal; however, full optimisation over a range of HRTs was not measured. Although the reactors were of the same design, the different operational conditions used makes it impossible to compare results between them.

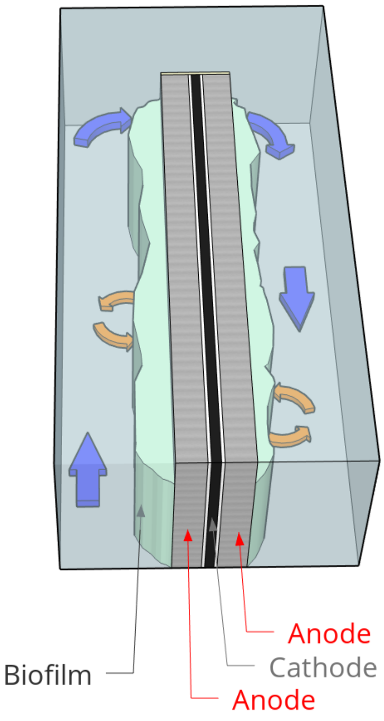


Figure 4.1: A simple diagram to represent wastewater flowing around the cassette style electrodes.

A HRT of 1 day has been most commonly used [1, 2, 3, 6, 13], while pilot reactors operating in batch have gone as long as 10 days [5]. The most thorough attempt to measure how HRT affects reactor performance was by Gill-Carrera et al. [8], measuring HRTs of 25, 23, 11, 7 and 4 hours in a two-chamber semi-pilot MEC, operated within a laboratory and kept at room temperature. Focusing on the first chamber, a longer HRT enabled greater coulombic efficiency (CE) (efficiency of anodic electron transfer), peaking at 94.3%. However, a shorter HRT showed increased cathodic conversion efficiency (CCE) (efficiency of electron recovery as hydrogen gas). The reactors used low strength wastewater as the influent substrate (<112 mg/L), and therefore were subject to OLRs well below typical AS loading rates, leading to low VTRs. Similarly, Gill-Carrera et al. [9] measured three HRTs and two voltages in a semi-pilot tubular MEC. It was determined that 4 hours and 1V were the optimum conditions in terms of hydrogen recovery and 10 hours and 1V in terms of pollutant removal. Using a pilot-scale multi-anode/cathode MFC, Jiang et al. [136] measured the performance at three HRTs, determining that COD removal efficiency increased from 66% to 80% from a 5 to 20 hour HRT.

This Chapter describes a pilot-scale MEC operated using high concentration RSL at Northumbrian Water Ltd.'s Howdon Wastewater Treatment Plant, June 2019 – February 2020. It aims to improve the effectiveness of the anode side of the cassette style electrodes by determining the optimal HRT with respect to COD removal efficiencies, VTRs and current densities. Using the data obtained and existing costing models, a direct analysis of the potential savings from the reduced loading on AS is then made.

4.2 Materials and methods

4.2.1 Reactor configuration

The reactor was built in the cassette design outlined by Heidrich et al. [2], with some modifications and improvements by BNC Solutions Ltd, a local engineering consultancy specialising in proof of concept development and new product design. It consisted of 10 cassettes set in a 72L glass tank (Figure 4.2). Electrodes were made using an internal cathodic chamber filled with 10g of stainless-steel wire, with stainless-steel mesh as a current collector (1mm diameter, 10mm × 10mm openings). A wire was attached to the current collector using a steel clamp and fed through a sample port in the top of the reactor to connect to the power supply. Rhinohide membrane (Entec ©, Harrogate, UK) was used as a divider on either side of the cathode chamber. Following this, another plastic frame was used to seal the membrane and provide a frame for the carbon-felt anodes (SGL Carbon, Wiesbaden, Germany). On top of each of the anodes, a stainless-steel mesh was added as the external current collector. Again, a wire was attached to the current collector to connect to the power supply. Anodic working volume of the tank, when containing all ten cassettes, was 36L. Anode surface area for each electrode was 0.0728m^2, giving the reactor a surface area to volume ratio of 20.2 m^2/m^3. Each cathodic compartment was filled with a phosphate buffer at a 0.5M concentration. This was not replenished after inoculation. The cathodic compartments were sealed using a gasbag (Supel™ Inert Foil 1L SCV Gas Sampling Bag with Thermogreen® LB-2 Septa, Merck, Gillingham, UK) and were attached using Saint-Gobain Tygon Flexible Tubing (Tygon S3™ E-3603, RS, Corby, UK), at 6.4mm external diameter.

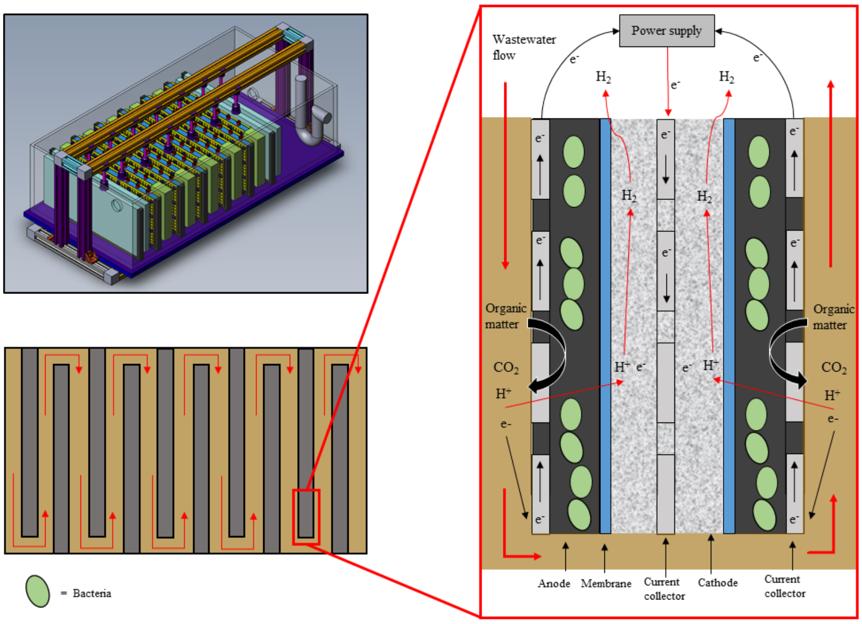


Figure 4.2: The pilot-scale MEC, showing the electrodes in the 'cassette' style design.

Electrodes were placed in the tank to create a serpentine flow (Figure 4.2). Computational Fluid Dynamic (CFD) modelling on the tank was performed by the contracted engineering company to identify the optimal cassette spacing of 5cm, and a false wall at the start of the flow was added to create laminar flow. Additionally, modelling showed that as the electrodes did not span the full width of the tank, PVC blocks needed to be added to the end of each cassette to provide a narrow gap for the water to travel around. The blocks were set on the outside of each cassette, minimising the ineffective areas at the corners as the RSL flowed around the electrodes.

4.2.2 Experimental site and operational conditions

The reactor was situated at Northumbrian Water Ltd.'s wastewater treatment site at Howdon, South Banks, in their pump control building. Wastewater was piped up from the RSL pipe into a 1m^3 intermediate bulk container (IBC), which was filled and replenished every 2 days with fresh wastewater. The room was not heated, so the RSL remained at ambient temperature. The reactor was run from June 2019 to March 2020, with full access to the site. However, due to global events in March 2020, we were suddenly locked out of this site and we have been unable to retrieve this reactor or perform any further experiments. This prevented us from doing pertinent post analysis work in our laboratories that may have been destructive during the reactor's operation. These include potentiostatic measurements, deconstruction of the reactor and taking biofilm samples.

4.2.3 Analytical methods

COD was measured using Merck COD cuvette tests (500 – 10,000 mg/L). Soluble COD was measured as previously stated but using a 0.45 μm-filtered sample. Phosphate was measured using Merck Phosphate cuvette tests (0.05 – 5 mg/L and 3 – 100 mg/L). Sulphate was measured using Merck Sulphate cuvette tests (5 – 250 mg/L). Ammonia was measured using Merck cuvette tests (0.2 – 8 mg/L and 2 – 75 mg/L) using a 0.45 μm-filtered sample. Nitrite and Nitrate was measured using Ion Chromatograph (IC) Dionex ICS-1000. All samples were measured in duplicate. VFAs were measured using Ion Chromatography System Dionex Aquinion and AS-AP auto sampler. Filtered (0.2 μm PES syringe) samples were mixed with 0.1M Octane Sulphonic Acid. Samples were then sonicated before analysis. All VFA samples were measured in triplicate.

Samples of the influent and effluent COD were measured three times a week during continuous flow mode. Voltage was measured over a 1-ohm resistor using Pico 6 software, with ADC-20 and ADC-24 PicoLogers for continuous measurement. The mean current density of all 10 electrodes was used for a total reactor current density. Hydrogen was measured in triplicates against an 80% hydrogen standard using a packed column in a Thermo Trace GC with a TCD, Argon carrier.

4.2.4 Inoculation and batch mode

The reactor was operated as an MEC for the entirety of the experiment, including start-up and acclimatisation. The inoculation of the reactor was done using 50% fresh RSL, 25% acetate mix (containing acetate, trace minerals and trace vitamins), and 25% effluent from laboratory-scale experimental work using MFCs, making up 36L and filling the reactor. These MFCs were run using the same RSL mixed with a 0.25 g/L acetate concentration. The COD for this combined inoculation mix was 2656 mg/L. Initially, the reactor was run with the inoculum using a 16-day HRT, which, due to such a low flow rate, was operated in batch. This long HRT was used to increase reactor start-up speed [4] and to enhance biofilm development [176]. The reactor was then replenished with fresh RSL for three different retention times of 11 days, 9 days, and 2 days, with no further acetate supplementation.

4.2.5 Experimental conditions

Each electrode was connected in parallel to a power supply, with 0.9V added. Initially, once switched to continuous flow, the HRT was set to 0.25 days. This was doubled to 0.5 days and run for a further two weeks. Following this, the reactor was operated using the different HRTs of 0.015 days, 0.1 days, 0.25 days, 0.5 days, 1 day, 2 days, 6 days, and 18 days. These cycles were performed in a randomised order to eliminate the effect of time and biofilm maturation.

4.2.6 Calculations

Voltage was measured using high resolution multi-channel data loggers from PicoTech [153]. This was converted to current density based on projected anode surface area. According to Ohms law, the current can be calculated as:

$$I = \frac{V}{R}$$

where I is the current (amps), V is the voltage (volts) and R is the resistance (Ohms). This was then divided by the surface area to get current density:

$$J = \frac{I}{A}$$

where A is the projected anode surface area (m^2) and J is the current density (A/m^2).

CE was calculated by:

$$E_c = \frac{C_p}{C_n} \times 100$$

where E_c is the CE, C_p is the total coulombs (current over time), and C_n is the theoretical coulombs that could be recovered from the COD removed. Theoretical coulombs were calculated based on Logan et al [61], and therefore CE was calculated by:

$$E_c = \frac{8 \times C_p}{F \times V_A n \times \mathit{\Delta} COD \times T} \times 100$$

where F is Faraday's constant (96,485 C/mol of electrons), 8 is a constant used for COD [61], $V_A n$ is the liquid volume in the anode chamber (L), $\mathit{\Delta} COD$ is the change in COD (g/L) and T is the number of days for the measurement.

Cathodic conversion efficiency (CCE) was calculated by:

$$C_c = \frac{N_{H_2}}{C_p/(F \times 2)} \times 100$$

where C_c is the CCE, N_{H2} the captured number of moles of H_2, C_p is the total coulombs (current over time), F is Faraday's constant and '2' is to give moles of H_2.

VTR has been calculated using the COD removed and the retention time of the RSL, where S is COD removed (mg/L), HRT is the retention time (days), and Ks is VTR (kgCOD/m^3·day).

$$Ks = \frac{S}{HRT \times 1000}$$

The energetic treatment cost (kJ/gCOD) was calculated by taking the average watts (W)

and converting this to kJ per day (multiplying by 86.4 incorporates the conversion of watt hours into kilojoules, then kilojoules into kilojoules/day), then dividing by the removal rate (gCOD/day).

$$kJ/gCODr = \frac{W \times 86.4}{gCOD/day}$$

Hydrogen production was measured in cubic meters of hydrogen, per cubic meter of reactor, per day. The total hydrogen produced from the whole reactor per week was divided by the reactor volume and then divided by the number of days over which it was collected.

$$\frac{m^3 H_2}{m^3 \cdot day} = \frac{\sum H_2(m^3)}{Days \times V(m^3)}$$

Energy recovered from hydrogen was based on a hydrogen volumetric density of 0.08988 kg/m^3 and an energy density of 142 MJ/kg, and was reported as kJ/gCOD.

$$kJ/gCOD = \frac{\sum H_2(m^3)}{Days} \times \frac{Days}{kgCODr} \times 0.08988 \times 142$$

The equivalent cost for AS was calculated by the grams of COD removed per day, multiplied by the energetic treatment cost for AS (2 kJ/gCOD).

$$kJ/day = \frac{gCOD}{day} \times 2$$

4.2.7 Tank design

The actual flow rate for the RSL at Howdon Wastewater Treatment Site (NWL) is on average 100 L/s, equivalent to 8640m^3 per day. At a HRT of 0.5-days, a capacity of 4320m^3 is needed for the wastewater for one day of flow. However, as the electrode cassettes used in this study took up around 50% of the total tank, the theoretical tank size needed is 8640m^3. Based on a nominal depth of 3m, which is similar dimensions to an AS lane [92, 113], and a suggested width of 36m, the VTR can then be modelled along the length of the tank, with 1 full day of flow requiring 80m length. The energetic cost savings between each measured COD have been calculated using the experimental energetic costs calculated and the assumed energetic treatment cost of 2 kJ/gCOD removed for AS. This has then been converted to actual cost saved using an energy price of £3.6 $\times 10^{-5}$/kJ [173, 174].

4.3 Results

4.3.1 Start-up and operation

Acclimatisation was rapid. Current was observed in two of the electrodes after two days, with seven more starting after five days. Gas was produced after 16 days. The final electrode produced a current after week six following wiring replacement (Electrode 8). Although there was variability between electrodes, average current density increased for the first 29 days before stabilising around 1.17 A/m^2. Gas production increased steadily throughout the first four weeks, reaching 0.007 m^{3}H$_2$/m^3·day. Following the 38 days of batch cycles, the reactor was switched to continuous flow. At the start, the reactor was run using a 0.25-day HRT. Due to an event on site termed 'core settling', high strength thick sludge was pumped into the reactor on day 57, causing it to flood and overflow. This reduced gas production and seemed to alter current densities for each electrode. In some cases, this was a reduction (e.g. Cassette 3), whereas in other cases it increased (e.g. Cassette 4). The HRT was then switched to 0.5 days for a further 2 weeks. Following this, the reactor was then deemed stable enough, and the different HRTs were tested over the following 6 months.

During 90 days of stable performance under continuous flow, gas production continued at all HRTs excluding the 0.015-day HRT. At day 134, another 'core settling' event occurred and completely flooded the cathode compartment of the electrodes. As a result, gas production stopped, most likely due to microbial contamination; however, current generation remained stable. As this is an immediate indication of the microbial substrate unitisation (hydrogen production being a secondary chemical process occurring at the cathode), the HRT optimisation experiment continued. Full wastewater components were measured prior to the experiment and can be found in Table 4.1. Spot samples of pH were taken throughout the experimental period and compared in different sections of the reactor; pH remained stable throughout the operation.

Table 4.1: Full wastewater components measured on spot samples of return sludge liquor (RSL) taken prior to running the reactor on site.

COD	**sCOD**	**Phosphate**	**Sulphate**	**Ammonia**	**Nitrate**	**Nitrite**	**Acetate**	**Butyric**	**Formic**
mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**
4535	1772	56.8	180.6	298.3	8.9	0.8	274.0	49.2	n/a

4.3.2 HRT optimisation

The eight HRTs varied between 0.015 days and 18 days. For each HRT, current densities from all 10 electrodes were recorded and the average used for the whole reactor. For HRTs under 6 days, the reactor was operated at that HRT for a minimum of 3 weeks, producing at least triplicate data. The longer cycles were operated in batch, producing triplicate 6-day results and duplicate 18-day HRT results. All the replicated HRTs were measured in a randomised order to reduce the confounding effect of a change in the biofilm over time. Influent COD was measured at the start of each cycle and the effluent at the end. The relationships between the performance indicators and HRT can be seen in Table 4.2.

The VTR shows a clear pattern with HRT, with the optimum being 0.5 days, as shown in Table 4.2 and Figure 4.3. The fastest HRT of 0.015-days achieved no measureable COD removal with a VTR of 0 kgCOD/m^3·day. Increasing the HRT increased VTRs until a 0.5-day HRT, where it peaked at 3.82 kgCOD/m^3·day. When compared to log HRT, the rise shows a logarithmic increase ($r^2 = 0.99$) and, following this peak, VTRs exponentially decrease ($r^2 = -0.862$). The hydrogen gas production appears as though it may have followed a similar trend, peaking at a HRT of 0.25-days; however, the incomplete data set makes this difficult to verify. The removal efficiency, i.e. the percentage of COD removed as the wastewater passes through the tank, predictably increases with HRT. However, it does not do so at a steady rate (Figure 4.3). Between HRTs of 0.015 and 0.5 days, removal efficiency sharply rises. Following this, for HRTs of 0.5, 1 and 2 days, it plateaus and the removal efficiency fluctuates between 52% and 58%. For the longest HRTs measured (6 and 18 days), a further increase is seen, with 18 days seeing the highest COD removal with 84%. Fitting a linear regression to log HRT and removal efficiency suggests a logarithmic increase as HRT increases ($r^2 = 0.965$). Visually on Figure 4.3, it can be seen that peak reactor performance for VTR sits at a HRT of 0.5-days. Doubling the 0.5-day HRT to a 1-day HRT halves the VTR from 3.82 to 1.81 kgCOD/m^3·day, but only rewards with a 5% increase in overall COD removal.

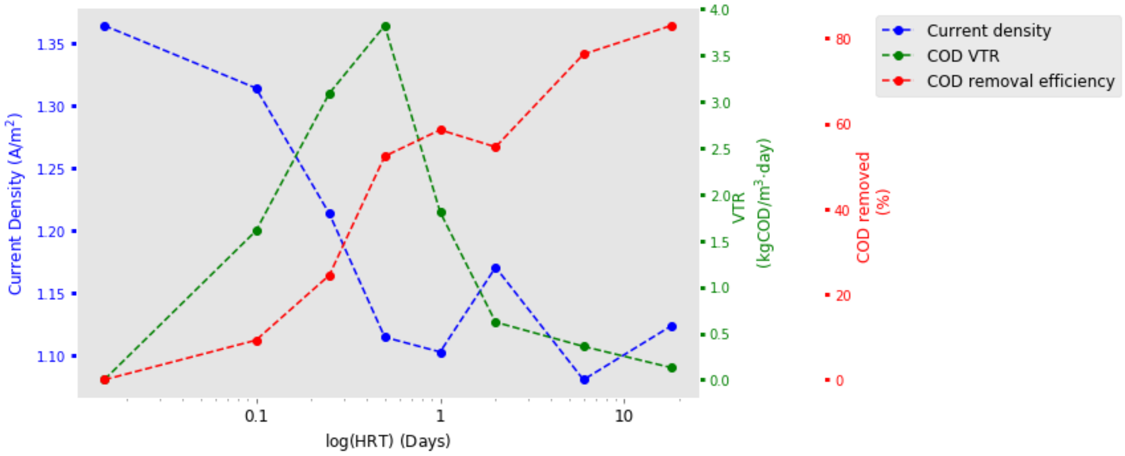


Figure 4.3: Current density, chemical oxygen demand (COD) removal efficiency and COD volumetric treatment rates (VTRs) with comparison to log (HRT).

Current density did not follow the expected optimisation curve, nor did it mirror the VTR data. The highest current density was at the fastest flow where there was no measurable wastewater treatment. Current density then declined with increasing HRT until 0.5 days (Figure 4.3). With longer HRTs, between a 0.5-day and an 18-day HRT, there was no

Table 4.2: Average performance indicators for each hydraulic retention time (HRT). For hydrogen production values, averages have been taken from HRTs prior to core settling. HRTs measured after core settling have had their hydrogen production removed and are denoted by a hyphen (-).

HRT	**Flow rate**	**Volumetric treatment rate**	**COD efficiency**	**Current density**	**Volumetric hydrogen production**	**Energetic treatment cost**	**Coulombic efficiency**	**Cathodic conversion efficiency**
(Days)	(L/hour)	(kgCOD/m^3·day)	(%)	(A/m^2)	(m^{3}H$_2$/m^3·day)	(kJ/gCOD)	(%)	(%)
0.015	100	0	0	1.36 ± 0.01	0	n/a	0	0
0.1	15	1.61 ± 2.27	9.3 ± 13.1	1.31 ± 0.07	0.0123 ± 0.0084	0.31 ± 0.44	2.9 ± 4.0	1.17 ± 0.86
0.25	6	3.09 ± 0.67	24.5 ± 1.6	1.21 ± 0.11	0.0155 ± 0.0076	0.64 ± 0.19	5.9 ± 1.8	1.16 ± 0.93
0.5	3	3.82 ± 1.94	52.4 ± 14.2	1.11 ± 0.08	0.0111 ± 0.0029	0.59 ± 0.42	5.6 ± 3.9	1.22 ± 0.25
1	1.5	1.81 ± 0.76	58.5 ± 7.4	1.10 ± 0.14	-	1.10 ± 0.46	9.9 ± 3.9	-
2	0.75	0.62 ±0.19	54.5 ± 2.3	1.17 ± 0.09	-	3.11 v 0.73	28.0 ±6.7	-
6	0.25	0.36 ± 0.14	76.2 ± 7.4	1.08 ± 0.04	-	5.25 ± 2.08	51.4 ± 20.5	-
18	0.083	0.13 ± 0.04	83.0 ± 12.9	1.12 ± 0.09	-	14.20 ± 5.04	131.0 ± 47.0	-

further decrease and current density fluctuated between 1.08 and 1.17 A/m^2. Fitting a linear regression to log (HRT) and current density gives an r^2 value of -0.832 and suggests current density exponentially decreases with increasing HRT. However, although there was a decline in average values over the HRTs, the range observed was very small (0.25 A/m^2). This is in comparison to the large range in current densities observed between the 10 cassettes (2.15 A/m^2) (see Appendix B, Figure B.1). Examining each cassette individually confirms that there was not a clear pattern between the HRT and the current density. Fitting regression lines to these resulted in a range of r^2 values from 0.142 (Cassette 4) to -0.96 (Cassette 2) (see Appendix B, Figure B.1). CE was shown to increase with HRTs. However, only at HRTs of greater than 1-day was there any reasonable recovery. No trends were seen with cathodic conversion efficiency (CCE) with respect to HRT.

4.3.3 Energetic cost

The average VTRs achieved at different HRTs, along with the voltage input and average current densities, have resulted in an energetic removal cost for this reactor at each HRT measured (Table 4.2). This can be compared to the equivalent cost of AS to achieve the same VTRs, using an energetic treatment cost of 2 kJ/gCOD for AS [58, 93]. For HRTs between 0.1 and 1 day, the MEC reactor removed the organics using less energy than would AS. At a HRT of 2 days or greater, this reactor was energetically more expensive than the AS equivalent. The reactor removed no pollutants when subject to a 0.015-day HRT and therefore used more energy than AS.

4.3.4 The effect of influent COD concentrations

In order to calculate the length of the tank that would be needed to remove fully the organics to legal discharge standards, and to understand if the optimal HRT was still relevant with lower strength wastewaters, a further experiment was conducted where the RSL was continually recirculated through the tank. Initially, the RSL flow rate was set to produce a 0.5-day HRT, and therefore was set to 3 L/hour. After the first fill of raw RSL, the effluent was piped back to the influent point of the reactor. The flow was continuously recirculated until the COD dropped to 280 mg/L COD. The rate of removal at these low COD values was so slow it was decided not to continue until UK discharge standards (<125 mg/L), as had been previously proposed. The COD was measured at the influent and effluent at the same time each day. In the case of the 0.5-day HRT, it was impossible to gain access to the site and sample at regular 12-hour intervals, and so measurements were taken every 24 hours. The VTRs were calculated based on the two circulations the RSL had achieved. This data was supplemented using the four repeated cycles for the initial HRT experiment, which due to the natural variation in the RSL, had influent CODs ranging between 4320 and 2186 mg/L (Table 4.3).

Table 4.3: Average VTRs from different influent CODs when subject to a flow rate of 3L/hour.

Influent COD (mg/L)	**Volumetric treatment rate (kgCOD/m^3·day)**	**Time of testing**	**Effluent COD (mg/L)**	**Energetic treatment cost (kJ/gCOD)**
4320	5.55	HRT optimisation	1548	0.285
4153	5.27	HRT optimisation	1519	0.36
3035	3.0	HRT optimisation	1538	0.584
2376	1.43	Tank re-circulation	950	1.20
2186	1.47	HRT optimisation	1449	1.13
950	0.282	Tank re-circulation	668	6.22
668	0.0985	Tank re-circulation	569	17.8
569	0.0653	Tank re-circulation	373	64.2
373	0.0275	Tank re-circulation	346	79.1
346	0.022	Tank re-circulation	324	52.1
324	0.0335	Tank re-circulation	290	94.1
290	0.0185	Tank re-circulation	270	79.1

It was possible to combine the two data sets from the replica 0.5-day HRT cycles (Figure 4.4, red line) and the recirculation data (Figure 4.4, blue line) as there is reasonably good agreement at the point they meet. It can be seen that the VTR clearly increases as the COD in the influent increases. Between the influent CODs of 4320 and 2186 mg/L from the HRT optimisation, VTRs have a linear increase with increasing COD (Figure 4.4, red line) ($r^2 = 0.994$). At lower influent CODs, VTRs increase exponentially with increasing COD in the influent (Figure 4.4, blue line) ($r^2 = 0.948$) when fitting a linear regression to log influent COD and VTRs.

Following this, the same recirculating technique was completed with a slower flow rate. It was hypothesised that as COD was reduced, the lower flow rate would be beneficial and result in higher VTRs due to the increased contact time between the biofilm and the RSL. The reactor was again filled with RSL, and then the effluent was pumped back to the top at 1.5 L/hour (the equivalent to a 1-day HRT). A similar pattern was observed, but the VTR was generally lower than at the faster flow, and at CODs of 2000 mg/L and less, VTR was virtually zero. The data shows the hypothesis to be incorrect: the slower flow offers no advantage over the faster one, especially at low COD (Figure 4.4, green line).

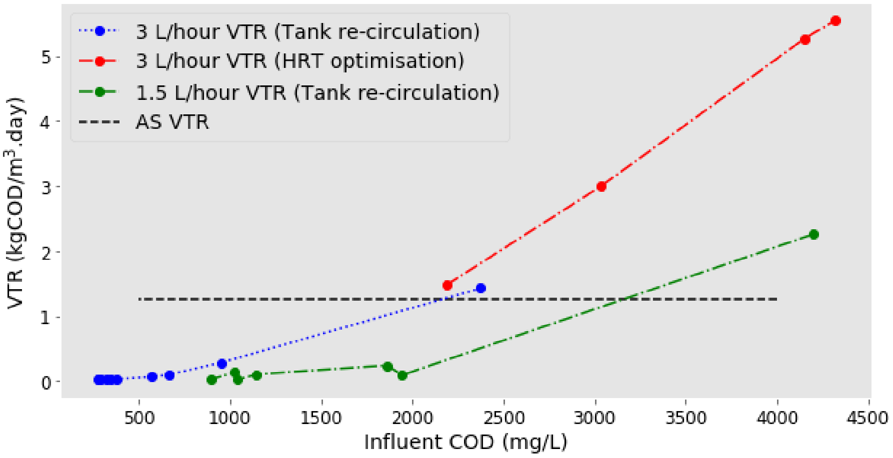


Figure 4.4: A graph to represent how the influent COD affects the VTR. Data collected during HRT optimisation is shown in red (3 L/h), and data collected from RSL recirculation can be seen in blue (3 L/h) and green (1.5 L/h).

4.3.5 Tank design

The data collected in Figure 4.4 can be used to extrapolate the design of the tank. It is seen that even for this small RSL waste stream, and an optimised HRT, the tank would need to be extremely large.

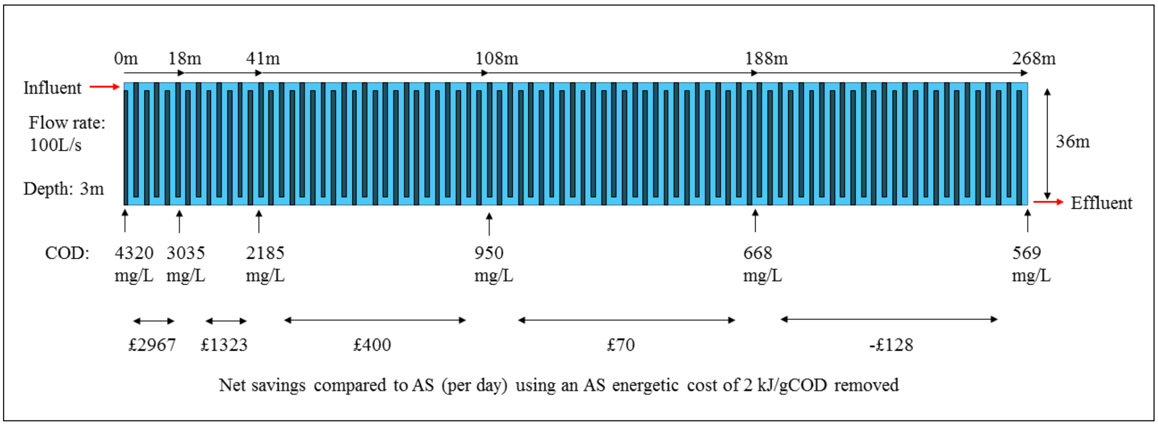


Figure 4.5: A theoretical tank design required for the actual flow rate found at Howdon Wastewater Treatment Plant (NWL). Values have been calculated based on experimental values from Section 4.3.4.

When running at the optimum HRT, energetic treatment costs were calculated for each individual influent COD. These can be compared to the energetic treatment costs for AS (which is currently the method used to treat this waste), modelling the difference in energetic costs of a full scale BES reactor. From Figure 4.5, it shows there is a high energetic cost saving of £2967 per day in the first 18m. Following this, savings decline and then turn negative, with the effluent only reaching 569 mg/L (higher than discharge standards) after a tank length of 268m and total size of 28,944m^3. Between an influent of 668 mg/L and 569 mg/L, a higher COD concentration than would be found in typical raw domestic wastewater, the MEC was energetically more expensive than AS (>2 kJ/gCOD) (Table 4.3).

To become a viable system, the savings to the energetic or running costs would need to be very high to overcome the high capital costs of BESs. A full analysis of the capital costs of this reactor was beyond the scope of this study and is covered elsewhere [34]. However, energetic cost savings in the first part of the tank, removing around 50% of the COD (0 – 41m), equate to over £2000 per day, making annual cost savings of £730K. If the infrastructure lasted over 5 years, it could cost £3.5 million and remain profitable. A recent cost–benefit analysis by Aiken et al. [34] states that in order for MECs to become viable, an increase in OLR and a 90% reduction in anode and current collector costs is needed. The proposed OLR targets of 0.8 – 1.4 kgCOD/m^3·day by Aiken et al. [34] were met by this reactor, though anode costs remained high at £285.9/m^2. Using the model produced by Aiken et al. [34], which was based on a similar pilot design, the capital costs of this reactor would be £61,754 per meter of tank. For the first 18m alone, the tank would cost £1,144,301, yet with a saving of £2967 per day, due to the high rate of COD removal by the BES, this requires a life span of just over a year (386 days) to break even (if performance seen in this study remained the same). Assuming a 2-year life span of the reactor, the first 41m of the tank (effluent quality of 2185 mg/L) would be cost effective. This will increase to 108m (effluent of 950 mg/L) with a 5-year life span. With a 10-year life span, the theoretical tank would be cost effective until an effluent of 668 mg/L was achieved (188m).

4.3.6 Variability

Within the reactor were 10 identical electrode cassettes, placed in sequence to generate a serpentine flow. The averages from all 10 of these electrode cassettes were taken to compare the whole reactor for the HRT experiments. However, there was extremely high variability between the electrodes in terms of their current density and hydrogen production. During the initial batch mode, all electrodes except Cassette 3 and Cassette 8 exhibited similar current densities. When the reactor was switched to continuous flow, current densities started to vary, and this continued until the end of HRT optimisation (Figure 4.6, A). Core-settling events also appeared to alter specific electrode current densities, although not in a systematic way. Following core-settling, Cassette 3, which previously was producing the highest current at 2.5 A/m^2, dropped down to 0.25 A/m^2, where it remained for the duration of the experiment. Comparatively, Cassette 4 increased at the same point, and remained the highest for the duration of the experiment, peaking at 3.4 A/m^2.

Similarly, this variability was seen in hydrogen production (Figure 4.6, B). During batch mode, the majority of the hydrogen gas was generated by Cassette 3. When switched to continuous flow, Cassette 2 generated the most and remained this way until day 113. From day 113, Cassette 6 started to produce similar amounts to Cassette 2 and then from day 127, Cassette 2 ceased hydrogen production. There was no correlation between high

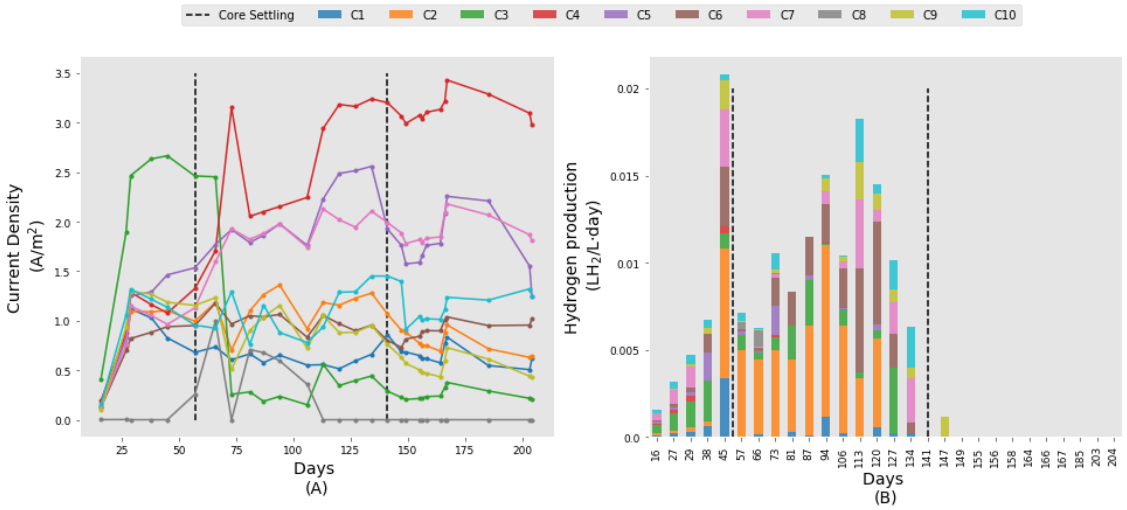


Figure 4.6: Current density (A) and hydrogen production (B) during start-up, batch mode and continuous flow in each individual electrode. Continuous flow started on day 39.

current density in certain electrodes and high hydrogen production ($r^2 = -0.296$). There also was no apparent trend between performance and positioning within the tank. At day 134, the second 'core-settling' event occurred, and hydrogen production stopped in all cassettes.

4.4 Discussion

If MECs could perform to the same level or greater than AS, the switch to a more sustainable wastewater treatment method would be both economically and environmentally beneficial. The performance that needs to be matched is that of COD removal, as the production of current or other products is likely to be a side issue. The VTRs of wastewater treatment systems is vital, as it is a function of both pollutant removal and wastewater flow. It is needed to calculate treatment cost and design treatment tanks. Previous work has highlighted that optimising the HRT of reactors could boost this performance [8, 9]; however, this has not been systematically attempted at pilot-scale.

The research presented here shows that the MEC design that has been used in several pilot studies [2, 3, 4, 5, 171] can match the treatment rates of AS. However, it can only do so with high concentration wastewaters with a COD of above ~2200 mg/L, or energy content of 35.4 kJ/L [21]. The HRT, or the speed of flow, was found to have a consequential effect on the VTRs. Eight different HRTs were measured in triplicate cycles, and it was found that 0.5-days was the optimal HRT. This equated to a flow of 3 L/hour, or 0.4 m/hour of flow past the electrode. At this speed, the average VTR was 3.82 kgCOD/m^3·day. The optimal HRT (0.5 days) was faster than the HRT that most previous pilot-scale experiments have chosen to use, meaning tank sizes would be smaller and costs therefore lower. Typical HRTs of AS are 0.3 days [112], slightly faster than the MEC HRT. However, for anaerobic digesters, where the upstream metabolic processes are putatively the same, these are much longer, between 12 and 30 days [172]. This optimal HRT and subsequent speed of flow seems to be relevant even at low COD concentrations, although here the VTRs are much slower.

It would be expected that the trend in current density would follow the trend of VTRs [126]. In a BES, complex wastes are sequentially broken down by different consortia of bacteria and are eventually funneled into the electrode as electrons. Although it is well documented that there are inefficiencies, dead end paths, and competitive reactions that occur in a BES [148, 177, 178, 179], COD removal should, to some degree, be represented by the current. In this study, this was not the case, which was observed in two distinct ways. Firstly, the optimal HRT with respect to current density was at 0.015-days, where no measureable COD was removed, and secondly, at lower HRTs most of the COD removed did not result in current. We hypothesise that the current recovered at high HRTs was that from the small amounts of the most readily available organics in the waste stream. In the raw RSL, the quantity of acetate measured was 274 mg/L (when the total COD was 4535 mg/L). At 100 L/h flow, this acetate could account for 12.2 coulombs of charge per second, which, distributed over the anodic surface area, would be 15.6 A/m^2. The current density of 1.36 A/m^2 at the fastest flow could have been produced by acetate consumption alone, yet as the acetate only makes up around 6% of the total COD, its removal could easily fall below the detectable COD removal values. At lower flows, acetate had to be provided by the breakdown of longer organics, producing a similar but slightly lower current. The results suggest that maintaining an adequate supply of acetate may be a critical factor in current production, as was observed by Fei et al. [177].

With lower flow rates, the disconnect between current production and COD removal was more surprising. At HRTs of between 0.5 and 2 days, over 50% of the COD was removed, yet only 5.6 – 27.9% of this was transferred to current. Oxygen diffusion into the surface waters is low [3], accounting for less than 1% of the COD removed (calculated using Henry's Law and an oxygen diffusion coefficient of 769.23 L·atm/mol [180] over the top surface area of the reactor). Sulphate reduction could account for between 3.3% and 7.3% of the COD removed at HRTs of 0.5 and 2 days respectively. This leaves between 63.8% and 90.1% of the COD removed unaccounted for. Some of this may have been converted to biomass, and although neither the build-up of sludge nor the thickness of the biofilm were measured quantitatively, there was no observed increases in these in the reactor. This suggests that much of the COD was anaerobically digested, with the resulting methane released from the open anode section. This is surprising: the reactor was run at ambient UK temperatures (wastewater remained between 10°C and 20°C [94]), which would normally prohibit anaerobic digestion; it was fed wastewater deemed too liquid to be suitable for the anaerobic digestion process; and it was operated at residence times much lower than typical anaerobic digestion [181]. Intriguingly, this suggests that the BES reactor facilitates high rates of anaerobic COD removal, yet this COD is not being converted into current, or subsequently hydrogen.

Although CEs were low, indicating a loss of electrons to other reactions, they did increase with increasing HRTs. This suggests that a greater amount of acetate, and then current, was produced the longer the wastewater remained within the reactor. Logically, this should also be seen throughout the reactor's serpentine flow, with electrodes set at the end being fed an increased amount of predigested wastewater compared to those at the beginning. The exponential decrease seen when comparing the average current density from the whole reactor with HRT should therefore be much stronger in these later cassettes. This was not the case. Cassette 2, 5 and 9 were the only electrodes to show the same significant trend as represented by the total reactor (see Appendix B, Figure B.1), with Cassette 2 being the most significant ($p = 0.0002$). Neither current density nor CE appear to be a good indicator of the optimum HRT with respect to wastewater treatment, and are in fact more representative of acetate uptake than organic removal.

The production of current from COD removed was likely to be limited by the development and function of the electrogenic biofilm, which was variable across the reactor. If acetate supply was the main driving force for current production, we would expect to see high current in Cassettes 1 and 2 where the acetate rich wastewater was fed in. We would also expect to see a second area of higher current towards the end of the reactor flow, once more acetate had been produced by the breakdown of longer organic chains. This pattern should be more significant when the flow was low. However, there was no such pattern; in fact, it was the cassettes in the mid-section of the reactor which appeared to perform better. High variability between replica cassettes has been observed previously at pilot scale [2, 4] and is a significant limitation of this technology. The results suggest that biofilm formation is critical to ongoing performance. In the first 25 days, current production was similar in all cassettes. However, once the reactor changed to continuous flow, this performance separated. Aside from Cassette 3 (which appears to have been harmed in the first core-settling event) this separation was maintained throughout. Devising strategies that can help engineer optimal biofilm development [176], which are workable at larger scales, will be necessary for future developments of this technology.

Such strategies should overcome the performance limitations of low current production from the wastes digested. However, it is also clear that higher efficiency of current conversion into hydrogen production is required to reach energy neutrality. During the hydrogen-producing period, peak production was 0.02 $m^3H_2/m^3 \cdot$day, higher than that achieved by Heidrich et al. [2], where hydrogen averaged 0.015 $m^3H_2/m^3 \cdot$day, although still only one-third that of Carlotta-Jones et al. [171], which achieved 0.066 $m^3H_2/m^3 \cdot$day. In this study, the higher average current densities drew more power, and the energy recovered did not offset the energy added. Events on site also led to contamination of the cathodic chamber, as has been reported in other studies [4], highlighting a potential problem with the reactor design or its operation. If run as an abiotic chamber, a mechanism to routinely sterilise the cathode compartment needs to be developed, such as heat sterilisation, or in situ caustic or peroxide production. It has been reported that hydrogen-scavenging bacteria, such as hydrogenotrophic methanogens, can result in the loss of hydrogen from the cathode chamber [1, 4]. This could be the case in this reactor, particularly as contamination was visible during the core-settling events. This would result in the production of methane. However, at the point of no hydrogen production, there was no gas produced at all within the cathode gasbags. It is therefore possible that gases diffused back into the anode side and released into the atmosphere. A further problem with hydrogen production is again the variability between electrode cassettes. This variability did not mirror that observed in current production. Cassette 2 was responsible for almost half of all the total hydrogen production, yet its current density was low throughout. With large-scale reactors, current at the anode is not the controlling factor for hydrogen production. It is possible that minor differences in the internal resistance in each cassette caused some of this variability, but further investigation into low hydrogen yields will be paramount for commercialisation.

High removal efficiency, i.e. the percentage of COD removed, is desirable and often a main focus in pilot-scale work. Wastewater treatment relies on the ability to meet discharge standards, which in the UK are 125 mg/L COD, or >75% removal [17]. The removal efficiency in this reactor at a 0.5-day HRT was around 50%. This is lower than several previous pilot studies, all of which use low strength wastewaters [1, 6, 11, 13], but with a longer HRT. This research showed that longer HRTs enabled greater breakdown of organics most likely in both the bulk liquid and in the biofilm, allowing the slower rate limiting steps, such as hydrolysis [98], to have occurred. This subsequently led to an increase of CE, as prolonged exposure to the biofilm enabled greater conversion of complex organics to electrons. The logarithmic increase between increasing removal efficiency and increasing HRT suggests that this reactor *could* reach discharge standards if left long enough; however, this would reduce the VTR and therefore increase costs.

The predicted energetic costs and forecasted tank size illustrate clearly the falling viability of MECs as COD concentration drops. In fact, with wastewaters with a COD below 600 mg/L (as would be typical of raw municipal or domestic wastewater [111]), the VTRs are extremely low, and HRTs would need to be very large to get organic removal down to the necessary level. However, with the high concentration wastewaters targeted in this pilot study, which are produced in all sites that dewater following anaerobic digestion, BES technology could be economically viable. The energetic cost savings versus AS could be huge when removing the first 50% of the COD load, and these savings may outweigh the large capital costs. It is acknowledged that performance would not stay the same when scaled up by such an order of magnitude, and maintenance costs have not been considered. However, the proposed OLR targets needed for economic viability [34] were met here, and elsewhere it has been shown that recycled carbon felt can effectively maintain or boost performance in a reactor design similar to this one, with a 88.6% reduction in cost [171].

Future pilot-scale research with this technology should seek to optimise performance, rather than demonstrate it. Improving CCE by optimising voltage input should help towards achieving energy neutrality. Improved understanding of, and ability to engineer biofilm formation processes could help improve CEs, generating more current. The exact nature of the biological processes and anaerobic metabolic pathways of BESs are unknown. They have been explored to an extent in laboratory studies [98, 148, 177] and can manifest themselves as strange observations when studies are carried out at scale, such as the disconnect between COD removal and current observed here. This in itself may not be a prohibiting factor in commercial uptake. The process of AS, discovered in 1913 [42], had revolutionised UK sanitation long before it was even known that the process was biological. However, the high variability within the reactor between identical commercially made electrode cassettes may be a greater challenge for industry to accept, as it introduces uncertainty and therefore risk. This variability has been documented in other pilot studies [1, 2, 4, 155, 182], and it shows that some electrodes are operating far below their potential. Major application of this technology cannot occur without further investigation into this electrode variability and analysis of the biofilm formation process. Finally, detailed understanding of how reactor components degrade over time will enable practical implementation at a larger scale.

4.5 Conclusion

This study is the first to rigorously optimise the HRT of a pilot-scale MEC treating real wastewater. VTRs were higher than ever previously reported, despite the fact that discharge standards were not reached. This was due to the use of RSL with a high organic content, rather than the use of raw wastewater. This study gives reason to view MECs differently. Instead of replacements for AS, further studies could look at using MECs as a pre-treatment technique for RSL to eliminate some of the AS cost. Theoretical calculations of the energetic cost savings indicates that the use of an MEC as a RSL treatment technique could result in an industrially viable system, despite the large capital costs. The use of a BES at this point in the treatment stream would remove the need to reach the legal discharge standards and reduce the need for large and high-risk industrial change. Using BESs here first could be an affordable transition phase that would help to improve the resilience, efficiency and reach of this technology.

Chapter 5

Investigating the natural variability in bioelectrochemical systems

5.1 Introduction

Throughout the previous chapters, the potential for bioelectrochemical systems (BESs) to be used for wastewater treatment has been reviewed extensively. However, in each of the experiments one major limitation has stood out. Large variability in performance has manifested in identically made reactors or electrodes at a seemingly random distribution. Initially, this was a 9-fold variability of current, produced from the same strength substrate in identical MFCs from Chapter 3 (Figure 3.2). Following this, in Chapter 4 there was a 12-fold variation in current from identical cassette electrodes within the same pilot reactor, and a 21-fold difference in the hydrogen recovered in each electrode (Figure 4.6). This variability reduces the overall performance and therefore increases costs, but more importantly, it highlights our lack of understanding of, and inability to control and engineer these systems. It introduces risk, moving the technology further away from commercialisation rather than towards it. The material costs for these systems are currently extremely high, and therefore, before being ready for industry it would be expected that all would perform to the same standard. This variability appears to stem from using fresh wastewater, which limits the reactors at a seemingly random distribution. Although this variability does not seem to affect the wastewater treatment capabilities of the BES, in order to become a viable source of renewable energy, reactors need to recover a valuable commodity at a predictable rate. Investigating this variability will be an opportunity to further understand the bacterial communities within BESs, and will be critical in advancing this technology.

Within a BES there is a biological anode, which consists of an electroactive biofilm on a conductive inorganic substance [183]. Typically, wastewater is used as an inoculum to establish this biofilm, which contains electrogenic bacteria capable of extracellular electron transfer. The electrogens act as a catalyst for the transfer of electrons from the breakdown of simple sugars, which are transferred to the anode via nanowires, electron shuttle mediators, or direct contact [184]. The electrons then travel from the anode to a cathode via an external pathway, while simultaneously hydrogen ions are produced from the breakdown of simple sugars, which flow to the cathode. This results in a reduction reaction occurring at the cathode, enabling the generation of current [183]. Therefore, BESs rely on an effective bioanode to produce electrons from the fed substrate.

A measure of this electron transfer is called the coulombic efficiency (CE), which compares the total number of electrons that could be produced from the removed organics (estimated using chemical oxygen demand (COD mg/L)) to the actual number transferred to the anode [143]. Wastewater fed reactors typically have a low CE of around 15 – 30% [158, 159] due to a large number of electrons lost to external pathways [148]. In comparison, reactors fed a simple substrate such as acetate have been known to reach CEs of 90+% [185]. This loss of electrons when using a more complex substrate may not affect the wastewater treatment, as the breakdown of complex organics is beneficial to COD removal regardless of the electron pathway. However, this technology is desirable due to the ability to recover energy. The formation and performance of the biofilm is therefore critical in the reactor performance.

Wastewater is highly complex, and these components vary over space and time. If the BES is treating wastewater, the biofilm will ideally contain hydrolytic, fermentative and electrogenic bacteria. Hydrolytic bacteria are required to break down complex carbohydrates into sugars, proteins into amino acids and fats into long chain fatty acids. Fermentative bacteria are then able to convert these products into simple compounds that can be useable for electrogens. For current production to be feasible from complex wastes there needs to be a syntrophic interaction between the bacteria found on the biofilm. However, energy recovery is affected by the presence of the competing bacteria that do not possess extracellular electron transfer capabilities. The presence of methanogens, specifically hydrogenotrophic and acetoclastic methanogens, can result in electron losses with the conversion of hydrogen or acetate into methane [186]. Similarly, the presence of sulphur reducing bacteria can result in sulphate reducing to hydrogen sulphide, which if deposited on the anode, can inhibit electrogenic growth [186]. If denitrifying bacteria are present, electron losses are possible due to the reduction of nitrate [187].

Other than the type of inoculum used, biofilm formation and microbial community composition can be influenced by a number of other factors. These include the anode potential [188], cell design [189], surface charge and hydrophobicity [190] and the temperature [191]. There is evidence to show that the more complex the substrate fed to the BES, the higher the diversity of bacteria found in the biofilm [187]. Additionally, a change in the type of substrate mid-run, such as a switch from acetate to glucose [5], or a change in the type of wastewater used [6], could also alter the microbial community [192]. Some of the factors that affect biofilm formation are controllable when setting up laboratory-based systems; however, the components and effect of wastewater are not. Kokko et al. [187] suggests that similar reactor configurations should be used in order to reduce variability, while also stressing the importance behind setting up identical experiments. Unfortunately, variability in performance in identically made reactors persists, which is often not discussed.

Previous research at Newcastle University has observed and reported this variability [3, 4], and it is expected to have occurred elsewhere. However, most published microbial fuel cell (MFC) and microbial electrolysis cell (MEC) studies use only duplicate or triplicate reactors, with a focus on factors that might influence performance. This means that the inter-reactor variability is not apparent and is not investigated. To investigate variability, it must be observed using a large number of replicates under identical conditions. Additionally, the difficulty in publishing results that show high variability may be another reason why it is underreported in the literature.

Pilot-scale reactors often use multiple identical electrodes and are fed real wastewater [3, 4, 5, 6], and therefore it is easier to see this variability. While running a pilot-scale MEC, Heidrich et al. [3] discovered that when using six identical electrodes within one reactor, hydrogen production from each electrode ranged from 38.5 – 175.6 ml/day. Additionally, power densities ranged from 0.49 – 0.88 mW/m^2, with one electrode failing to start up at all. Cotterill et al. [4] observed a similar event. With three identically made 1m^2 electrodes, average current production varied from 192 – 252mA, and average hydrogen production ranged from 24 - 474.6 ml/day. If you take the best performing electrodes from the pilot-scale MECs, and assume hydrogen gas recovery from that electrode is the peak potential, Heidrich et al. [2] reactor was only performing at 53.1%, while Cotterill et al. [4] was as low as 38.8%. Additionally, although it was not explicitly discussed, this variability can be seen in some other studies. Current densities were shown to range from 2 – 5 A/m^3 in a 200 litre modulated MFC treating municipal wastewater [155] and in a stacked MFC design treating residential wastewater, a range of 0.5 – 3.5mA in current was seen between 9 electrode configurations [182]. There was no pattern seen in performance with respect to wastewater flow in any of the pilot-scale studies.

At a laboratory based scale, Koch et al. [193] ran 5 identical reactors fed real wastewater, and from inoculation, each exhibited a different performance in terms of CE (10% - 38%) and COD removal (37% – 65%). This variability in performance continued over six runs. Understanding the root cause of this variability will be key to achieving improved biofilms, capable of high pollutant removal and electron transfer rates, and boosting the performance of BESs overall. If this technology is to become industrially viable, the prevention of this variability is needed before there are any serious attempts to compete with current wastewater treatment.

When using a mixed culture as the inoculum, it is likely to cause a mixed population of bacteria on the biofilm, of which some are beneficial and some are not. Due to this variability, the performance of bioanodes using mixed cultures is still insufficient for industrial application [55]. Pure cultures of electrochemically active microorganisms (EAMs) have been used as inoculum for BESs instead of mixed cultures and it would be reasonable to assume that the use of a pure culture would improve the performance. The most commonly known EAMs are *Geobacter sulfurreducens* and *Shewanella oneidensis* [194], which have been studied extensively. *G. sulfurreducens* is an anaerobic bacterium, found naturally in soils where reduction of Iron III oxides is required, whereas *S. oneidensis* is a facultative bacterium capable of anaerobically reducing metals, sulphates, nitrates and chromates [195]. Although pure cultures of EAMs have been successfully used within BESs [196, 197, 198, 199, 200], it is recognised that mixed cultures tend to produce higher current densities than identical reactors inoculated with pure cultures. For example, *G. sulfurreducens* produced 22% less power than a mixed culture from anaerobic digester sludge [201], and *S. oneidensis* produced 56% less power than an air cathode MFC inoculated with wastewater [202]. When the digestion of complex organics is required, a pure culture biofilm containing only electrogens is unsuitable. Thus, although the use of a pure culture can lead to a high CE with simple substrates, as there are fewer electrons lost to alternative pathways, using a mixed culture is necessary and often results in a more robust community that is less prone to contamination [203]. The use of a pure culture may remove variability between identically made reactors; however, for BESs to act as a wastewater treatment technology, the biofilm would need to cope with continuous fresh wastewater as an influent. The solution therefore needs to incorporate the continued use of a mixed culture and the use of fresh wastewater as a substrate.

For BES technology to act as a renewable energy source, this variability needs to be removed. This study therefore first investigates the variability that occurred in the previous chapters, where a large number of replica reactors were operated under identical conditions. Additionally, it is hypothesised that higher performing biofilms could be created artificially by inoculating the reactors with the effluent from previous high performing reactors, removing this variability. Therefore, a further experiment was undertaken that again used a large number of replica reactors, with attempts to remove this variability. Finally, inoculation with a pure culture was attempted to determine if this variability persists when using only *G. sulfurreducens.* Unfortunately, growth of the pure culture was unsuccessful.

5.2 Materials and methods

5.2.1 Reactor configuration

During Chapter 3, the variability was initially observed, and was apparent when running the pilot-scale in Chapter 4. This experiment was designed to investigate this variability, and it became clear that modification to the MFC design would be needed to enable easier access to the biofilm. Therefore, some of the results in this Chapter will refer back to the initial design, termed Type–A, and an explanation on the reactor design can be found in Section 3.2.1, Chapter 3. The following design, which was used for the re-seeding experiments, has been termed Type–B.

Type-B reactor design

The new design, termed Type-B, consisted of 18 identical MFCs. These reactors were made using inexpensive plastic containers designed for food storage (LocknLock Ltd, Seoul, Korea). They have the advantage of being cheap, and therefore enabling a high number of replicates. They are also simple to open, resulting in easy access to the biofilm, while maintaining water tightness when closed. The reactor set-up can be seen in Figure 5.1.

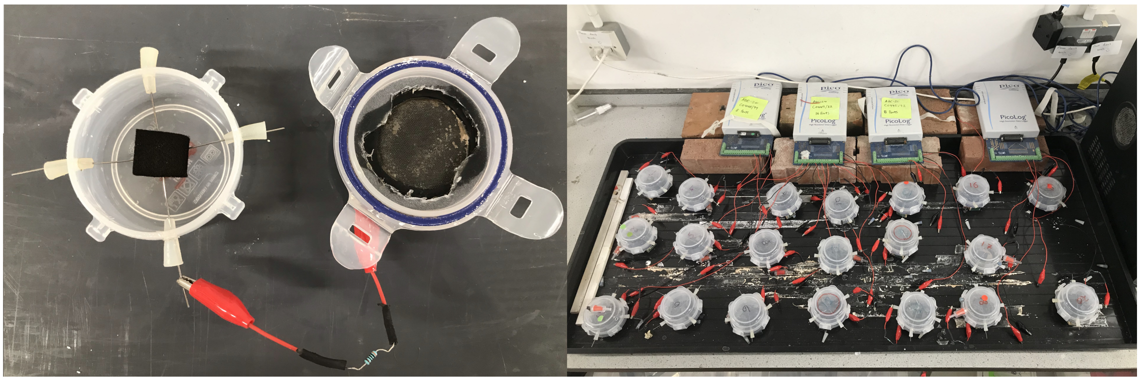

(A) (B)

Figure 5.1: A singular open Type-B MFC design (A) and reactor set-up for the 18 Type-B MFCs (B).

The reactors were cylindrical chambers with two sample ports, resulting in a 100ml working volume (Figure 5.2). The anode was 20mm x 20mm x 3mm carbon felt (SGL Carbon, Wiesbaden, Germany) connected to 0.6mm^2 stainless steel MIG welding wire (Clarke Tools ©, Chronos Ltd, Dunstable, UK) fed out of a sample port through a rubber bung. The air cathode was 0.2 mg/cm^2 20% platinum on Vulcan carbon cloth electrode (Fuel Cell Store ©, Texas, USA), platinum side facing the air. A 300Ω resistor connected the anode wire and the carbon cloth cathode, secured by crocodile clips. All wire connections were soldered to maintain high connection. The carbon cloth cathode was 50mm x 50mm, with an extra 10mm x 20mm section cut at the top, which was folded at 90 degrees to allow crocodile clips to connect. This was glued to a rubber circle (outer diameter 60mm) to aid its seal to the reactor and limit damage to the air cathode. A 40mm diameter circle was cut out of the rubber to fit around the 40mm diameter hole cut out of the reactor lid, with the cathode glued on top. Epoxy Resin (Gorilla glue Ltd, Chorley, UK) was used to glue both the cathode and rubber together, and the rubber to the reactor lid, to provide a watertight seal (Figure 5.2).

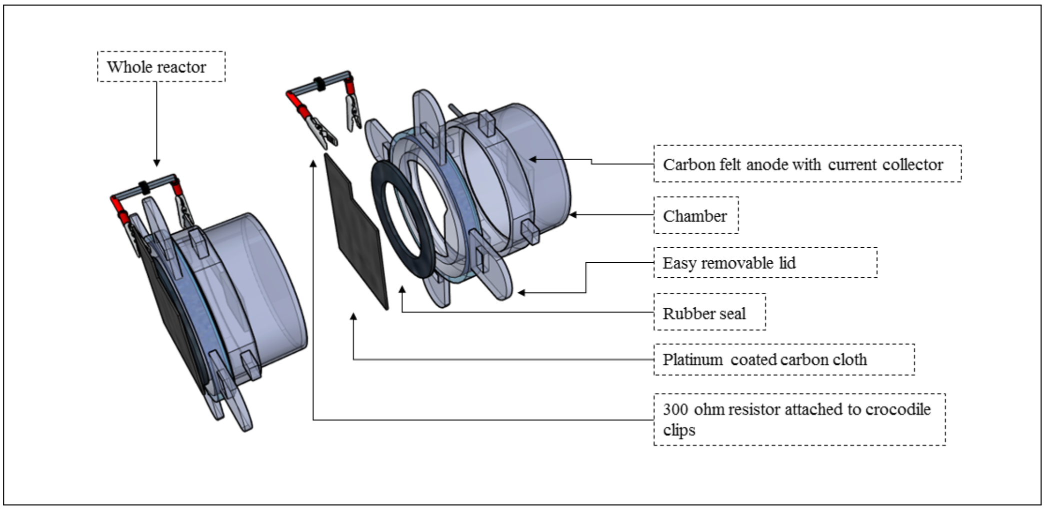


Figure 5.2: Type-B air-cathode MFC configuration (left) with individual components in an exploded view (right).

5.2.2 Multiple runs

This Chapter refers to experiments from Chapter 3, along with new re-seeding experiments, all using air-cathode MFCs. Therefore, the multiple runs of each set of reactors, and their experimental conditions, can be seen in Table 5.1. All reactors were operated within a laboratory at ambient temperatures around 22°C. Inoculation refers to clean sterile reactors with a fresh anode, being filled with either a mixed culture or pure culture of bacteria. For all mixed cultures, reactors were inoculated with an acetate mix combined with either raw wastewater or return sludge liquor (RSL). Raw wastewater was collected from Birtley Wastewater Treatment Works (NWL) and RSL was collected from Howdon Wastewater Treatment Works (NWL). Standardising runs refers to having a new fresh substrate added without change to the anode. The COD is the total COD measured in the inoculum or substrate, while the type of reactor refers to the design of MFC used (see Section 5.2.1).

Runs 1 – 8 were results from a previous experiment that used the Type-A design for the air-cathode MFC. Initially the reactors were inoculated with fresh raw wastewater and acetate, before being subject to three standardising runs using a raw wastewater-acetate mix. Following this, the reactors were then cleaned and re-built, before being inoculated again with a fresh raw wastewater-acetate mix (runs 5 - 8, Chapter 3). These were again subject to three standardising runs, however they were fed a pure acetate solution. For runs 9 - 13, the Type-B design was used to enable easier biofilm access. The 18 reactors were inoculated with a RSL-acetate mix. The two highest performing reactors and the two lowest performing reactors in terms of charge production then had their anodes removed. The biofilm was then extracted and mixed with a sterile wastewater-acetate mix to be used to inoculate 9 reactors each. This was repeated twice more (runs 10 – 12) and the final run was using fresh RSL-acetate mix.

Table 5.1: The order of runs for the air-cathode MFCs, including the type of run, the substrate used and the inoculum, along with the final mixtures COD and the reactor configuration used.

Run	**Type**	**Substrate**	**Inoculum**	**COD (mg/L)**	**Type of reactor**
1	Inoculation	WW-acetate mix	Mixed culture	420	Type A
2	Standardising	WW-acetate mix	n/a	442.5	Type A
3	Standardising	WW-acetate mix	n/a	665	Type A
4	Standardising	WW-acetate mix	n/a	320	Type A
5	Inoculation	WW-acetate mix	Mixed culture	431	Type A
6	Standardising	Sterilised acetate mix	n/a	269	Type A
7	Standardising	Sterilised acetate mix	n/a	211	Type A
8	Standardising	Sterilised acetate mix	n/a	182	Type A
9	Inoculation	RSL-acetate mix	Mixed culture	727	Type B
10	Inoculation	Sterilised RSL-acetate	Biofilms from run 9	495 / 505	Type B
11	Inoculation	Sterilised RSL-acetate	Biofilms from run 10	707/ 714	Type B
12	Inoculation	RSL-acetate mix	Biofilms from run 11	696 / 670	Type B
13	Inoculation	Sterilised acetate mix	*G. sulfurreducens*	n/a	Type B

5.2.3 Analytical methods

Following the end of each run, all reactors were sampled and measured for COD. COD removal was measured using Merck COD cuvette tests (25 - 1500 mg/L) in duplicates according to standard methods. Voltage was measured using Pico 6 software across a 300Ω resistor, with ADC-20 and ADC-24 PicoLogers for continuous measurement. Biofilms were removed from the reactors following runs 9, 10 and 11. Careful handling ensured no damage to the biofilm, which was placed in the effluent from the reactor it came from. This was then mixed using a magnetic stirrer, before being used as inoculum for the following run.

5.2.4 Substrate sterilisation

Sterilisation of the wastewater was accomplished by filtration and UV light. The RSL was first allowed to settle, before the soluble fraction was passed through a 0.2μm filter. This was then left under a UV light for 30 minutes prior to being used as a substrate. Acetate mixes were sterilised using an autoclave.

5.2.5 Calculations

Current density, COD removal, VTR, CE, peak current density and total coulombs were calculated as follows:

Voltage was measured using high resolution multi-channel data loggers from PicoTech [153]. This was converted to current density based on projected anode surface area. According to Ohms law, the current can be calculated as:

$$I = \frac{V}{R}$$

where I is the current (amps), V is the voltage (volts) and R is the resistance (Ohms). This was then divided by the surface area to get current density:

$$J = \frac{I}{A}$$

where A is the projected anode surface area (m^2) and J is the current density (A/m^2).

Volumetric treatment rate has been calculated using the COD removed and the retention time of the substrate, where S is COD removed (mg/L), HRT is the retention time (days), and Ks is VTR (kgCOD/m^3·day).

$$Ks = \frac{S}{HRT \times 1000}$$

Total coulombs (C_p) was calculated by the sum of the total current. As the Pico Log software recorded the Volts over a 300Ω resistor every minute, this was multiplied by 60 to convert to seconds, and then summed.

$$C_p = \sum (I \times 60)$$

CE was calculated by:

$$E_c = \frac{C_p}{C_n} \times 100$$

where E_c is the CE, C_p is the total coulombs (current over time), and C_n is the theoretical coulombs that could be recovered from the COD removed. Theoretical coulombs were calculated based on Logan et al [61], and therefore CE was calculated by:

$$E_c = \frac{8 \times C_p}{F \times V_A n \times \Delta COD} \times 100$$

where F is Faraday's constant (96,485 C/mol of electrons), 8 is a constant used for COD [61], V_{An} is the liquid volume in the anode chamber (L), ΔCOD is the change in COD (g/L) that has occurred over the batch cycle.

There is a tacit assumption that the increase in current represents growth rates of electrogens. Therefore, the average increase in amps per second has been taken from each reactor for the period of exponential rise in current, and the rise in current is reported in mA·s.

Additionally, the point at which the current started to increase exponentially, once above a threshold of 0.02 A/m^2, was labelled the time of initiation, and is recorded in Days.

5.2.6 Pilot-scale results

The pilot-scale data refers to Chapter 4, which was collected over a period of 204 days. The data discussed represents the changes in current density and hydrogen production over time in each individual electrode from start-up in batch mode, through to continuous flow.

5.2.7 Pure culture

Sterilisation

Keeping the reactors sterile was critical in setting up a pure culture experiment. The following precautions were taken. Anode carbon felt, once cut, was heated in a furnace at 500°C, and kept in sealed sterile bags. The cathode, reactor chamber, rubber seals, wire connections and resistors were placed in a UV cabinet for 1 hour prior to use. The substrate mix made primarily of acetate was autoclaved. Each component was swabbed before and after the sterilisation process to be grown on agar plates to confirm decontamination. If bacteria were shown to be present, further sterilisation would take place. Reactors were then built within the anaerobic cabinet, and kept sealed until addition of *G. sulfurreducens* [204].

Growth medium

Growth medium for the *G. sulfurreducens* was made according to DSMZ (DSMZ ©, Braunschweig, Germany) guidelines and was made up of the components found in Table 5.2.

All ingredients except fumarate, bicarbonate and vitamins solution were dissolved and sparged with nitrogen/CO_2 gas mix to create an anoxic environment. Bicarbonate was then added and sparged with a gas mix to reach pH 6.8 before being dispensed into serum bottles and autoclaved. Fumarate, vitamins and minerals were then added under 100% nitrogen atmosphere using an anaerobic hood.

Incubation

Growth of *G. sulfurreducens* was done following the guidelines from DSMZ in a sterile environment. A freeze-dried pellet was re-hydrated for 30 minutes in 10ml growth medium

Table 5.2: Growth medium as described by DSMZ guidelines for G. *sulfurreducens*

Component	**Amount**	**Unit**
Ammonium chloride (NH_4Cl)	1.5	g
Di-sodium hydrogen phosphate (Na_2HPO_4)	0.6	g
Potassium chloride (KCl)	0.1	g
Sodium Acetate ($NaC_2H_3O_2$)	0.82	g
Trace element solution (ATCC™)	10	ml
Sodium bicarbonate ($NaHCO_3$)	2.5	g
Sodium fumarate ($Na_2C_4H_2O_4$)	8	g
Vitamin solution (ATCC™)	10	ml
Distilled water	980	ml

under anaerobic conditions. This was then transferred to the ∼1L growth medium (Table 5.2) and incubated at 37°C for 7 days.

Substrate mix

An acetate mix with a phosphate buffer, trace element (ATCC™, Teddington, UK) and vitamin solution (ATCC™, Teddington, UK) was used for the substrate. Acetate mix was made to give a COD of 500 mg/L, and the components can be seen in Table 5.3.

Table 5.3: Acetate mix: components per litre.

Component	**Amount**	**Unit**
Sodium acetate ($NaC_2H_3O_2$)	1	g
Sodium di-hydrogen phosphate di-hydrate ($NaH_2PO_4 \cdot H_2O$)	0.528	g
Di-sodium hydrogen phosphate (Na_2HPO_4)	0.984	g
Vitamin solution	15	ml
Trace element solution	15	ml
Distilled water	1.5	L

Reactor set-up

All reactor set up was done under completely sterile anaerobic conditions using an anaerobic chamber. To keep the amount of *G. sulfurreducens* the same in each MFC, the mixture was homogenised before adding a pre-determined amount. Two different methods were used for *G. sulfurreducens* addition. Fourteen sprays directly onto the anode using a dispenser equated to 1.04ml of bacteria mixture. Each side of the anode was sprayed seven times and then the reactor sealed by the bung. The acetate mix was then pipetted in via sample ports. In the other 10 reactors, the acetate mix was added via the sample ports into the reactor, and then 1.04ml of *G. sulfurreducens* mix was pipetted in. This resulted in 10 identical reactors for both methods.

Biofilm analysis

If biofilm analysis were possible, cell count for the pure-cultures would have been measured by flow cytometry. Three identical cores would have been taken from 10 reactors (5 from reactors with each different *G. sulfurreducens* addition method) to give an average of each reactor. This would have been done anaerobically to reduce the impact removing the cores will have on the MFCs. Samples would then have been sonicated to break up the biofilm, with the carbon felt removed. Special care would have been taken to ensure all of the biofilm was detached. Serial dilutions for each sample repeat would have been done in 1/10, 1/100, and 1/1000, fixed in 'ETCS', then 10μl of FITC would have been added to each sample, followed by incubation at room temperature in the dark for 10 minutes. Samples would then have been put through a flow cytometer for cell count.

5.3 Results

5.3.1 Results from experiments in Chapter 3

In Chapter 3, 28-replica air-cathode MFCs (Type–A design) were inoculated with a wastewater-acetate mix and left in identical conditions until current production ceased. It was anticipated that all 28 reactors would have a similar performance, and they could then be used to perform the COD saturation experiments detailed in Chapter 3. However, high variation in current was observed across 27 reactors in the first inoculation run, with one reactor failing to start (Figure 5.3). Peak current ranged from 0.05 – 0.28 A/m^2 and total coulombs ranged from 33 – 165 A·s.

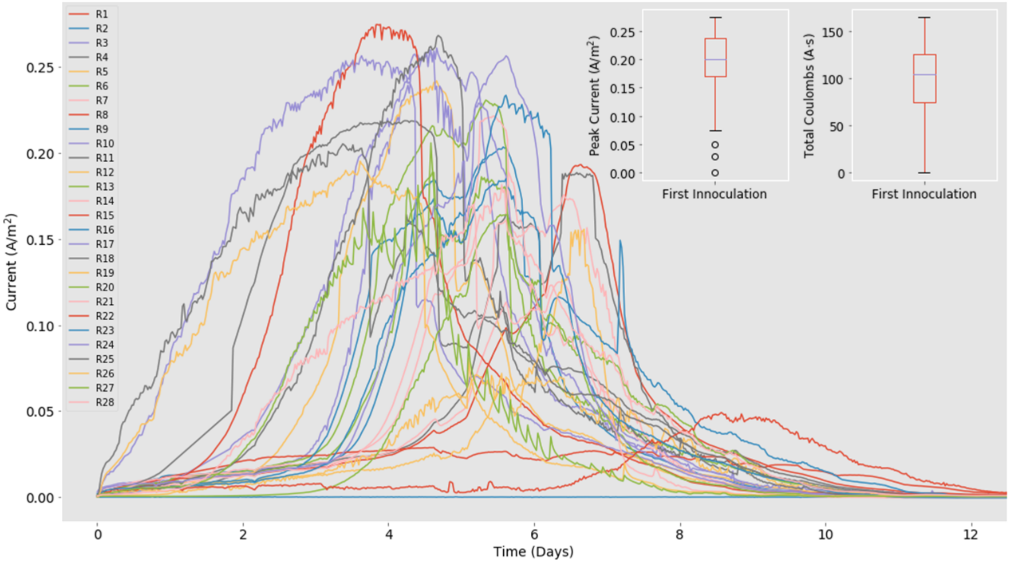


Figure 5.3: Current production during initial inoculation and standardising runs, all with a wastewater-acetate mix.

This variability and, in particular the different shaped curves of current production and different peaks of current production, led to the experimental work outlined in this Chapter. Specifically the following research questions were formed:

1. Is this high variability among large numbers of replica reactors reproducible?
2. Is this variability related to the time taken until initiation of current is observed? I.e. are electrogenic biofilms that initiate more quickly able to exploit more of the COD available, and produce a higher peak current?
3. Is the variability related the rate of exponential increase in current, which can be used as a proxy for growth [205]? I.e. are electrogenic biofilms that grow more quickly able to exploit more of the COD available, and produce a higher peak current?

In order to overcome this issue and engineer these systems to perform better, three further questions were formed:

4. Can initial variability be overcome by subsequent feeding of simple or complex substrates?
5. Can the variability be overcome by artificially seeding new reactors using high performing reactors?
6. Can this variability be overcome by inoculating with a pure-culture of *G. sulfurreducens*?

5.3.2 Pilot-scale variability

Variability was also present during Chapter 4, when running a pilot-scale MEC at a wastewater treatment site using RSL. Throughout continuous flow mode, each individual electrode showed highly variable current densities and hydrogen production. Some electrodes never produced any gas, while others produced gas sporadically. Total reactor cathodic conversion efficiency was around 1.2% and there was no correlation in electrodes when comparing the distance along the flow-path with performance (see Figure 4.6). There was also no relationship between high current densities and high hydrogen production.

5.3.3 Natural variation during inoculation with wastewater

Following the toxic RSL used prior to the experimental results in Chapter 3, the reactors were dismantled, cleaned and re-inoculated with a wastewater-acetate mix. Due to damage caused to three reactors, 25 replicas were used. To answer the first research question, and determine if the high variability among large numbers of replica reactors was reproducible, the reactors were again inoculated with a wastewater-acetate mix. This was shown to be reproducible, as the current produced for each reactor showed high variability (Figure 5.4), with peak current ranging from 0.04 – 0.36 A/m^2 and total coulombs ranging from 46 – 183 A·s.

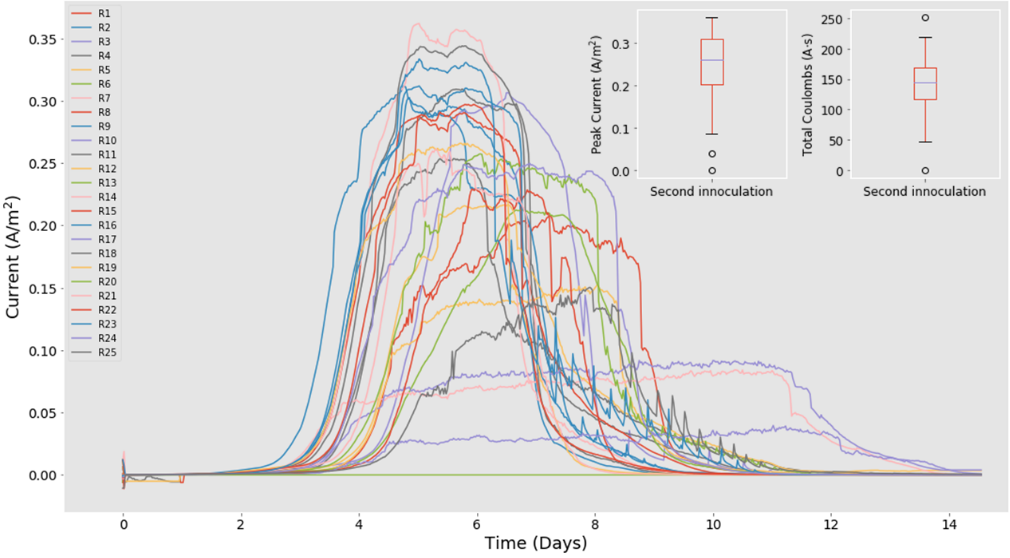


Figure 5.4: Current production during inoculation of the 25 reactors from the second attempt.

The current production curves during the second inoculation seemed to visually separate into three different groups (Figure 5.5). Growth curve A increased exponentially before reaching its peak current (average 0.31 A/m^2), which maintained at the peak current for 2 – 3 days before an exponential decline. Growth curve B was similar, however, reached lower peak currents of on average 0.24 A/m^2, and typically remained at this level for 4 – 6 days before an exponential decline. Comparatively growth curves C had much lower peak currents of 0.1 A/m^2 and remained at this peak current for between 5 – 10 days.

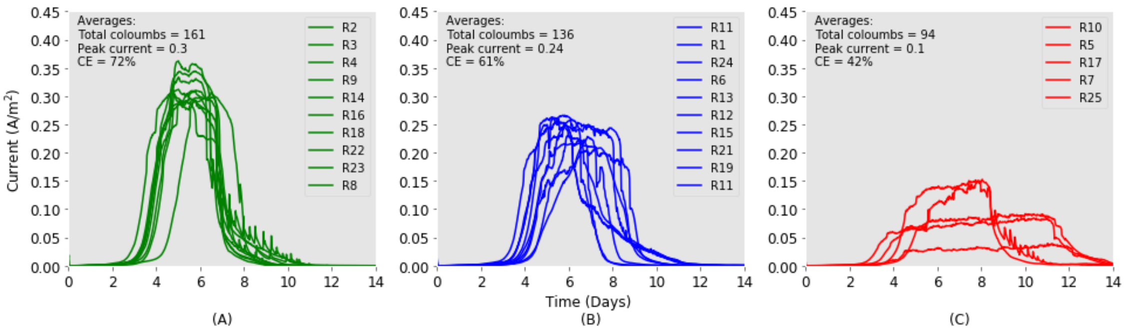


Figure 5.5: Current production during inoculation of 25 air-cathode MFCs from the second attempt, split into three groups.

These three groups have been based primarily on the peak current values. Groups are statistically different from each other (p-values of 0.000 and 0.002 when comparing groups A and B, and B and C respectively) (Figure 5.6). When comparing total coulombs there is greater overlap; however, the averages still show the same decline as seen in peak current and groups are still statistically different (p-values of 0.007 and 0.025 when comparing groups A and B, and B and C respectively).

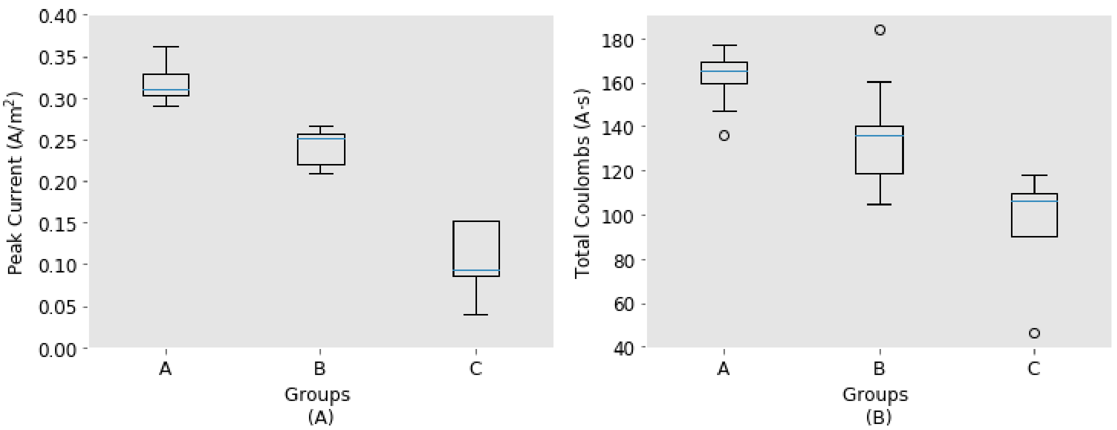


Figure 5.6: Box plots representing Groups A, B and C during inoculation for peak current (A) and total coulombs (B).

To answer the second research question, the time of initiation, defined as the time current started to increase exponentially, was calculated for each reactor. During this second inoculation, the reactors took between 3 – 4 days to start producing current. It was seen that time for initiation showed slight negative correlations with both total coulombs (r^2 = -0.618) and peak current (r^2 = -0.473) (Figure 5.7, A and B), signifying that those that started more quickly produced more charge than those that started more slowly. Additionally, the current produced during this initiation shows a similar pattern to exponential growth, which you would expect to observe in microbial systems. Therefore to answer the third research question, this rate was calculated for each reactor and ranged from 0.0015 –

0.017 mA·s, which correlated slightly with total coulombs ($r^2 = 0.653$) and strongly with peak current ($r^2 = 0.916$) (Figure 5.7, C and D), indicating that those that grew quicker were able to exploit more of the COD.

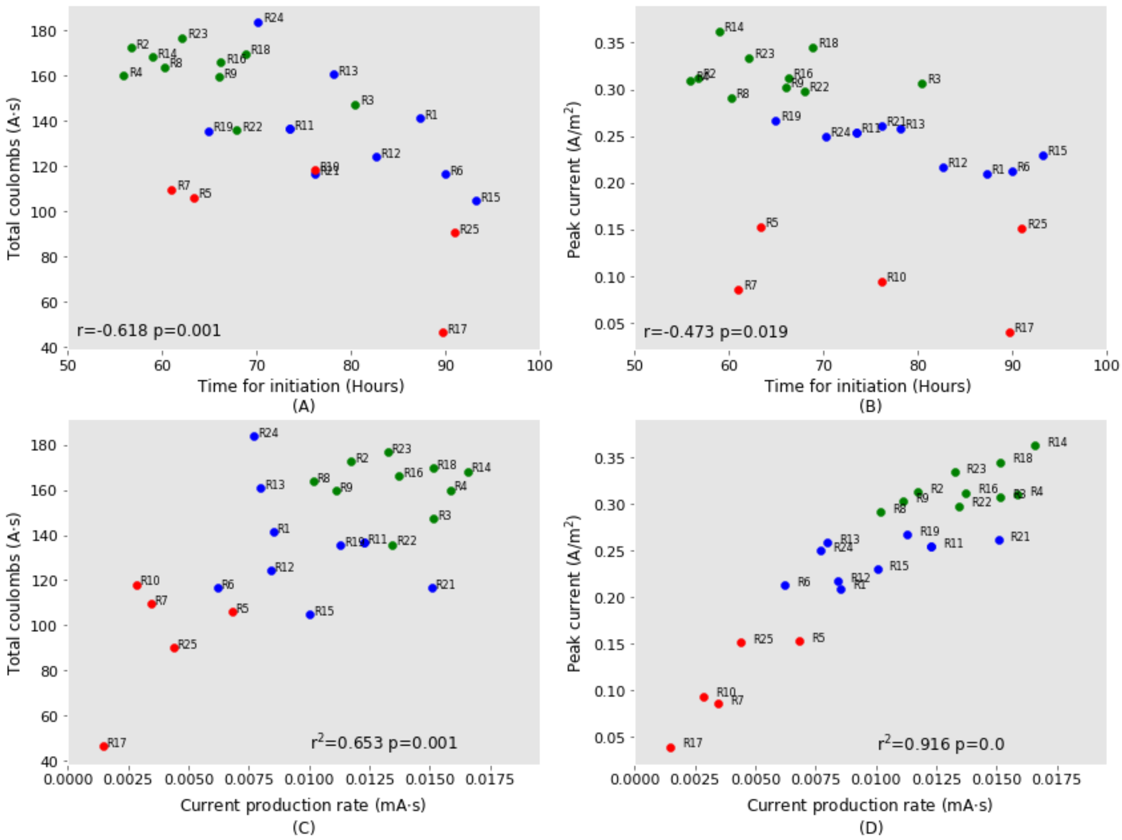


Figure 5.7: Total coulombs vs time for initiation (A), peak current vs time for initiation (B), total coulombs vs growth rates (C) and peak current vs growth rates (D) during inoculation of 25 air-cathode MFCs fed a wastewater-acetate mix.

5.3.4 Standardising runs

In an attempt to engineer a better system, the fourth research question set out to see if subsequent feeding of the reactors for three runs would help remove the variability. Initially reactors were fed a wastewater-acetate mix to standardise the current following inoculation (runs 2 - 4 in Table 5.1). It was anticipated that these further runs would stabilise the performance of the reactors so that experiments could start. However, the variability in current production seen during inoculation continued (Figure 5.8). There was no reduction in the range of values across the 28 reactors when comparing peak current (ranges of 0.26, 0.20 and 0.21 A/m^2) and only a small reduction when comparing total coulombs (ranges of 103, 139 and 78 A·s).

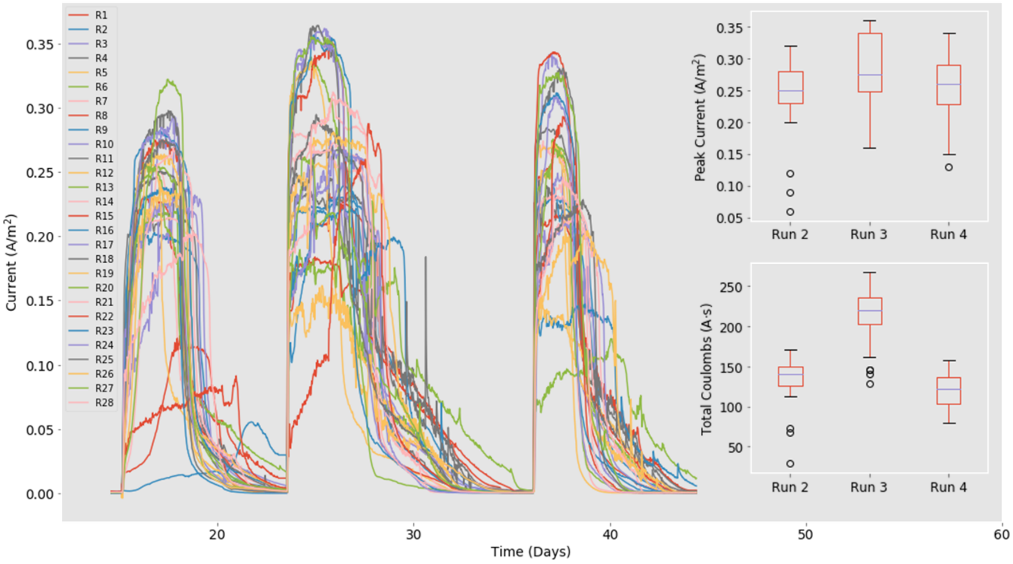


Figure 5.8: Current production for the initial three standardising runs using a wastewater-acetate mix, with box plots to represent the variation in peak current and total coulombs.

Therefore, for the repeat experiment in Chapter 3, the 25 reactors were fed a pure acetate mix for the three standardising runs to try to remove the variability (Figure 5.9) (runs 6 - 8 in Table 5.1). As discussed in Chapter 3, variability continued between the reactors in terms of peak current (ranges of 0.17, 0.19 and 0.17 A/m^2); however, when comparing total coulombs, this range appeared to lessen (ranges of 57, 55 and 34 A·s) (also see Figure 3.2). As discussed in Chapter 3, the decrease in average total coulombs was due to subjecting reactors in each consecutive run with less acetate within the substrate.

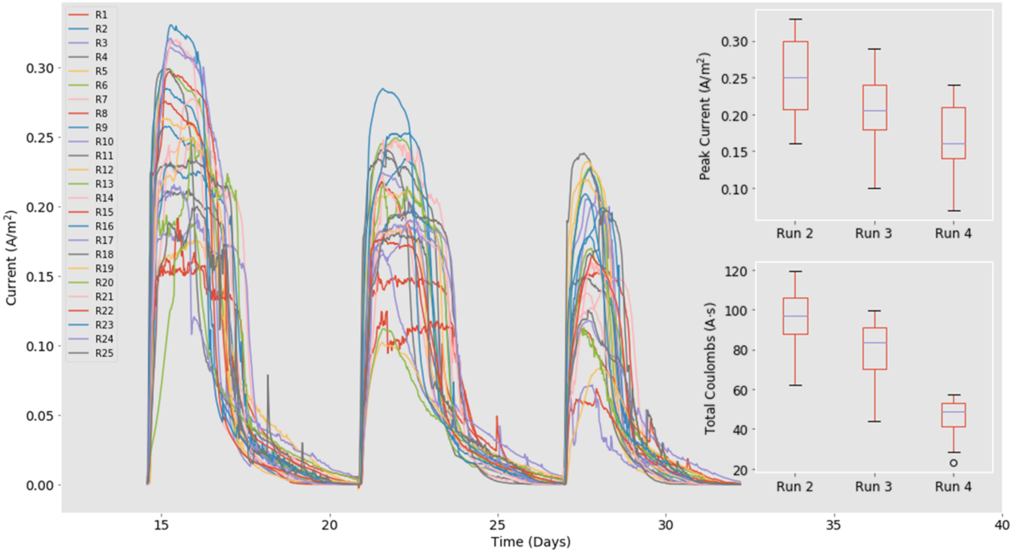


Figure 5.9: Current production for the repeated three standardising runs using a pure acetate mix, with box plots to represent the variation in peak current and total coulombs.

5.3.5 Controlled inoculation

In order to answer the fifth research question, and determine if the variability could be overcome by artificially seeding new reactors using the biofilm and effluent from high performing reactors, the following experiment was undertaken. The reactors were switched to the Type-B design, due to easy access to the anode/biofilm, and 18 reactors were inoculated with a RSL-acetate mix. All reactors produced a current, and again different current production curves were seen (Figure 5.10). Time to initiate ranged from 1.4 – 2.7 days, peak current ranged from 0.04 A/m^2 to 0.3 A/m^2, total coulombs ranged from 18.5 – 167 A·s and CE ranged from 2.4% to 26.6%.

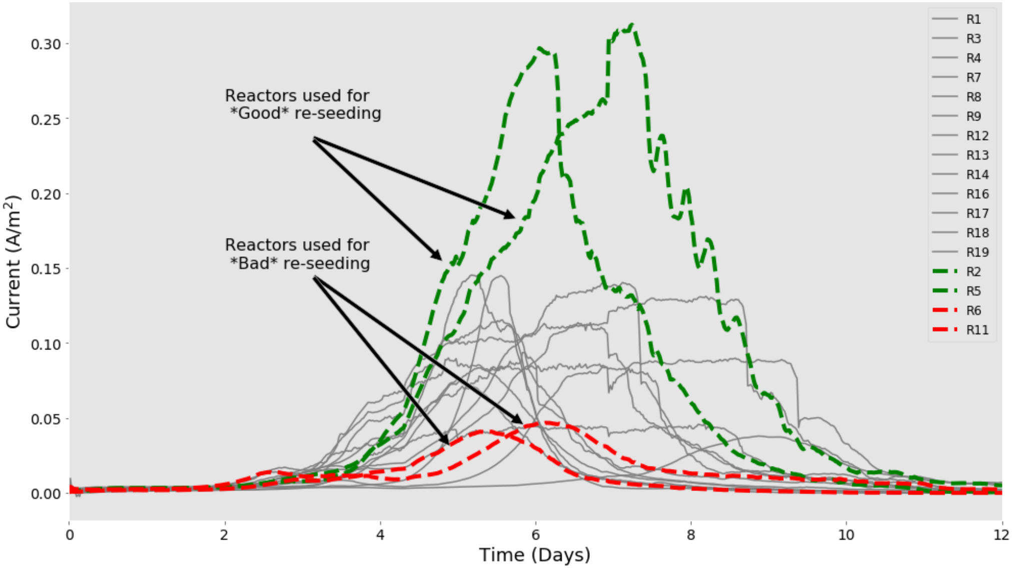


Figure 5.10: Current production during inoculation of 18 Type-B air-cathode MFCs using a RSL-acetate mix.

Following this first inoculation run, the effluent and biofilm from two high performing reactors (R2, R5, green dotted line in Figure 5.10) and two low performing reactors (R6, R11, red dotted line Figure 5.10) were removed. This was then used to re-inoculate half of the reactors each, i.e. 9 'Good' and 9 'Bad' reactors, with a sterile RSL-acetate mix. Prior to re-inoculation, the reactors were cleaned, sterilised and had their anodes replaced with the fresh carbon felt. A visual difference was seen between 'Good' and 'Bad' reactors, however, with high variation in both groups still being seen. For 'Good' reactors, time to initiate ranged from 1.2 – 1.4 days, peak current from 0.04 - 0.25 A/m^2, total coulombs from 11.4 – 80.2 A·s and CE from 2.8 – 16.2% (Figure 5.11, green lines). For 'Bad' reactors, time to initiate ranged from 1.6 – 2.0 days, peak current from 0.01 - 0.11 A/m^2, total coulombs from 1.2 – 24.3 A·s and CE from 0.2 – 5.0% (Figure 5.11, red lines).

Due to one reactor out-performing the rest by a considerable amount, this was repeated using just one reactor for both the 'Good' and 'Bad' groups. These were the biofilm and effluent from R2 ('Good') and R13 ('Bad') (Figure 5.11), and again fed with a sterile RSL–acetate mix (Figure 5.12). Again, there was a visual difference between the 'Good'

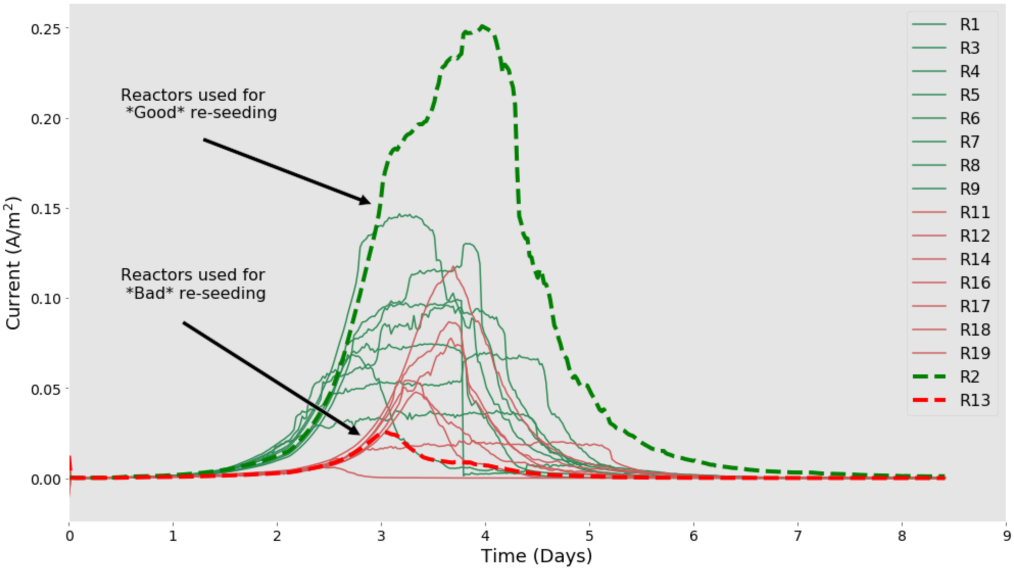


Figure 5.11: Current production during inoculation of 18 Type-B air-cathode MFCs using a sterilised RSL–acetate mix. Green reactors were inoculated using Reactors 2 and 5, and red reactors were inoculated using Reactors 6 and 11 from run 9.

reactors and the 'Bad' reactors, yet there was still high variability within each group. For 'Good' reactors, time to initiate ranged from 1.1 – 2.8 days, peak current from 0.02 - 0.19 A/m^2, total coulombs from 3.5 – 62.0 A·s and CE from 0.5 – 8.8% (Figure 5.12, green lines). For 'Bad' reactors, time to initiate ranged from 2.6 – 3.5 days, peak current from 0.003 - 0.03 A/m^2, total coulombs from 1.0 – 7.3 A·s and CE from 0.2 – 1.0% (Figure 5.12, red lines).

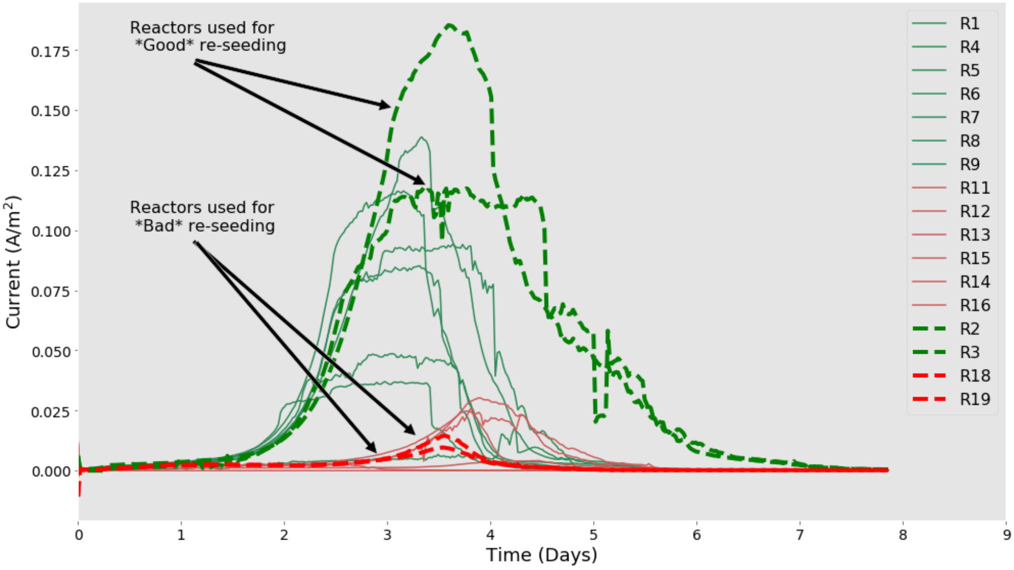


Figure 5.12: Current production during inoculation of 18 Type-B air-cathode MFCs using a sterilised RSL–acetate mix. Green reactors were inoculated using Reactor 2, and red reactors were inoculated using Reactor 13 from run 10.

Finally, using the effluent and biofilms from R2 and R3 ('Good'), and R18 and R19

(‘Bad’), the reactors were re-inoculated, this time with a fresh RSL – acetate mix (Figure 5.13). Two more reactors were made available, and therefore this run totalled 20 identical air-cathode MFCs. This time, although all the ‘Good’ reactors started before the ‘Bad’ reactors, variation within the groups appeared to increase, with much higher overlap seen when comparing peak current, total coulombs and CE. For ‘Good’ reactors, time to initiate ranged from 0.8 – 1.2 days, peak current from 0.04 - 0.39 A/m^2, total coulombs from 9.6 – 89.2 A·s and CE from 1.8 – 16.9% (Figure 5.13, green lines). For ‘Bad’ reactors, time to initiate ranged from 1.4 – 1.8 days, peak current from 0.04 - 0.20 A/m^2, total coulombs from 7.5 – 105.4 A·s and CE from 1.3 – 16.0% (Figure 5.13, red lines).

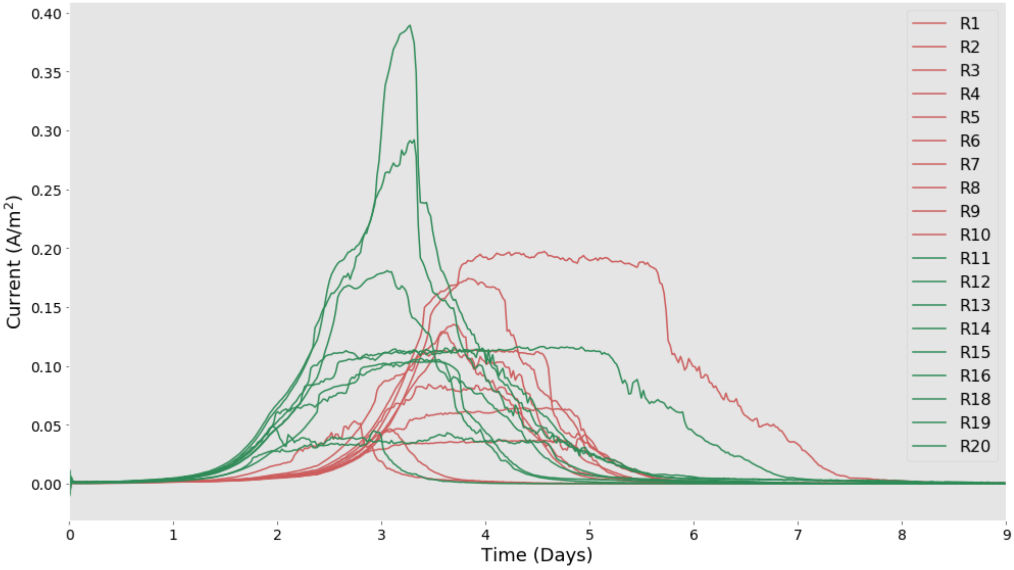


Figure 5.13: Current production during inoculation of 20 Type-B air-cathode MFCs using a sterilised RSL–acetate mix. Green reactors were inoculated using effluent and biofilm from Reactors 2 and 3, and red reactors were inoculated using effluent and biofilm from Reactors 18 and 19 from run 11.

In all three runs, ‘Good’ reactors inoculated quicker than ‘Bad reactors’ (Table 5.4). A significant difference in runs 10 and 11 was seen when comparing ‘Good’ and ‘Bad’ reactors with respect to total coulombs (p-values = 0.013, 0.004), peak current (p-values = 0.049, 0.002), and CE (p-values = 0.015, 0.004). However, in run 12 the use of non-sterile wastewater resulted in high overlap between both groups, with p-values of 0.250, 0.220 and 0.196 for total coulombs, peak current and CE respectively. Additionally, although there were significant differences between ‘Good’ and ‘Bad’ reactors in runs 10 and 11, reactor performance was still highly variable within each group.

Table 5.4: Average performance indicators for each re-inoculating run for both 'Good' and 'Bad' reactors.

Run	**Type**	**Influent COD (mg/L)**	**Effluent COD (mg/l)**	**Initiation (Days)**	**COD removal (%)**	**VTR (kgCOD/m^3·day)**	**Total coulombs (A·s)**	**Peak current (A/m^2)**	**CE (%)**
10	Good	505.0	105.9	1.3	79.0	0.10	32.7	0.11	6.6
	Bad	496.0	92.0	1.9	81.5	0.16	11.5	0.06	2.4
11	Good	706.5	121.9	1.5	82.7	0.19	29.4	0.09	4.1
	Bad	713.5	125.6	2.9	82.4	0.42	3.6	0.01	0.5
12	Good	672.6	195.0	0.9	71.0	0.12	49.5	0.15	8.8
	Bad	693.4	171.0	1.6	75.4	0.15	34.9	0.10	5.8

5.3.6 Pure culture

The final research question was to see if inoculating all 20 reactors using a pure-culture of *G. sulfurreducens* with a pure substance of acetate would result in reduced variability. An anaerobic growth medium was made up from the components in Table 5.2, and under anaerobic conditions, the *G. sulfurreducens* strain from DZSM was added. Unfortunately, after the required period, the *G. sulfurreducens* did not grow. This was attempted four more times with no success, and the growth of *G. sulfurreducens* was not accomplished.

5.4 Discussion

BESs are a form of microbial technology that aims to rival current wastewater treatment methods. They rely upon the combined effect of different species of bacteria to remove pollutants while simultaneously recovering energy [187]. However, due to the lack of control in bacteria abundance within wastewater, a natural variability occurs. This variability is often not discussed, or even obvious, due to the small number of replicates studies use. BESs are falling short of their maximum potential to be a renewable source of energy due to this variability, which is stopping this technology from advancing.

Analysis of the variability seen in the previous Chapters saw the variability was most obvious during inoculation, when the 28 and then 25 air cathode MFCs were inoculated with a wastewater–acetate mix. Assuming current generation is a function of the number of electrogenic bacteria, the different current curves can be explained by classic bacterial growth under favourable conditions [205]. Typically, this contains four stages: the lag phase, exponential phase, stationary phase and death phase. There are a number of factors that can affect each of these four stages, giving rise to the different growth curves seen across all the reactors [140]. Although in theory these should all be the same in identically made BESs, subtle differences in the amount of organics available for food, the number of electrogens present and loss of biological space to competing species of bacteria may lead to the variability seen.

The lag phase is represented by the time taken for the electrogenic bacteria to establish an electro-active biofilm on the anode. A greater initial number of electrogens in the inoculum will start to produce a current sooner, and if the proportion of electrogens in the established biofilm is therefore higher, there will be increased current due to fewer electrons lost to external pathways. This was seen with the negative correlation between time to initiate versus both peak current and total coulombs during inoculation. Additionally, assuming the generation time of electrogens is the same, growth will be represented by a steeper current production curve in reactors with more electrogens. Current production rates correlated positively with peak current, which will be higher due to a greater proportion of the biological space on the anode taken by electrogens. Finally, the organics available in the system will deplete more quickly, resulting in a shorter stationary phase and a steeper death phase. All of these trends were characteristics of Group A. Fewer electrogens on the anode will result in more of the biological space proportionally containing more competing bacteria, resulting in a more gentle growth curve and a lower peak current. A lower peak current symbolises fewer established electrogens, but the food available would last longer, resulting in a longer stationary phase, which is the characteristic seen in Group C. It can therefore be hypothesised that the majority of the differences seen between identical reactors are symbolic of the number of electrogenic bacteria within the system. Unless you know the exact number of electrogens in the inoculum, and keep this the same, the reactors are likely to show variation. This could be tested by artificially adding a known amount of pure-culture; however, this was unable to be accomplished in this study.

When subject to standardising runs with wastewater (which attempted to remove this variability), the variability between identical reactors continued. Comparatively, further runs with a pure acetate mix showed a general decrease in this variability when comparing the total charge produced; however, the peak current remained highly variable. When fed pure acetate, hydrolytic and fermentative bacteria may die off due to lack of food, increasing the space for electrogenic bacteria and decreasing the number of electron competitors overall. This is useful for laboratory scale work; however, it holds no practical use when scale-up and actual wastewater treatment is attempted.

In an attempt to control the variability, reactors that showed high performance were used to inoculate further reactors, and simultaneously the same was done with lower performing reactors as a control. The rationale behind this was that it would be possible to transfer a known good community of bacteria into further reactors. Often studies have adopted a form of this technique, inoculating with an already enriched culture from previous biofilms [206], with the conviction that electrogens will be present in the sample. With the addition of the sterilised wastewater, the electrogens that did not die during the ‘death’ phase were successfully transferred into the following reactor, increasing performance in all aspects. However, even with a significant difference in performance between ‘Good’ and ‘Bad’ reactors, the variability within these groups was again characteristic of the variability seen in the previous inoculation stages, which was a result of being unable to control the exact distribution of electrogens. Unfortunately, the final addition of non-sterilised wastewater removed this successful difference in all aspects, excluding time to initiate, and this addition of fresh bacteria re-established the variation between the two groups. The use of a potentiostat may have enabled precise measurements of the different anode potentials, and this technique has successfully contributed to the understanding of electrochemically active biofilms [207]. However, these were not used as they have little practicality at a larger scale, and the discovery of an engineering solution that did not require a potentiostat was desired.

The effect on variability seen when adding fresh wastewater in both experiments will have consequential problems, as it is representative of the continuous flow at a wastewater treatment site. When running the pilot-reactor in Chapter 4, there was disconnect between COD removal and energy recovered, while also showing highly variable hydrogen production. This is the same with previous pilot-scale attempts [2, 4, 155, 182]. However, periods of stable current generation were common in each individual electrode even with constantly changing wastewater. These were altered when key events occurred, including a change to the HRT and speed of the influent, or core-settling events and the inflow of thick sludge. Therefore, these changes in current could have been a result of a greater supply of food, the biofilm washing away, or new bacteria entering the community. This gives reason to suspect that an established high performing biofilm could theoretically remain high performing when subject to continuously flowing wastewater, as long as the speed of flow or solid content did not cause any physical disturbances. If high performing electrodes could be established, it would be possible to maintain this performance with a pilot-scale system. Pre-treatment measures to remove the risk of physical changes would be needed, and a reactor setup that enabled the removal of low performing electrodes to sterilise and re-inoculate would improve the chance of a well-balanced reactor.

Due to the level of variability seen in small identical reactors when inoculated with real wastewater, hypothesis testing should include a number of repeats, otherwise the hypothesis could be shown to be correct or incorrect purely from the random effect of the inoculum. For example, a study investigating if reactors can recover more energy if they were run with pure substrate made of solely acetate, compared to a complex substrate made up of both acetate and wastewater, can be both positive and negative when looking at reactors from run 6 and run 4 respectively. If R19 (80 A·s, run 4) was compared to R24 (119 A·s, run 6), acetate recovers more charge than wastewater. However, the opposite of this is seen if comparing R11 (158 A·s, run 4) and R11 (97 A·s, run 6). Even if the reactors were in triplicates, each scenario was still possible.

The variability seen in this study and across literature appears primarily due to the proportion of electrogens established on the anode. Unfortunately, the use of a pure-culture was unable to test this hypothesis. If variability still existed even with a pure-culture, then removal of the variability might be too hard a challenge for this technology to overcome, as variability would be due to the interactions between bacteria at a microscopic scale. Comparatively, if this was not the case, then the use of a pure-culture could remove the variability. Many pure-culture experiments only use one reactor [196, 197, 198, 199, 201, 202], and therefore this variability is hard to examine. However, Dumas et al. [200] ran duplicate reactors when using *G. sulfurreducens*, and this experiment suggested there was little variation between the two, with good reproducibility of the results. Unfortunately, variability may not be seen even when running reactors in duplicate, so it cannot be determined that in this experiment variability would not be present when using more reactors.

The use of a pure culture at a pilot-scale may be futile. Mixed cultures are more robust to environmental changes than pure-cultures [203], yet the mixed cultures seen in Chapter 4 were still affected by continuously flowing wastewater. A pure culture would be even more susceptible, which is highlighted by the repeated unsuccessful attempts to grow up *G. sulfurreducens*, and therefore not a realistic solution to the problem. One such way would be to adopt a similar technique to that described by Chatterjee et al. [206], who suggested that incrementally increasing the strength of the wastewater might help reduce the effect of the indigenous bacteria. Alternatively, a pure-culture biofilm could be established as a base for each electrode in a pilot-scale system, reducing the variability and enabling high CEs when using acetate [203]. Slow addition of more complex substrates and bacteria could then be added until the system is ready to accept wastewater. The electrogens would have established a high proportion of the biological space, while still enabling the syntrophic interactions required to break down complex wastes. Although some variability might still appear, all the electrodes would be capable of high performance. However, in practice this may be too hard to achieve.

5.5 Conclusion

This study has shown the extent that variability in performance occurs in BESs. It is seen most obviously during inoculation with the use of fresh wastewater, and appears to be caused by the total number of electrogens able to establish onto the anode. The use of acetate or sterilised wastewater removes the variability to some extent; however, it does not completely disappear. With the addition of fresh wastewater, either as a subsequent run in batch mode or continuously flowing, this variability appears to continue. It was seen that variability could not be overcome by artificially seeding new reactors using high performing reactors and therefore this does not appear to be the solution. Periods of stable current when running a pilot reactor in continuous flow give hope that modification to the design and operational conditions may reduce the impact of this variability. However, the ability to create multiple high performing biofilms in one reactor is required before this technology is ready for industry.

Chapter 6

Conclusion

The overall aim of this research was to improve the wastewater treatment and energy recovery capabilities of BESs, closing the gap between pilot-scale and industry. It can be concluded that the use of return sludge liquor (RSL) from Howdon wastewater treatment plant enabled high pollutant removal, at a similar or greater rate than the estimated rate for activated sludge (AS), helping to decrease the gap between pilot-scale BESs and being industry-ready.

Initially, this research reviewed all the published pilot-scale MECs and set them against the performance parameters that should be met in order to be competitive with current treatment techniques. This was used to identify areas where performance significantly lagged behind industry standards. The criteria for success was therefore based primarily on the performance of AS, as it is the most popular treatment process used in the UK. No single MEC reviewed was able to match all the criteria. However, all but two of the criteria were met by at least one study, and in four out of the twelve pilot studies examined, the MECs were energy neutral or even positive. Surprisingly, it was seen that the previously thought 'Achilles heels' of this technology were not detrimental to the operation: reactors successfully produced hydrogen while treating complex substrates, with natural conductivities and at low temperatures. It was concluded that the expensive supplementation or amendment that is often used to by-pass these limitations was not necessary. Instead, the volumetric treatment rates (VTRs) the reactors have achieved appear to be a limiting factor. An assumed average VTR for AS of 1.25 kgCOD/m^3·day was not met by any reactor, with the closest resulting in 1.06 kgCOD/m^3·day [4]. Additionally, it was seen that the size of the reactors are still far too small. As many modular electrodes have been developed, depth appears to be the critical factor, especially if retrofitting into existing infrastructure.

Following this conclusion, it was clear that achieving high VTRs could help bridge the gap between this technology and industry standards. Achieving the optimal VTR would allow the correct size of the reactor to be designed and modelled for the given flow rate, while treatment costs could also be accurately estimated. Previous research had highlighted that higher strength substrates increased VTRs, and so RSL was used with laboratory scale MFCs. As hypothesised, it was shown that an increase in the strength of the substrate increased the VTRs in both acetate and RSL fed reactors. The VTRs increased with a logarithmic trend, plateauing when the strength of the substrate appeared to exceed the biofilms' capabilities to break down the organics. This also may have been influenced by both the anode potential and the buffer capacity of the substrate. There was no discernible difference between the treatment capabilities of the MFCs when run with either acetate or RSL and it was concluded that the high soluble COD boosted the wastewater treatment performance in a similar way to when a pure substance is used. Finally, the performance of both acetate and RSL reactors once half of the anode was removed gave further evidence that hydrolysis is the rate limiting step in the conversion of complex wastes to current in BESs. It appeared that the soluble nature of RSL was able to by-pass this step to some extent.

Following on from the successful use of RSL in the laboratory, we were able to conduct a pilot scale study with this wastewater, on site at Howdon Wastewater Treatment Works (NWL). This was run for a 7-month period. During the reactor operation, optimisation of the HRT resulted in the highest VTR achieved by a pilot-scale BES treating real wastewater that can be found in publications to date (3.82 kgCOD/m^3·day), even though discharge standards were not reached. The optimal HRT for this reactor with respect to VTRs was 0.5-days, and, in addition, it was shown that increasing the strength of wastewater increased the VTRs. The theoretical calculations on cost savings based on the measured energetic treatment rates, compared to assumed energetic rates of AS, gave reason to view this technology differently. Rather than a direct replacement for AS, BES could be used as a pre-treatment method for RSL, removing up to 50% of the initial COD at a high rate. The use of a BES at this point in the treatment process would remove the need to reach the legal discharge standards, while reducing the need for large and high-risk industrial change.

Considering the original aim of this research, it can be concluded that the use of RSL substantially improves the wastewater treatment side of BESs. However, energy recovery was not as successful as initially hoped. During batch tests in Chapter 3, total coulombs were seen to increase with a linear trend with increasing COD. Unfortunately, this level of energy recovery and coulombic efficiency was not achieved at pilot-scale, unless the HRT was set to 6 days or greater. At faster HRTs, it appeared that due to the speed of flow, the biofilm was only able to utilise the most readily available organics within the wastewater. When analysing the composition of the RSL, there was more than enough acetate supplied to the reactor to account for the observed current. Therefore, the level of organic removal was not represented by current or hydrogen production; instead, the reactor facilitated high rates of anaerobic digestion under conditions that usually inhibit such a process. As this removal of COD did not coincide with high levels of gas production in the cathode, it has been assumed that gases diffused back into the anode side and released into the atmosphere.

High variation in the performance of the individual cassette-style electrodes affected the success of this reactor. This variation was also seen in Chapter 3, where identical batch-fed MFCs exhibited a wide range of power outputs, which has been reported in other lab and pilot-scale work. In an attempt to remove the variability seen in previous research, the pilot reactor was built by a local engineering company, which resulted in 10 identically made cassette style electrodes. Unfortunately, this was not enough, and the high variation in hydrogen production prevented the reactor from being energy positive.

The final chapter therefore aimed to understand this variability. This natural variation appears to be the major drawback in bioelectrochemical technology. It was concluded that in identically made systems, the overriding factor appears to be the ability to establish an effective bacterial community onto the anode. As the nature of wastewater is so variable, this is a seemingly random and uncontrollable event. Chapter 5 highlighted that this variability could be reduced using standardising runs of either acetate or sterilised wastewater, and when operational conditions at a pilot-scale were kept the same, periods of stable current were seen. This gives hope that modification of the design may reduce the impact of this variability. The ability to create multiple high performing biofilms in one reactor is required before this technology is ready for industry.

Energy recovery from wastewater treatment using BESs is possible, and has been demonstrated in a number of studies. However, this study suggests that future pilot-scale research should seek to optimise performance, rather than just demonstrate it. It has shown that high strength domestic wastewater, in the form of RSL, can improve the wastewater treatment side of BESs. Using BESs as a pre-treatment for RSL, rather than a replacement for AS, could be an affordable transition phase that would help to improve the resilience, efficiency and reach of this technology. Assuming that other dimensions will be overcome by the use of modular electrodes, depth remains a major challenge, and even if this is accomplished, the natural variability from using wastewater may prevent this technology from ever being implemented into industry. However, if these issues can be solved, the switch to a more sustainable wastewater treatment method would be both economically and environmentally beneficial.

Chapter 7

Future research

The overall aim of this research was to boost the performance of BESs for wastewater treatment and energy recovery. Most of the research detailed here has improved the performance, however, in the process more questions have been unearthed, and remain unanswered. Each Chapter in this thesis indicate a specific area of research that should be pursued.

Chapter 2: The size of the reactors remains a significant problem. The largest microbial electrolysis cell (MEC) to date still only stands at 1000L, with a hydraulic retention time (HRT) of 1 day [1]. This is far from the size required to manage actual flow-rates at wastewater treatment sites. Many BESs have therefore been designed with modular electrodes, which increases the capacity of the volume of the tank indefinitely. It has therefore been theorised that an affordable way into this industry could be the use of old AS lanes. Depending on the treatment site, these will range significantly in volume, but with an optimal depth of between 3 - 6m to enhance aeration [86, 115]. Creating modular electrodes that could fit to the same depth would allow implementation of this technology without large-scale industrial change. The length and width of these tanks can be solved by the use of more electrodes; however, designing an electrode that can span to the depth of 3m will need to overcome the effect that hydrostatic pressure has on both the biofilm formation, performance and structural integrity. It will also need to cope with the changes in the thermodynamic and kinetic properties of the biological and electrochemical processes. In Chapter 2, it was seen that no reactor matched the required depth of 3m, with the largest at 0.98m [6], which highlights how far this technology still has to go.

Additionally, although the authors determined that MECs have a greater capacity to be scaled up than microbial fuel cells (MFCs), which is discussed in Chapter 2, the potential for MFCs still needs to be reviewed. Regardless of energy recovery, MFCs are still energy neutral, criteria in which most pilot-scale MECs fall short. Therefore, the review could take a different angle than the one described here. Instead of viewing all the performance parameters as one whole criteria that must be met, they could be split in two. A review could compare firstly the wastewater treatment capabilities of the MFCs in comparison to AS, including size, temperature, OLR and VTRs. As they are at minimum energy neutral, this would in-itself be representative of the distance from industry. Following this, the ability for MFCs to be a source of renewable energy could be reviewed for each study. There is clearly a gap in the literature for this research, as to the author's knowledge there are no in-depth reviews comparing all pilot-scale MFCs in a systematic way.

Chapter 3: It was concluded that using RSL increased COD removal in BESs, and it was assumed that the soluble nature of the COD aided this. However, this was by no means proven. The complexity and random nature of wastewater makes determining the direct effect that the composition of wastewaters have on BESs very difficult, which makes it impossible to compare studies which use different wastewaters. The effect of this difference was stressed by an observation while running the experiments in Chapter 3. The COD dilutions were originally attempted with a different return sludge liquor (RSL), this time from Bran Sands Wastewater Treatment plant (NWL) on the North East coast of England. All the MFC reactors at every dilution failed, producing no current. However, the acetate reactors that were run simultaneously performed as described in the reported experiment. Bran Sands RSL and Howdon RSL are inherently the same style of waste stream, with the major difference being that Brans Sands treats both industrial and domestic wastewater. Therefore, the individual components will be different.

A number of small experiments were attempted to produce current from Bran Sands (BS) wastewater. These included adding BS wastewater into already working reactors, using different dilutions of BS wastewater, and inoculation of reactors with a mix of both BS and raw wastewater, which had been used successfully before. However, all failed. BS wastewater has high ammonia (>1400 mg/L), which was initially thought to be the source of toxicity. Unfortunately, even when this was removed, which was done using a pilot-scale algae cascade reactor at the Bran Sands treatment plant, the MFC reactors were still unable to produce current. In fact, biofilms that were exposed to BS wastewater became unusable. An MSc project was developed to determine the cause of this toxicity, unfortunately as this was the summer of 2020, this became a desk-based study. From this literature review, it was suggested that the combined effect of the heavy metals present within the BS wastewater, although independently not reported to cause toxicity, may be the reason for the MFC failure. However, this has not been proven. Further research into the critical components of BS wastewater would shed light into the cause of toxicity.

Additionally, although further evidence was provided to support the hypothesis that hydrolysis is the rate-limiting step, the metabolic processes with BESs are far from certain. The understanding of these processes will be required to enable better reactor optimisation.

Chapter 4: Following the success of optimising VTRs by modifying the HRT, the same process could be used to maximise cathodic conversion efficiency. A few studies have attempted something similar on a smaller scale [9]; however, as with HRT, voltage has not been optimised at pilot-scale. Typically, the supplied voltage to enable hydrogen production varies from study to study, as it is required to drive the electrons to the cathode, and to overcome the over potentials within the system. Applied voltage in previous studies has ranged from 0.2 [11] – 1.5V [5] and the use of a higher voltage can encourage more hydrogen production, although at a greater energetic cost to the reactor. Therefore, optimising the voltage for a reactor may find a similar relationship as seen with HRT and VTRs in Chapter 4, with an optimum cost effective voltage for that system. The hydrogen recovery in this reactor was also severely affected by contamination of the cathode compartment, which is an event reported in other pilot-scale MECs [4]. Development of sterilisation techniques that could be performed during reactor operation will be needed for reactor development, and although a number were planned, these were unable to take place in this study.

In addition, breakdown of the reactor components to determine the long-term effect that continuous flow had on reactor materials was unable to be completed. Analysis of the membrane would determine how it coped with continuous flowing wastewater, and if the permeability changed throughout the experiment. Additionally, it would be interesting to see if the contamination of the cathode compartment affected the catholyte composition and the stainless steel wire wool cathode. Finally, biofilm analysis for each electrode may determine the cause of the variability with respect to specific bacteria; however, in previous studies this was not beneficial [2, 3]. Detailed understanding of how reactor components degrade over time could therefore enable practical implementation at a larger scale.

Furthermore, this study has successfully shown the benefit of using RSL. The increase in wastewater treatment, the lack of discharge requirements and the fact that it is a resource laden waste stream, with currently no economically attractive solution, makes it an excellent location for future pilot-scale BESs. Further research is needed to build on this success.

One interesting observation during the start-up and acclimatisation of the pilot-scale MEC was the infestation of Rat Tail maggots. These are the larvae of a species of hoverfly, and are known to live in stagnant and oxygen-deprived water with a high organic content [208]. They gained some media coverage when they were seen in the composting toilets of the Glastonbury festival [209] and are relatively tolerant of pollution. At its peak, the pilot-scale MEC was estimated at containing over 100 of these creatures. In an attempt to understand the effect they have on wastewater treatment, the reactor was drained, with the maggots safely captured. The reactor was then refilled with fresh RSL and divided into two, with the maggots deposited in one side. This was left in batch for a week, and then the process was repeated. Unfortunately, due to the seasonal hatching of these, there was significantly less maggots in the reactor the second time, and when a third repeat was tried there was barely any left. An observed increase in hover flies suggested that they had hatched by this point. When comparing the two halves of the reactors, both with maggots and without, there was evidence that COD removal was higher in the reactor with the maggots. However, a detailed experiment was not completed to confirm this. Should this event occur again, it would be interesting to determine if there is a symbiotic relationship to aid pollutant removal with rat-tail maggots and MECs. It is hypothesised that these maggots would increase the pollutant removal due to an increased mixing of the wastewater, along with digestion of longer chain organics into shorter chain organics, which would then be more biodegradable for the biofilm.

Chapter 5: Throughout this thesis, the natural variability has been a limitation in every experiment, and it is the author's belief that this remains the biggest milestone with this technology, which is fundamental to the success of BESs. The uncertainty and therefore risk associated with the variability at pilot-scale may prevent industry from ever accepting this technology as a realistic possibility. Unfortunately, the findings from Chapter 5 were incomplete. Although the reason behind the variation was discussed, there was no conclusive determination of the exact cause. Major application of this technology cannot occur without further investigation into the biofilm community structure and bacterial interactions when using fresh wastewater. Additionally, there was no successful prevention of the variability when using fresh wastewater and the pure-culture experiment was unable to be accomplished. This should be completed, as it would determine if the variability exists during the electrogenic step in the process, rather than during the upstream steps of hydrolysis and fermentation, and help determine how best to proceed with removing or lowering the variability. Finally, monitoring of the biofilms during continuous flow is needed before advanced scale-up occurs.

Overall next step: From this study, it is clear there are two areas of research required before BESs can be implemented into industry. Firstly, assuming that other dimensions will be overcome by the use of modular electrodes, depth remains a major challenge. In areas where land availability is low and increased reactor depth is crucial, the impact of increased hydrostatic pressure will prevent these systems from working at scale. Secondly, even if this is accomplished, the natural variability from using wastewater may prevent this technology from ever being implemented into industry. In-depth analysis of biofilm formation and bacteria community dynamics will be required before these systems are fully understood.

Appendix A

Supplementary information for Chapter 2

For each of the pilot studies used, the values presented in the manuscript were analysed and standardised using the methods described below.

A.1 Performance of a pilot-scale continuous flow microbial electrolysis cell fed winery wastewater [1].

A.1.1 Complexity

A value of 26 for winery wastewater has been taken from Mosse et al. [122].

A.1.2 Conductivity

Two different conductivities were reported, 0.7 mS/cm when boiler water was used for dilution, and 1.8 mS/cm when phosphate buffer was added. The results used were during the period of 0.7 mS/cm.

A.1.3 OLR

COD of the influent was highly variable and reported in soluble COD (SCOD). Data has been taken during the period when current density increased to 7.4 A/m^2. Removal averaged 70%, effluent decreased to 0.15 g SCOD/L, indicating influent was 500 mgSCOD/L. An OLR of 0.5 $kgSCOD/m^3{\cdot}day$ was calculated from the provided information (HRT of 1 day, influent of 500 mgSCOD/L). Typical soluble COD loading in an activated sludge plant based on average influent of 200 mgSCOD/L (0.67 $kgSCOD/m^3{\cdot}day$).

A.1.4 Depth

Anodes were fixed to a 0.7 × 0.6 perforated plastic frame.

A.1.5 VTR

Reported 70% SCOD removal once the reactor was enriched, resulting in 0.35 $kgSCOD/m^3{\cdot}day$.

A.1.6 Effluent quality

COD removal was reported on average to reach 70%.

A.1.7 Energy balance

Reported values of 0.9V inputted and an average of 7.4 A/m^3 results in an energetic treatment cost of 1.64 kJ/gCOD removed. Taking the reported value of biogas production (0.16 L/L·day), COD removal rate (350 g/day) and methane content (86%) at peak reactor performance, (energy density of 55.6 MJ/kg for methane), the energy recovered is 14.34 kJ/gCOD removed.

A.1.8 Temperature

Reactor was reported to be heated to 31°C.

A.2 2. Production of hydrogen from domestic wastewater in a pilot-scale microbial electrolysis cell [2, 3].

A.2.1 Complexity

Use of raw wastewater fits the criteria and rewards a value of 90.

A.2.2 Conductivity

A reported value of 1.8 mS/cm was given in Heidrich et.al [3].

A.2.3 OLR

The OLR was not reported, however the average influent was reported at 450 mgCOD/L, along with a HRT of 1 day, a volumetric treatment rate of 0.14 kgCOD/m^3·day and a removal rate of 30% discounting anomalies. From this, a loading rate can be calculated at 0.45 kgCOD/m^3·day.

A.2.4 Depth

Anodes were 0.2m wide and 0.3m high.

A.2.5 VTR

Reported as 0.14 kgCOD/m^3·day.

A.2.6 Effluent quality

Discounting anomalous values of COD influent, the removal rate was reported to be on average 30%, which results in an average effluent concentration to be 315 mgCOD/L.

A.2.7 Energy balance

The reported energetic treatment rate is given as 2.3 kJ/gCOD. From recent calculations, a value of 1.37 kJ/gCOD has been calculated. This is using the value of 1.1V inputted, a surface area of each anode as 0.2m × 0.3m (with 12 anodes) and a current density reported of 0.27 A/m^2. Confirmation with Dr Heidrich is that this is now the correct value. Using the average hydrogen production rate of 0.015 LH$_2$/L·day and with 13.5 gCOD/day, this results in an energy recovery of 1.373 kJ/gCOD, and energy balance results in 0.005 kJ/gCOD.

A.2.8 Temperature

Wastewater minimum temperature was reported as 8.5 ± 2.3°C.

A.3 Low temperature domestic wastewater treatment in a microbial electrolysis cell with 1m^2 anodes: Towards system scale-up [4].

A.3.1 Complexity

Used primary wastewater for the entire experiment, which rewards a value of 90.

A.3.2 Conductivity

Reported an average value of 812 μS/cm, which is 0.812 mS/cm.

A.3.3 OLR

Not reported, however an average influent concentration of 347 mg COD/L and a HRT of 0.208 days (6 hours) is given, and therefore an organic loading rate of 1.67 kgCOD/m^3·day has been calculated.

A.3.4 Depth

Reported dimensions of the electrodes were 0.8 high and 1.2m wide.

A.3.5 VTR

The treatment rate is not directly reported, however a removal rate of 63.5% is given, which when calculated with the calculated organic loading rate results in a volumetric treatment rate of 1.06 kgCOD/m^3·day.

A.3.6 Effluent quality

Reports an average removal of 63.5%, which results in an effluent quality of 126.7 mg-COD/L. However, the paper reports that the average effluent results were below the discharge requirements (<125 mg COD/L), leading to uncertainty.

A.3.7 Energy balance

Calculated from given values of 0.9V, anode surface area of 6m^2 and a current density of 0.29 A/m^2, which results in a value of 0.73 kJ/gCOD. Using the average hydrogen production rate of 0.0046 LH_2/L·day, at a 93% hydrogen content and with 185.4 gCOD/day, this results in an energy recovery of 0.0359 kJ/gCOD

A.3.8 Temperature

Wastewater temperatures during start up were reported to average 9.9 ± 1.2°C.

A.4 Bioelectrochemical hydrogen production from urban wastewater on a pilot-scale [5].

A.4.1 Complexity

Used domestic wastewater for the third part of the experiment, which rewards a value of 90. All following results are from this period.

A.4.2 Conductivity

The conductivity of the wastewater was not given.

A.4.3 OLR

Initially given as 0.5 kgCOD/m^3·day, however a switch to 0.25 kgCOD/m^3·day occurred, and all subsequent results were obtained operated under these conditions. This is using an influent with an average of 500 mgCOD/L, and a HRT of 2 days.

A.4.4 Depth

Reported dimensions of the electrodes were 3cm wide, 36cm in length and 46cm in height.

A.4.5 VTR

The volumetric removal rate has been determined as 0.06 kgCOD/m^3·day, which has been calculated based on the 25% COD removal and the 0.25 kgCOD/m^3·day loading rate.

A.4.6 Effluent quality

The effluent quality has assumed to have an average of 375 mgCOD/L. This has been calculated based on the 25% COD removal reported.

A.4.7 Energy balance

Values for anodic surface area (1.63m^2) and volts inputted (1.5V) have been given; however, no current density has been reported specifically for the period of testing using domestic wastewater. Using the given value for 'the second period' and by careful analysis of Figure 4C, a current density of 300 mA/m^2 has been used. This gives an energetic treatment rate of 7.838 kJ/gCOD removed. Using the hydrogen production rate of 4.2 LH$_2$/day per day and calculated removal of 8.125 gCOD/day, this results in energy recovered to be 6.60 kJ/gCOD removed, leading to a net energy cost of 1.24 kJ/gCOD removed.

A.4.8 Temperature

Stated that they conducted all experiments at room temperature (T = 22 ± 2°C).

A.5 Pilot-scale bioelectrochemical system for simultaneous nitrogen and carbon removal in urban wastewater treatment plants [6].

A.5.1 Complexity

Values taken from Stage 1, when fed urban wastewater, and so results in a value of 90.

A.5.2 Conductivity

The conductivity for the wastewater was reported to be 0.8 mS/cm.

A.5.3 OLR

Results are given in TOC values; however, these have been converted to COD by the following formula, which was given by the author.

$$COD = 49.2 + (3 \times TOC)$$

With the calculated COD of the influent being 319.2 mg/L, and a HRT of 1 day, organic loading rate has been calculated at 0.32 kgCOD/m^3·day

A.5.4 Depth

Reported dimensions of the anodes were 0.98m × 0.48m

A.5.5 VTR

With the reported TOC removal in stage 1 as “almost 100% efficiency for most of the time, except for the first 10 days (out of 39), where it averaged 40%”, the average removal has been determined as 84%. This is using an average of 40% for 10 days, and 99% for the remaining 29 days. Using the conversion given, this is a volumetric treatment rate of 0.27 kgCOD/m^3·day.

A.5.6 Effluent quality

The effluent quality has assumed to have an average of 51 mgCOD/L. This has been calculated based on the 84% COD removal reported.

A.5.7 Energy balance

Taking the calculated removal rate of 20.12 gCOD/day, an inputted voltage of 1V and average current densities resulting in 0.224 A/m^2 results in an energetic treatment cost of 1.131 kJ/gCOD removed. It was also reported that although gas was produced, comprising of methane, carbon dioxide and hydrogen, the gas was in such small amounts it was considered barely usable. Considering this, no energy was recovered from the system.

A.5.8 Temperature

Stated that they conducted all experiments at room temperature and so awarded a value of 22°C.

A.6 Microbial electrolysis cell scale-up for combined wastewater treatment and hydrogen production [7].

A.6.1 Complexity

Pilot-scale MEC were fed with raw municipal wastewater and discussed in Section 3.3, and so complexity results in a value of 90.

A.6.2 Conductivity

The conductivity for the wastewater was not given.

A.6.3 OLR

Multiple OLR were used, however results have been taken from the 10 hour HRT, as this was deemed best performing and closest to AS operating conditions. Influent described as 250 - 300 mgCOD/L, using 275 mgCOD/L results in an OLR of 0.66 kgCOD/m^3·day (also stated in the paper).

A.6.4 Depth

Reported dimensions of the anodes were not given.

A.6.5 VTR

Described as reaching 76% removal at a HRT of 10 hours, resulting in a VTR of 0.5 kgCOD/m^3·day

A.6.6 Effluent quality

With 76% removal and an average influent of 275 mgCOD/L results in 66 mgCOD/L in the effluent.

A.6.7 Energy balance

Reports the energy cost as 0.9 kWh/kgCOD removed, which can be converted to 3.24 kJ/gCOD. Then using reported hydrogen production of 0.045 LH_2/L·day, and 5.016 gCOD/day, results in energy recovery of 1.16 kJ/gCOD.

A.6.8 Temperature

MECs were reported to operate at 23 - 25°C, and so a value of 23°C has been used.

A.7 Reduced energy consumption during low strength domestic wastewater treatment in a semi-pilot tubular microbial electrolysis cell [8]; Performance of a semi-pilot tubular microbial electrolysis cell (MEC) under several hydraulic retention times and applied voltages [9].

Although both these papers operate at various HRTs and voltages, the same values are taken from both papers. This is at a HRT of 4 hours, as in terms of hydrogen production, this is the best. The following paper regarding voltages suggests that 1V is still ideal.

A.7.1 Complexity

MECs were fed domestic wastewater and so a value of 90 is rewarded.

A.7.2 Conductivity

The conductivity for the wastewater was reported as 0.605 mS/cm.

A.7.3 OLR

Multiple OLR were used, however results have been taken from the 4-hour HRT due to successful hydrogen production, and the choice from the author to use these results to compare to previous pilot-scale papers. Reported as 1.32 kgCOD/m^3·day; however this was just the first module, and it was actually two in series. Combined OLR was 0.67 kgCOD/m^3·day.

A.7.4 Depth

Reported dimensions of the anodes were not given.

A.7.5 VTR

Effluent from the second module reported as 40.3 mgCOD/L. Back calculating this gives a VTR of 0.43 kgCOD/m^3·day.

A.7.6 Effluent quality

Reported effluent quality given as 40.3 mgCOD/L, which results in 64% removal.

A.7.7 Energy balance

This paper does not report the energy consumption, volts inputted or anodic surface area. States that at 1.32 kgCOD/m^3·day loading rate, net energy consumption fell below 0.5 kWh/kgCOD removed. Calculation of energy recovered from hydrogen was possible using 0.45 LH$_2$/L·day and 1.72 gCOD/day removed, resulting in 1.34 kJ/gCOD. Adding this energy recovery to the net energy stated (0.5 kWh/kgCOD) results in an energetic cost of 3.14 kJ/gCOD.

A.7.8 Temperature

MECs were reported to operate at 20°C.

A.8 Scaling-up of membraneless microbial electrolysis cells (MECs) for domestic wastewater treatment: Bottlenecks and limitations [10].

Multiple operational configurations including batch and continuous flow. Results are taken from Test C4, when the influent wastewater was closest to our ideal value (401 mg/L), and when hydrogen production occurred. Additionally two MECs were used; results are taken from MEC$_1$ due to greater performance.

A.8.1 Complexity

MECs were fed primary effluent and so awarded a value of 90.

A.8.2 Conductivity

The conductivity for the wastewater was reported as 1.78 mS/cm.

A.8.3 OLR

Multiple OLR were used, however results have been taken from Test C4. With a combined volume of 3.3L, influent of 401 mg/L and HRT of 17.9 hours, these results in an OLR of 0.54 kgCOD/m^3·day.

A.8.4 Depth

Reported dimensions of the anodes were 42 × 53cm.

A.8.5 VTR

Effluent reported as 341 mgCOD/L. Back calculating this gives a VTR of 0.08 kgCOD/m^3·day

A.8.6 Effluent quality

Reported effluent quality given as 341 mgCOD/L, resulting in 15.0% removal.

A.8.7 Energy balance

Energy consumption was described as high, and from analysis of Figure 5 within the paper, for MEC$_1$ at OLR 0.54 kgCOD/m^3·day, it seems this was roughly 7 kWh/kgCOD removed, resulting in an energetic cost of 25.2 kJ/gCOD. There was a reported 20% hydrogen recovery, but it is unsure what this 20% is attributed to, and therefore the energy recovered is left blank.

A.8.8 Temperature

MECs were reported to operate at room temperature, which was kept at 19.2°C.

A.9 Evaluating the effects of scaling up on the performance of bioelectrochemical systems using a technical scale microbial electrolysis cell [11].

A.9.1 Complexity

MECs were fed primary effluent and so awarded a value of 90.

A.9.2 Conductivity

The conductivity for the wastewater was not reported

A.9.3 OLR

OLR reported as 0.5 gCOD/L·day, or 0.5 kgCOD/m^3·day.

A.9.4 Depth

Reported dimensions of the anodes were not given.

A.9.5 VTR

Effluent reported as 210 mgCOD/L. Calculating gives a VTR of 0.35 kgCOD/m^3·day.

A.9.6 Effluent quality

Reported COD removal of 67%.

A.9.7 Energy balance

With 0.2V inputted and a current density of 0.72 A/m^2, along with 5.55 gCOD/day removed results in an energetic cost of 0.874 kJ/gCOD. There was no discussion of energy recovery.

A.9.8 Temperature

Operating temperature reported to vary between 25 - 36°C, a value of 25°C is therefore given.

A.10 Long-term continuous production of H_2 in a microbial electrolysis cell (MEC) treating saline wastewater [12].

Results are taken from Phase 2, due to influent COD being closer to real wastewater.

A.10.1 Complexity

A synthetic medium was fed to the MEC, primarily acetate, and so a value of 1 is given.

A.10.2 Conductivity

The conductivity for the wastewater was reported at 9 S/m which is 90 mS/cm.

A.10.3 OLR

OLR reported as 0.64 - 1.28 kgCOD/m^3·day, results are taken at 1.28 kgCOD/m^3·day.

A.10.4 Depth

Reported dimensions of the anodes were 1cm × 20cm high.

A.10.5 VTR

COD described as stabilising at 75% from day 30 onwards, from this a VTR of 0.963 kgCOD/m^3·day is used.

A.10.6 Effluent quality

Reported removal of 75%.

A.10.7 Energy balance

With 0.2V inputted and a current density of 2.3 A/m^2, along with 3.85 gCOD/day removed results in an energetic cost of 0.778 kJ/gCOD. At this point hydrogen production was reported to be 0.2 LH$_2$/L·day, which results in an energy recovery of 2.65 kJ/gCOD removed.

A.10.8 Temperature

The temperature of the MEC was reported to be kept at 37°C.

A.11 Effective control of biohythane composition through operational strategies in an innovative microbial electrolysis cell [13].

This study examined different operational conditions. Results have been taken when the MEC was set at a HRT of 24 hours, recirculation rates of 800 ml/min and external resistance of 1Ω, as discussed in the abstract.

A.11.1 Complexity

A synthetic medium was fed to the MEC, primarily granular sucrose, and so is awarded the value of 1.

A.11.2 Conductivity

The conductivity for the medium was reported at 1.7 mS/cm.

A.11.3 OLR

Influent reported as 200 mgCOD/L with a HRT of 24 hours results in 0.2 kgCOD/m^3·day.

A.11.4 Depth

Reported dimensions of the anodes were not given, used a novel configuration that was not modular.

A.11.5 VTR

COD removal at a HRT of 24 hours reported to be 60.9%. This results in a VTR of 0.122 kgCOD/m^3·day.

A.11.6 Effluent quality

Reported removal of 60.9%.

A.11.7 Energy balance

Reported 'biohythane production of 0.64 L/day (16.5% hydrogen, 83.5% methane), with a net energy recovery of 1.52 kWh/day'. Using both higher heating values for hydrogen and methane, and 2.19 gCOD/day removed this result in an energy recovery of 0.615 kJ/gCOD from hydrogen, and 8.89 kJ/gCOD from methane. 1.52 kWh/day or 5472 kJ/day is 2494 kJ/gCOD removed, which is a 100-fold difference. A value of 0.0175A has been used for energy inputted. This is from values taken from Figure 2 during the period of no anolyte recirculation, and the comment of 'anolyte recirculation only slightly increased current'. This results in a treatment cost of 0.552 kJ/gCOD. The value of 8.954 kJ/gCOD has thus been used for energy balance as attempts to contact the author was unsuccessful, and it still results in a 'full petal' for this specific rose diagram.

A.11.8 Temperature

The temperature of the MEC was reported to be kept at room temperature and so awarded a value of 22°C.

Appendix B

Supplementary information for Chapter 4

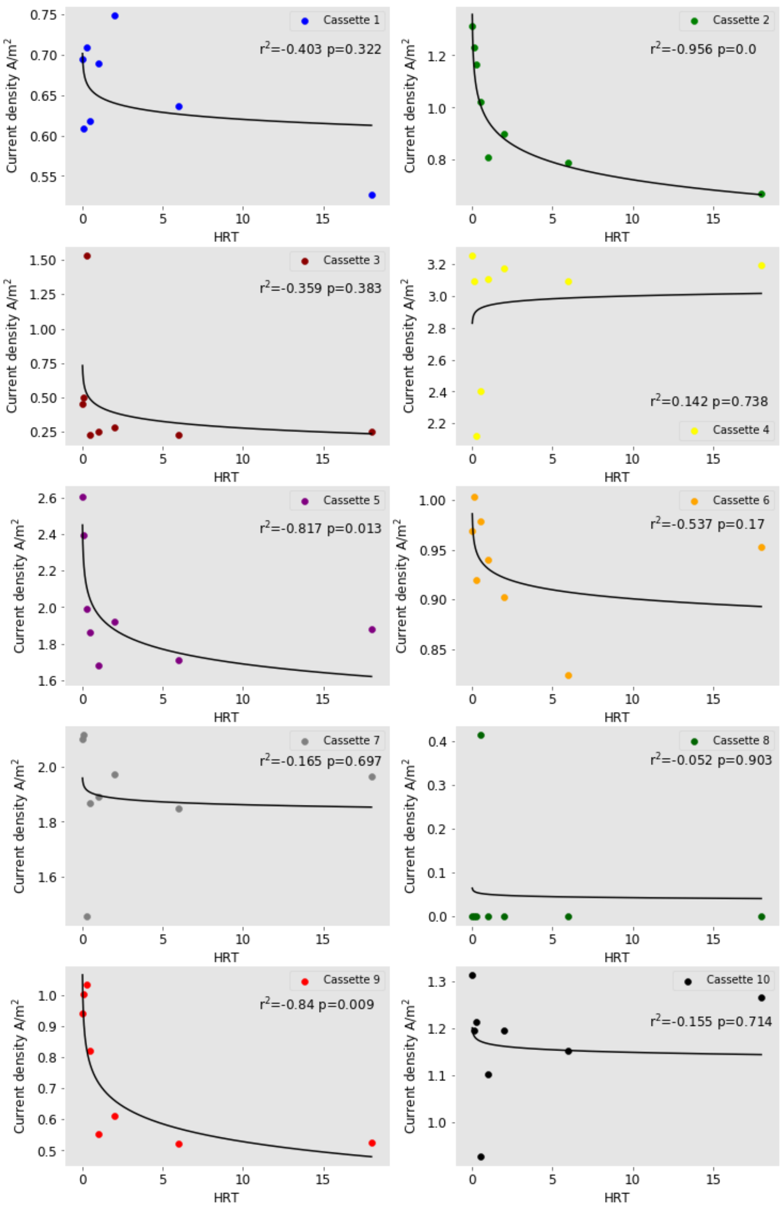


Figure B.1: The average current densities compared to HRT for the 10-cassette style electrodes.

Bibliography

[1] R. D. Cusick, B. Bryan, D. S. Parker, M. D. Merrill, M. Mehanna, P. D. Kiely, G. Liu, and B. E. Logan, "Performance of a pilot-scale continuous flow microbial electrolysis cell fed winery wastewater," *Applied microbiology and biotechnology*, vol. 89, no. 6, pp. 2053–2063, 2011.

[2] E. S. Heidrich, J. Dolfing, K. Scott, S. R. Edwards, C. Jones, and T. P. Curtis, "Production of hydrogen from domestic wastewater in a pilot-scale microbial electrolysis cell," *Applied microbiology and biotechnology*, vol. 97, no. 15, pp. 6979–6989, 2013.

[3] E. S. Heidrich, S. R. Edwards, J. Dolfing, S. E. Cotterill, and T. P. Curtis, "Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12month period," *Bioresource technology*, vol. 173, no. Supplement C, pp. 87–95, 2014.

[4] S. E. Cotterill, J. Dolfing, C. Jones, T. P. Curtis, and E. S. Heidrich, "Low Temperature Domestic Wastewater Treatment in a Microbial Electrolysis Cell with 1 m ² Anodes: Towards System Scale-Up," *Fuel Cells*, vol. 17, pp. 584–592, oct 2017.

[5] J. A. Baeza, À. Martínez-Miró, J. Guerrero, Y. Ruiz, and A. Guisasola, "Bioelectrochemical hydrogen production from urban wastewater on a pilot scale," *Journal of Power Sources*, vol. 356, pp. 500–509, jul 2017.

[6] M. Isabel San-Martín, R. Mateos, B. Carracedo, A. Escapa, and A. Morán, "Pilot-scale bioelectrochemical system for simultaneous nitrogen and carbon removal in urban wastewater treatment plants," *Journal of Bioscience and Bioengineering*, vol. 126, pp. 758–763, dec 2018.

[7] L. Gil-Carrera, A. Escapa, P. Mehta, G. Santoyo, S. R. Guiot, A. Moran, and B. Tartakovsky, "Microbial electrolysis cell scale-up for combined wastewater treatment and hydrogen production," *Bioresour Technol*, vol. 130, pp. 584–591, 2013.

[8] L. Gil-Carrera, A. Escapa, R. Moreno, and A. Morán, "Reduced energy consumption during low strength domestic wastewater treatment in a semi-pilot tubular microbial electrolysis cell," *Journal of Environmental Management*, vol. 122, no. Supplement C, pp. 1–7, 2013.

[9] L. Gil-Carrera, A. Escapa, B. Carracedo, A. Morán, and X. Gómez, "Performance of a semi-pilot tubular microbial electrolysis cell (MEC) under several hydraulic retention times and applied voltages," *Bioresource Technology*, vol. 146, pp. 63–69, 2013.

[10] A. Escapa, M. San-Martín, R. Mateos, and A. Morán, “Scaling-up of membraneless microbial electrolysis cells (MECs) for domestic wastewater treatment: Bottlenecks and limitations,” *Bioresource Technology*, vol. 180, pp. 72–78, mar 2015.

[11] R. K. Brown, F. Harnisch, S. Wirth, H. Wahlandt, T. Dockhorn, N. Dichtl, and U. Schröder, “Evaluating the effects of scaling up on the performance of bioelectrochemical systems using a technical scale microbial electrolysis cell,” *Bioresource Technology*, vol. 163, pp. 206–213, 2014.

[12] A. A. Carmona-Martínez, E. Trably, K. Milferstedt, R. Lacroix, L. Etcheverry, and N. Bernet, “Long-term continuous production of H2 in a microbial electrolysis cell (MEC) treating saline wastewater,” *Water Research*, vol. 81, pp. 149–156, sep 2015.

[13] S. Luo, A. Jain, A. Aguilera, and Z. He, “Effective control of biohythane composition through operational strategies in an innovative microbial electrolysis cell,” *Applied Energy*, vol. 206, pp. 879–886, 2017.

[14] U. N. (UN), “United Nations Sustainable Development – 17 Goals to Transform Our World,” 2015.

[15] U. N. (UN), “Water and Sanitation - United Nations Sustainable Development,” 2016.

[16] M. Scholz, “Activated Sludge Processes,” in *Wetlands for Water Pollution Control*, pp. 91–105, Elsevier, jan 2015.

[17] EU, “Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC). Council of European Communities (CEC),” *Official Journal of the European Communities*, vol. 135, pp. 40–52, 1991.

[18] S. E. Williams, “Energy usage comparison between activated sludge treatment and rotating biological contactor treatment of municipal wastewater,” *Williams & Works, Inc*, pp. 1–11, 2011.

[19] W. I. F. (WIF), “2050 Water Innovation Strategy,” 2020.

[20] W. I. F. (WIF), “2050 Water - Draft Innovation Strategy Launched,” 2020.

[21] Z. Dai, E. S. Heidrich, J. Dolfing, and A. P. Jarvis, “Determination of the Relationship between the Energy Content of Municipal Wastewater and Its Chemical Oxygen Demand,” *Environmental Science and Technology Letters*, vol. 6, pp. 396–400, jul 2019.

[22] DEFRA, “Anaerobic Digestion Strategy and Action Plan. A commitment to increasing energy from waste through Anaerobic Digestion,” tech. rep., Department of Energy and Climate Change, Department of Energy and Climate Change, London, UK, 2011.

[23] S.-Q. Ni and J. Zhang, “Anaerobic Ammonium Oxidation: From Laboratory to Full-Scale Application,” *BioMed Research International*, vol. 2013, pp. 1–10, 2013.

[24] T. H. Pham, P. Aelterman, and W. Verstraete, “Bioanode performance in bioelectrochemical systems: recent improvements and prospects,” *Trends in Biotechnology*, vol. 27, pp. 168–178, mar 2009.

[25] B. E. Logan, D. Call, S. Cheng, H. V. M. Hamelers, T. H. J. A. Sleutels, A. W. Jeremiasse, and R. A. Rozendal, “Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter,” *Environmental Science & Technology*, vol. 42, pp. 8630–8640, dec 2008.

[26] D. Call and B. E. Logan, “Hydrogen Production in a Single Chamber Microbial Electrolysis Cell Lacking a Membrane,” *Environmental Science & Technology*, vol. 42, pp. 3401–3406, may 2008.

[27] H. Wang and Z. J. Ren, “A comprehensive review of microbial electrochemical systems as a platform technology,” *Biotechnology Advances*, vol. 31, pp. 1796–1807, dec 2013.

[28] S. Cheng, D. Xing, D. F. Call, and B. E. Logan, “Direct biological conversion of electrical current into methane by electromethanogenesis,” *Environmental Science and Technology*, vol. 43, pp. 3953–3958, may 2009.

[29] D. Ki, S. C. Popat, B. E. Rittmann, and C. I. Torres, “H2O2 Production in Microbial Electrochemical Cells Fed with Primary Sludge,” *Environmental Science and Technology*, vol. 51, pp. 6139–6145, jun 2017.

[30] F. Aulenta, A. Canosa, M. Majone, S. Panero, P. Reale, and S. Rossetti, “Trichloroethene dechlorination and H2 evolution are alternative biological pathways of electric charge utilization by a dechlorinating culture in a bioelectrochemical system,” *Environmental Science and Technology*, vol. 42, pp. 6185–6190, aug 2008.

[31] H. Luo, P. Xu, T. M. Roane, P. E. Jenkins, and Z. Ren, “Microbial desalination cells for improved performance in wastewater treatment, electricity production, and desalination,” *Bioresource Technology*, vol. 105, pp. 60–66, 2012.

[32] R. Rossi, D. Jones, J. Myung, E. Zikmund, W. Yang, Y. A. Gallego, D. Pant, P. J. Evans, M. A. Page, D. M. Cropek, and B. E. Logan, “Evaluating a multi-panel air cathode through electrochemical and biotic tests,” *Water Research*, vol. 148, pp. 51–59, jan 2019.

[33] B. Logan, “Scaling up microbial fuel cells and other bioelectrochemical systems,” *Applied Microbiology and Biotechnology*, vol. 85, no. 6, 2010.

[34] D. C. Aiken, T. P. Curtis, and E. S. Heidrich, “Avenues to the financial viability of microbial electrolysis cells [MEC] for domestic wastewater treatment and hydrogen

production," *International Journal of Hydrogen Energy*, vol. 44, pp. 2426–2434, jan 2019.

[35] Z. Dai, *Developing Energy and Nutrient Mass Balances to Inform Value Recovery Options in Municipal Wastewater Treatment Systems.* Phd, PhD, Newcastle University, Newcastle, UK, 2019.

[36] Anaerobic Digestion and Bioresource Association, "Anaerobic digestion map," 2020.

[37] S. E. Cotterill, *Scale up and Development of a Microbial Electrolysis Cell for Domestic Wastewater Treatment and Energy Recovery.* Engd thesis, 2017.

[38] J. B. Zimmerman, J. R. Mihelcic, and J. Smith, "Global stressors on water quality and quantity," jun 2008.

[39] H. Ritchie and M. Roser, "Fossil Fuels," 2018.

[40] EIA, *International Energy Outlook 2016 whith Projections to 2040.* No. May, may 2016.

[41] A. Boretti and L. Rosa, "Reassessing the projections of the World Water Development Report," *npj Clean Water*, vol. 2, dec 2019.

[42] U. Wiesmann, I. S. Choi, and E.-M. Dombrowski, "Historical Development of Wastewater Collection and Treatment," in *Fundamentals of Biological Wastewater Treatment*, pp. 1–23, Berlin, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, dec 2006.

[43] W.-W. Li, H.-Q. Yu, and B. E. Rittmann, "Chemistry: Reuse water pollutants," *Nature*, vol. 528, no. 7580, pp. 29–31, 2015.

[44] A. B. UN, "Wastewater Management," *Geneva, Switzerland*, 2015.

[45] A. Kadier, Y. Simayi, and P. Abdeshahian, "A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production," *Elsevier*, no. November, pp. 427–443, 2015.

[46] A. J. Slate, K. A. Whitehead, D. A. Brownson, and C. E. Banks, "Microbial fuel cells: An overview of current technology," mar 2019.

[47] A. Kundu, J. N. Sahu, G. Redzwan, and M. Hashim, "An overview of cathode material and catalysts suitable for generating hydrogen in microbial electrolysis cell," *International Journal of Hydrogen Energy*, vol. 38, pp. 1745–1757, feb 2013.

[48] Y. Hindatu, M. S. Annuar, and A. M. Gumel, "Mini-review: Anode modification for improved performance of microbial fuel cell," 2017.

[49] A. Kadier, M. S. Kalil, P. Abdeshahian, K. Chandrasekhar, A. Mohamed, N. F. Azman, W. Logroño, Y. Simayi, and A. A. Hamid, “Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals,” aug 2016.

[50] P. Pandey, V. N. Shinde, R. L. Deopurkar, S. P. Kale, S. A. Patil, and D. Pant, “Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery,” 2016.

[51] L. He, P. Du, Y. Chen, H. Lu, X. Cheng, B. Chang, and Z. Wang, “Advances in microbial fuel cells for wastewater treatment,” 2017.

[52] J. Wei, P. Liang, and X. Huang, “Recent progress in electrodes for microbial fuel cells,” 2011.

[53] B. E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, and K. Rabaey, “Microbial Fuel Cells: Methodology and Technology,” *Environmental Science & Technology*, vol. 40, no. 17, pp. 5181–5192, 2006.

[54] R. Rousseau, L. Etcheverry, E. Roubaud, R. Basséguy, M. L. Délia, and A. Bergel, “Microbial electrolysis cell (MEC): Strengths, weaknesses and research needs from electrochemical engineering standpoint,” jan 2020.

[55] A. Escapa, R. Mateos, E. J. Martínez, and J. Blanes, “Microbial electrolysis cells: An emerging technology for wastewater treatment and energy recovery. from laboratory to pilot plant and beyond,” mar 2016.

[56] Y. Zhang, M. Liu, M. Zhou, H. Yang, L. Liang, and T. Gu, “Microbial fuel cell hybrid systems for wastewater treatment and bioenergy production: Synergistic effects, mechanisms and challenges,” apr 2019.

[57] C. Y. Shi, “Mass Flow and Energy Efficiency of Municipal Wastewater Treatment Plants,” *Water Intelligence Online*, vol. 10, p. 9781780400907, 2015.

[58] D. Pant, A. Singh, G. Van Bogaert, Y. A. Gallego, L. Diels, and K. Vanbroekhoven, “An introduction to the life cycle assessment (LCA) of bioelectrochemical systems (BES) for sustainable energy and product generation: Relevance and key aspects,” *Renewable and Sustainable Energy Reviews*, vol. 15, pp. 1305–1313, feb 2011.

[59] EPA, “Urban Waste Water Treatment in 2017,” tech. rep., 2017.

[60] I. Bodík, S. Sedláček, M. Kubaská, and M. Hutňan, “Biogas production in municipal wastewater treatment plants - Current status in EU with a focus on the Slovak Republic,” 2011.

[61] B. E. Logan, *Microbial fuel cells.* State College, Pennsylvania, USA: Wiley-Interscience, 2008.

[62] R. Dinsdale, K. Bryne, and D. Tucker, "The Anaerobic Digestion of Textile Desizing Wastewater," in *Ecotextiles: The Way Forward for Sustainable Development in Textiles*, pp. 163–167, Elsevier Ltd., 2007.

[63] EPA, "Wastewater Technology Fact Sheet Trickling Filters," tech. rep., 2000.

[64] C.-D. Henrich and M. Marggraff, "Energy-efficient Wastewater Reuse – The Renaissance of Trickling Filter Technology," *Proc. 9th International Conference on Water Reuse*, pp. 27–31, 2013.

[65] USEPA, "Wastewater Technology Fact Sheet Trickling Filter Nitrification," tech. rep., 2000.

[66] E. Articles, "Trickling Filter - Classification and Mechanism - Engineering Articles," 2015.

[67] Y. Deng, "Improvements to the performance of trickling filters by inclusion of alternative surface active media," tech. rep., 2018.

[68] S. B. Velasquez-Orta, O. Heidrich, K. Black, and D. Graham, "Retrofitting options for wastewater networks to achieve climate change reduction targets," *Applied Energy*, vol. 218, pp. 430–441, may 2018.

[69] M. P. Rittmann, BE., *Enviromental biotechnology: Principles and applications.* 2001.

[70] G. Tchobanoglous, *Wastewater Engineering : Treatment and Resource Recovery -, Volume 2.* 2014.

[71] L. Liu, G. Hall, and P. Champagne, "Disinfection processes and mechanisms in wastewater stabilization ponds: A review," dec 2018.

[72] G. E. Alexiou and D. D. Mara, "Anaerobic waste stabilization ponds: A low-cost contribution to a sustainable wastewater reuse cycle," in *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology*, vol. 109, pp. 241–252, apr 2003.

[73] D. Mara, *Low-cost urban sanitation.* 1996.

[74] A. J. Ward, P. J. Hobbs, P. J. Holliman, and D. L. Jones, "Optimisation of the anaerobic digestion of agricultural resources," *Bioresource technology*, vol. 99, no. 17, pp. 7928–7940, 2008.

[75] G. Marsh, "Rise of the Anaerobic Digester," *Renewable Energy Focus*, vol. 9, pp. 28–34, nov 2008.

[76] R. Tanaka, S. Kowase, and M. Unno, "Chiral cyclotrisiloxanes," *Dalton Transactions*, vol. 39, no. 39, pp. 9235–9237, 2010.

[77] E. Petropoulos, J. Dolfing, R. J. Davenport, E. J. Bowen, and T. P. Curtis, "Developing cold-adapted biomass for the anaerobic treatment of domestic wastewater at low temperatures (4, 8 and 15 °C) with inocula from cold environments," *Water Research*, vol. 112, pp. 100–109, apr 2017.

[78] I. Martin Garcia, M. Mokosch, A. Soares, M. Pidou, and B. Jefferson, "Impact on reactor configuration on the performance of anaerobic MBRs: Treatment of settled sewage in temperate climates," *Water Research*, vol. 47, pp. 4853–4860, sep 2013.

[79] J. A. Álvarez, E. Armstrong, M. Gómez, and M. Soto, "Anaerobic treatment of low-strength municipal wastewater by a two-stage pilot plant under psychrophilic conditions," *Bioresource Technology*, vol. 99, pp. 7051–7062, oct 2008.

[80] I. A. Ieropoulos, A. Stinchcombe, I. Gajda, S. Forbes, I. Merino-Jimenez, G. Pasternak, D. Sanchez-Herranz, and J. Greenman, "Pee power urinal-microbial fuel cell technology field trials in the context of sanitation," *Environmental Science: Water Research and Technology*, vol. 2, no. 2, pp. 336–343, 2016.

[81] A. N. Ghadge, D. A. Jadhav, and M. M. Ghangrekar, "Wastewater treatment in pilot-scale microbial fuel cell using multielectrode assembly with ceramic separator suitable for field applications," *Environmental Progress and Sustainable Energy*, vol. 35, pp. 1809–1817, nov 2016.

[82] J. Kretzschmar, S. Riedl, R. K. Brown, U. Schröder, and F. Harnisch, "eLatrine: Lessons Learned from the Development of a Low-Tech MFC Based on Cardboard Electrodes for the Treatment of Human Feces," *Journal of The Electrochemical Society*, vol. 164, no. 3, pp. H3065–H3072, 2017.

[83] C. J. Castro, J. E. Goodwill, B. Rogers, M. Henderson, and C. S. Butler, "Deployment of the microbial fuel cell latrine in Ghana for decentralized sanitation," *Journal of Water Sanitation and Hygiene for Development*, vol. 4, no. 4, pp. 663–671, 2014.

[84] H. Peavy, D. Rowe, and G. Tchobanoglous, "Water and Wastewater Pumping," *Environmental Engineering*, 1986.

[85] J. M. Foley, R. A. Rozendal, C. K. Hertle, P. A. Lant, and K. Rabaey, "Life cycle assessment of high-rate anaerobic treatment, microbial fuel cells, and microbial electrolysis cells," *Environmental Science and Technology*, vol. 44, pp. 3629–3637, may 2010.

[86] J. Drewnowski, A. Remiszewska-Skwarek, S. Duda, and G. Łagód, "Aeration process in bioreactors as the main energy consumer in a wastewater treatment plant. Review of solutions and methods of process optimization," *Processes*, vol. 7, no. 5, 2019.

[87] M. I. San-Martín, D. D. Leicester, E. S. Heidrich, R. M. Alonso, R. Mateos, and A. Escapa, "Bioelectrochemical Systems for Energy Valorization of Waste Streams," in *Energy Systems and Environment*, InTech, sep 2018.

[88] EPA, “Parameters of Water Quality,” vol. 74, no. October, pp. 177–186, 2001.

[89] Y. Zhou, N. Duan, X. Wu, and H. Fang, “COD discharge limits for urban wastewater treatment plants in China based on statistical methods,” *Water (Switzerland)*, vol. 10, no. 6, 2018.

[90] M.-h. Huang, Y.-m. Li, and G.-w. Gu, “Chemical composition of organic matters in domestic wastewater,” *Desalination*, vol. 262, pp. 36–42, nov 2010.

[91] M. Henze, P. Harremoes, J. l. C. Jansen, and E. Arvin, *Wastewater Treatment: Biological and Chemical Processes.* Springer, 2002.

[92] W. Eckenfelder, *Activated sludge: process design and control.* Lancaster, Pennsylvania, USA: Technomic Publishing Company, Inc, 1998.

[93] W. W. Li, H. Q. Yu, and Z. He, “Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies,” mar 2014.

[94] S. F. Ali, “Determining the UK’s potential for heat recovery from wastewater using steady state and dynamic modelling - preliminary results,” *WEENTECH Proceedings in Energy*, vol. 5, no. December 2018, pp. 107–121, 2019.

[95] T. P. Curtis, I. M. Head, M. Lunn, S. Woodcock, P. D. Schloss, and W. T. Sloan, “What is the extent of prokaryotic diversity?,” in *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 361, pp. 2023–2037, Royal Society, nov 2006.

[96] T. P. Curtis, W. T. Sloan, and J. W. Scannell, “Estimating prokaryotic diversity and its limits,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, pp. 10494–10499, aug 2002.

[97] I. Ferrera and O. Sánchez, “Insights into microbial diversity in wastewater treatment systems: How far have we come?,” 2016.

[98] S. B. Velasquez-Orta, E. Yu, K. P. Katuri, I. M. Head, T. P. Curtis, and K. Scott, “Evaluation of hydrolysis and fermentation rates in microbial fuel cells,” *Applied Microbiology and Biotechnology*, vol. 90, pp. 789–798, apr 2011.

[99] K. F. Adekunle and J. A. Okolie, “A Review of Biochemical Process of Anaerobic Digestion,” *Advances in Bioscience and Biotechnology*, vol. 06, pp. 205–212, mar 2015.

[100] T. V. Fernandes, G. J. Klaasse Bos, G. Zeeman, J. P. Sanders, and J. B. van Lier, “Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass,” *Bioresource Technology*, vol. 100, pp. 2575–2579, may 2009.

[101] A. Valo, H. Carrère, and J. P. Delgenès, “Thermal, chemical and thermo-chemical pre-treatment of waste activated sludge for anaerobic digestion,” *Journal of Chemical Technology and Biotechnology*, vol. 79, pp. 1197–1203, nov 2004.

[102] L. Zhang, R. Gao, A. Naka, T. L. Hendrickx, H. H. Rijnaarts, and G. Zeeman, “Hydrolysis rate constants at 10–25 °C can be more than doubled by a short anaerobic pre-hydrolysis at 35 °C,” *Water Research*, vol. 104, pp. 283–291, nov 2016.

[103] R. C. Wagner, J. M. Regan, S.-E. Oh, Y. Zuo, and B. E. Logan, “Hydrogen and methane production from swine wastewater using microbial electrolysis cells,” *Water Research*, vol. 43, no. 5, pp. 1480–1488, 2009.

[104] X. Zhang, W. He, L. Ren, J. Stager, P. J. Evans, and B. E. Logan, “COD removal characteristics in air-cathode microbial fuel cells,” *Bioresource Technology*, vol. 176, pp. 23–31, jan 2015.

[105] M.-F. Dignac, P. Ginestel, A. Bruchet, J.-M. Audic, S. Derenne, and C. Largeau, “Changes in the organic coposition of wastewater during bilogical treatment as studied by NMR and IR spectroscopies,” *Water Science & Technology*, vol. 43, pp. 51–58, jan 2001.

[106] E. Eriksson, K. Auffarth, M. Henze, and A. Ledin, “Characteristics of grey wastewater,” *Urban Water*, vol. 4, pp. 85–104, mar 2002.

[107] T. Colin, A. Bories, Y. Sire, and R. Perrin, “Treatment and valorisation of winery wastewater by a new biophysical process (ECCF®),” *Water Science and Technology*, vol. 51, no. 1, pp. 99–106, 2005.

[108] C. Gimkiewicz and F. Harnisch, “Waste water derived electroactive microbial biofilms: growth, maintenance, and basic characterization.,” *Journal of visualized experiments : JoVE*, no. 82, p. 50800, 2013.

[109] J. R. Ambler and B. E. Logan, “Evaluation of stainless steel cathodes and a bicarbonate buffer for hydrogen production in microbial electrolysis cells using a new method for measuring gas production,” *International Journal of Hydrogen Energy*, vol. 36, pp. 160–166, jan 2011.

[110] L. Verea, O. Savadogo, A. Verde, J. Campos, F. Ginez, and P. J. Sebastian, “Performance of a microbial electrolysis cell (MEC) for hydrogen production with a new process for the biofilm formation,” *International Journal of Hydrogen Energy*, vol. 39, no. 17, pp. 8938–8946, 2014.

[111] M. Oakley, “Environmental engineering,” 2018.

[112] B. S. Yildiz, “Water and wastewater treatment: Biological processes,” in *Metropolitan Sustainability: Understanding and Improving the Urban Environment*, pp. 406–428, Cambridge, UK: Woodhead Publishing, jan 2012.

[113] Japan Sewage Works Association, “Design Standard For Municipal Wastewater Treatment Plants,” 2013.

[114] IWA, “Activated Sludge Process — IWA Publishing,” 2012.

[115] R. Snyder and D. Wyant, “Activated sludge process control, training manual for wastewater treatment plant operators,” tech. rep., Department of Environmental Quality, State of Michigan, 2009.

[116] A. Xia, C. Herrmann, and J. D. Murphy, “How do we optimize third-generation algal biofuels?,” *Biofuels, Bioproducts and Biorefining*, vol. 9, pp. 358–367, jul 2015.

[117] E. J. Bowen, J. Dolfing, R. J. Davenport, F. L. Read, and T. P. Curtis, “Low-temperature limitation of bioreactor sludge in anaerobic treatment of domestic wastewater,” *Water Science and Technology*, vol. 69, no. 5, pp. 1004–1013, 2014.

[118] D. J. Sanderson and D. C. Peacock, “Making rose diagrams fit-for-purpose,” feb 2020.

[119] Z. Wang and Z. He, “Demystifying terms for understanding bioelectrochemical systems towards sustainable wastewater treatment,” feb 2020.

[120] M. Sugnaux, M. Happe, C. P. Cachelin, A. Gasperini, M. Blatter, and F. Fischer, “Cathode deposits favor methane generation in microbial electrolysis cell,” *Chemical Engineering Journal*, vol. 324, pp. 228–236, 2017.

[121] A. Kadier, Y. Simayi, M. S. Kalil, P. Abdeshahian, and A. A. Hamid, “A review of the substrates used in microbial electrolysis cells (MECs) for producing sustainable and clean hydrogen gas,” 2014.

[122] K. P. Mosse, A. F. Patti, E. W. Christen, and T. R. Cavagnaro, “Review: Winery wastewater quality and treatment options in Australia,” 2011.

[123] B. Tartakovsky, P. Mehta, G. Santoyo, and S. R. Guiot, “Maximizing hydrogen production in a microbial electrolysis cell by real-time optimization of applied voltage,” *International Journal of Hydrogen Energy*, vol. 36, pp. 10557–10564, aug 2011.

[124] K. Rabaey, G. Lissens, S. D. Siciliano, and W. Verstraete, “A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency,” *Biotechnology Letters*, vol. 25, pp. 1531–1535, sep 2003.

[125] A. P. Borole, G. Reguera, B. Ringeisen, Z.-W. Wang, Y. Feng, and B. H. Kim, “Electroactive biofilms: Current status and future research needs,” *Energy & Environmental Science*, vol. 4, no. 12, p. 4813, 2011.

[126] D. Recio-Garrido, M. Perrier, and B. Tartakovsky, “Modeling, optimization and control of bioelectrochemical systems,” apr 2016.

[127] Y. Feng, Q. Yang, X. Wang, and B. E. Logan, "Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cells," *Journal of Power Sources*, vol. 195, pp. 1841–1844, apr 2010.

[128] L. Wang, W. Liu, L. Kang, C. Yang, A. Zhou, and A. Wang, "Enhanced biohydrogen production from waste activated sludge in combined strategy of chemical pretreatment and microbial electrolysis," *International Journal of Hydrogen Energy*, vol. 39, pp. 11913–11919, aug 2014.

[129] M. Di Lorenzo, K. Scott, T. P. Curtis, and I. M. Head, "Effect of increasing anode surface area on the performance of a single chamber microbial fuel cell," *Chemical Engineering Journal*, vol. 156, pp. 40–48, jan 2010.

[130] S. Cheng, D. Xing, and B. E. Logan, "Electricity generation of single-chamber microbial fuel cells at low temperatures," *Biosensors and Bioelectronics*, vol. 26, pp. 1913–1917, jan 2011.

[131] European Commission, "H2020 - Work Programme 2018-2020 13. Europe in a changing world-Inclusive, innovative and reflective societies," tech. rep., 2018.

[132] K. Y. Kim, W. Yang, P. J. Evans, and B. E. Logan, "Continuous treatment of high strength wastewaters using air-cathode microbial fuel cells," *Bioresource Technology*, vol. 221, pp. 96–101, 2016.

[133] D. D. Leicester, J. M. Amezaga, A. Moore, and E. S. Heidrich, "Optimising the Hydraulic Retention Time in a Pilot-Scale Microbial Electrolysis Cell to Achieve High Volumetric Treatment Rates Using Concentrated Domestic Wastewater," *Molecules (Basel, Switzerland)*, vol. 25, p. 2945, jun 2020.

[134] I. Ivanov, L. Ren, M. Siegert, and B. E. Logan, "A quantitative method to evaluate microbial electrolysis cell effectiveness for energy recovery and wastewater treatment," *International Journal of Hydrogen Energy*, vol. 38, pp. 13135–13142, oct 2013.

[135] B. Khoshnevisan, M. Tabatabaei, P. Tsapekos, S. Rafiee, M. Aghbashlo, S. Lindeneg, and I. Angelidaki, "Environmental life cycle assessment of different biorefinery platforms valorizing municipal solid waste to bioenergy, microbial protein, lactic and succinic acid," *Renewable and Sustainable Energy Reviews*, vol. 117, p. 109493, jan 2020.

[136] D. Jiang, M. Curtis, E. Troop, K. Scheible, J. McGrath, B. Hu, S. Suib, D. Raymond, and B. Li, "A pilot-scale study on utilizing multi-anode/cathode microbial fuel cells (MAC MFCs) to enhance the power production in wastewater treatment," *International Journal of Hydrogen Energy*, vol. 36, no. 1, 2011.

[137] L. Zhuang, Y. Yuan, Y. Wang, and S. Zhou, "Long-term evaluation of a 10-liter serpentine-type microbial fuel cell stack treating brewery wastewater," *Bioresource Technology*, vol. 123, pp. 406–412, nov 2012.

[138] S. Luo, H. Sun, Q. Ping, R. Jin, and Z. He, "A review of modeling bioelectrochemical systems: Engineering and statistical aspects," feb 2016.

[139] R. M. Jr, *Environmental engineering: principles and practice.* 2014.

[140] R. M. Maier and I. L. Pepper, "Bacterial Growth," in *Environmental Microbiology: Third Edition*, pp. 37–56, Elsevier Inc., jan 2015.

[141] J. M. Pisciotta, Z. Zaybak, D. F. Call, J. Y. Nam, and B. E. Logan, "Enrichment of microbial electrolysis cell biocathodes from sediment microbial fuel cell bioanodes," *Applied and Environmental Microbiology*, vol. 78, pp. 5212–5219, aug 2012.

[142] A. R. Hari, K. Venkidusamy, K. P. Katuri, S. Bagchi, and P. E. Saikaly, "Temporal microbial community dynamics in microbial electrolysis cells - Influence of acetate and propionate concentration," *Frontiers in Microbiology*, vol. 8, jul 2017.

[143] T. Sleutels, S. Molenaar, A. Heijne, and C. Buisman, "Low Substrate Loading Limits Methanogenesis and Leads to High Coulombic Efficiency in Bioelectrochemical Systems," *Microorganisms*, vol. 4, p. 7, jan 2016.

[144] H.-S. Lee and B. E. Rittmann, "Significance of biological hydrogen oxidation in a continuous single-chamber microbial electrolysis cell," *Environmental Science & Technology*, vol. 44, no. 3, pp. 948–954, 2009.

[145] R. Moscoviz, E. Desmond-Le Quéméner, E. Trably, and N. Bernet, "Bioelectrochemical Systems for the Valorization of Organic Residues," in *Biorefinery*, pp. 511–534, Springer International Publishing, 2019.

[146] B. Tartakovsky, M.-F. Manuel, H. Wang, and S. Guiot, "High rate membrane-less microbial electrolysis cell for continuous hydrogen production," *International Journal of Hydrogen Energy*, vol. 34, no. 2, 2009.

[147] B. R. Dhar and H. S. Lee, "Evaluation of limiting factors for current density in microbial electrochemical cells (MXCs) treating domestic wastewater," *Biotechnology Reports*, vol. 4, no. 1, pp. 80–85, 2014.

[148] F. Zhao, E. S. Heidrich, T. P. Curtis, and J. Dolfing, "Understanding the complexity of wastewater: The combined impacts of carbohydrates and sulphate on the performance of bioelectrochemical systems," *Water Research*, vol. 176, p. 115737, jun 2020.

[149] V. Vavilin, S. Rytov, L. Lokshina, and J. Rintala, "Description of hydrolysis and acetoclastic methanogenesis as the rate-limiting steps during anaerobic conversion

of solid waste into methane," in *Second International Symposium on Anaerobic Digestion of Solid Wastes*, pp. 1–4, 1999.

[150] J. Mata-Alvarez, S. Macé, and P. Llabrés, "Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives," *Bioresource Technology*, vol. 74, no. 1, pp. 3–16, 2000.

[151] V. A. Vavilin, B. Fernandez, J. Palatsi, and X. Flotats, "Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview," *Waste Management*, vol. 28, no. 6, pp. 939–951, 2008.

[152] J. R. Asztalos and Y. Kim, "Enhanced digestion of waste activated sludge using microbial electrolysis cells at ambient temperature," *Water Research*, vol. 87, pp. 503–512, dec 2015.

[153] PicoTech, "High-Resolution Data Acquisition — Pico Technology," 2014.

[154] Y. Ahn and B. E. Logan, "Domestic wastewater treatment using multi-electrode continuous flow MFCs with a separator electrode assembly design," *Applied Microbiology and Biotechnology*, vol. 97, pp. 409–416, jan 2013.

[155] Z. Ge and Z. He, "Long-term performance of a 200 liter modularized microbial fuel cell system treating municipal wastewater: treatment, energy, and cost," *Environmental Science: Water Research & Technology*, vol. 2, no. 2, pp. 274–281, 2016.

[156] F. Zhang, Z. Ge, J. Grimaud, J. Hurst, and Z. He, "Long-term performance of liter-scale microbial fuel cells treating primary effluent installed in a municipal wastewater treatment facility," *Environmental Science and Technology*, vol. 47, pp. 4941–4948, may 2013.

[157] K. Y. Kim, W. Yang, and B. E. Logan, "Impact of electrode configurations on retention time and domestic wastewater treatment efficiency using microbial fuel cells," *Water Research*, vol. 80, pp. 41–46, 2015.

[158] D. F. Juang, P. C. Yang, H. Y. Chou, and L. J. Chiu, "Effects of microbial species, organic loading and substrate degradation rate on the power generation capability of microbial fuel cells," *Biotechnology Letters*, vol. 33, pp. 2147–2160, nov 2011.

[159] A. Larrosa-Guerrero, K. Scott, I. M. Head, F. Mateo, A. Ginesta, and C. Godinez, "Effect of temperature on the performance of microbial fuel cells," *Fuel*, vol. 89, pp. 3985–3994, dec 2010.

[160] B. Min and B. E. Logan, "Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell," *Environmental Science and Technology*, vol. 38, pp. 5809–5814, nov 2004.

[161] P. Kaewkannetra, W. Chiwes, and T. Y. Chiu, "Treatment of cassava mill wastewater and production of electricity through microbial fuel cell technology," *Fuel*, vol. 90, pp. 2746–2750, aug 2011.

[162] Y. Y. Choi, S. R. Baek, J. I. Kim, J. W. Choi, J. Hur, T. U. Lee, C. J. Park, and B. J. Lee, "Characteristics and biodegradability of wastewater organic matter in municipal wastewater treatment plants collecting domestic wastewater and industrial discharge," *Water (Switzerland)*, vol. 9, no. 6, 2017.

[163] W. He, X. Zhang, J. Liu, X. Zhu, Y. Feng, and B. E. Logan, "Microbial fuel cells with an integrated spacer and separate anode and cathode modules," *Environmental Science: Water Research and Technology*, vol. 2, no. 1, pp. 186–195, 2016.

[164] Z. Ge, L. Wu, F. Zhang, and Z. He, "Energy extraction from a large-scale microbial fuel cell system treating municipal wastewater," *Journal of Power Sources*, vol. 297, pp. 260–264, 2015.

[165] M. Behera, P. S. Jana, T. T. More, and M. M. Ghangrekar, "Rice mill wastewater treatment in microbial fuel cells fabricated using proton exchange membrane and earthen pot at different pH," *Bioelectrochemistry*, vol. 79, pp. 228–233, oct 2010.

[166] F. Guo, G. Fu, Z. Zhang, and C. Zhang, "Mustard tuber wastewater treatment and simultaneous electricity generation using microbial fuel cells," *Bioresource Technology*, vol. 136, pp. 425–430, may 2013.

[167] D. Jiang, B. Li, W. Jia, and Y. Lei, "Effect of inoculum types on bacterial adhesion and power production in microbial fuel cells," *Applied Biochemistry and Biotechnology*, vol. 160, pp. 182–196, jan 2010.

[168] I. Gajda, J. Greenman, and I. A. Ieropoulos, "Recent advancements in real-world microbial fuel cell applications," oct 2018.

[169] S. Cheng, W. Liu, J. Guo, D. Sun, B. Pan, Y. Ye, W. Ding, H. Huang, and F. Li, "Effects of hydraulic pressure on the performance of single chamber air-cathode microbial fuel cells," *Biosensors and Bioelectronics*, vol. 56, pp. 264–270, 2014.

[170] M. I. San-Martín, A. Sotres, R. M. Alonso, J. Díaz-Marcos, A. Morán, and A. Escapa, "Assessing anodic microbial populations and membrane ageing in a pilot microbial electrolysis cell," *International Journal of Hydrogen Energy*, vol. 44, pp. 17304–17315, jun 2019.

[171] D. I. Carlotta-Jones, K. Purdy, K. Kirwan, J. Stratford, and S. R. Coles, "Improved hydrogen gas production in microbial electrolysis cells using inexpensive recycled carbon fibre fabrics," *Bioresource Technology*, vol. 304, p. 122983, may 2020.

[172] N. Mills, P. Pearce, J. Farrow, R. B. Thorpe, and N. F. Kirkby, "Environmental & economic life cycle assessment of current & future sewage sludge to energy technologies," *Waste Management*, vol. 34, pp. 185–195, jan 2014.

[173] UKPower, "Compare Energy Prices Per kWh — Gas & Electric Per Unit — UKPower," 2020.

[174] Business Electricity Prices, "Business Electricity Rates and Unit Prices per kWh 2019," 2019.

[175] D. Akman, K. Cirik, S. Ozdemir, B. Ozkaya, and O. Cinar, "Bioelectricity generation in continuously-fed microbial fuel cell: Effects of anode electrode material and hydraulic retention time," *Bioresource Technology*, vol. 149, pp. 459–464, dec 2013.

[176] O. Ichihashi, T. A. Vishnivetskaya, and A. P. Borole, "High-Performance Bioanode Development for Fermentable Substrates via Controlled Electroactive Biofilm Growth," *ChemElectroChem*, vol. 1, pp. 1940–1947, nov 2014.

[177] F. Zhao, E. S. Heidrich, T. P. Curtis, and J. Dolfing, "The effect of anode potential on current production from complex substrates in bioelectrochemical systems: a case study with glucose," *Applied Microbiology and Biotechnology*, 2020.

[178] J. Ma, Z. Wang, D. He, Y. Li, and Z. Wu, "Long-term investigation of a novel electrochemical membrane bioreactor for low-strength municipal wastewater treatment," *Water Research*, vol. 78, pp. 98–110, jul 2015.

[179] D. A. Jadhav, M. M. Ghangrekar, and N. Duteanu, "Recent progress towards scaling up of MFCs," in *Microbial Fuel Cell: A Bioelectrochemical System that Converts Waste to Watts*, pp. 443–457, New Delhi, India: Springe, Cham, dec 2017.

[180] R. Sander, "Compilation of Henry's law constants (version 4.0) for water as solvent," *Atmospheric Chemistry and Physics*, vol. 15, pp. 4399–4981, apr 2015.

[181] O. Emmanuel Alepu and Z. Li, "Effect of Hydraulic Retention Time on Anaerobic Digestion of Xiao Jiahe Municipal Sludge," *International Journal of Waste Resources*, vol. 6, no. 3, 2016.

[182] R. V. Linares, J. Domínguez-Maldonado, E. Rodríguez-Leal, G. Patrón, A. Castillo-Hernández, A. Miranda, D. D. Romero, R. Moreno-Cervera, G. Camara-chale, C. G. Borroto, and L. Alzate-Gaviria, "Scale up of microbial fuel cell stack system for residential wastewater treatment in continuous mode operation," *Water (Switzerland)*, vol. 11, p. 217, jan 2019.

[183] N. Khan, M. D. Khan, S. Sultana, M. Z. Khan, and A. Ahmad, "Bioelectrochemical systems for transforming waste to energy," in *Modern Age Environmental Problems and their Remediation*, pp. 111–128, Springer International Publishing, jan 2017.

[184] B. E. Logan, "Exoelectrogenic bacteria that power microbial fuel cells," *Nature Reviews Microbiology*, vol. 7, no. 5, pp. 375–381, 2009.

[185] P. Aelterman, S. Freguia, J. Keller, W. Verstraete, and K. Rabaey, "The anode potential regulates bacterial activity in microbial fuel cells," *Applied Microbiology and Biotechnology*, vol. 78, pp. 409–418, mar 2008.

[186] S. Jung and J. M. Regan, "Influence of external resistance on electrogenesis, methanogenesis, and anode prokaryotic communities in microbial fuel cells," *Applied and Environmental Microbiology*, vol. 77, no. 2, pp. 564–571, 2011.

[187] M. Kokko, S. Epple, J. Gescher, and S. Kerzenmacher, "Effects of wastewater constituents and operational conditions on the composition and dynamics of anodic microbial communities in bioelectrochemical systems," jun 2018.

[188] C. I. Torres, R. Krajmalnik-Brown, P. Parameswaran, A. K. Marcus, G. Wanger, Y. A. Gorby, and B. E. Rittmann, "Selecting anode-respiring bacteria based on anode potential: Phylogenetic, electrochemical, and microscopic characterization," *Environmental Science and Technology*, vol. 43, pp. 9519–9524, dec 2009.

[189] S. Sevda, S. Sharma, C. Joshi, L. Pandey, N. Tyagi, I. Abu-Reesh, and T. R. Sreekrishnan, "Biofilm formation and electron transfer in bioelectrochemical systems," jan 2018.

[190] K. Guo, S. Freguia, P. G. Dennis, X. Chen, B. C. Donose, J. Keller, J. J. Gooding, and K. Rabaey, "Effects of surface charge and hydrophobicity on anodic biofilm formation, community composition, and current generation in bioelectrochemical systems," *Environmental Science and Technology*, vol. 47, no. 13, pp. 7563–7570, 2013.

[191] S. A. Patil, F. Harnisch, B. Kapadnis, and U. Schröder, "Electroactive mixed culture biofilms in microbial bioelectrochemical systems: The role of temperature for biofilm formation and performance," *Biosensors and Bioelectronics*, vol. 26, pp. 803–808, oct 2010.

[192] J. Yu, J. Seon, Y. Park, S. Cho, and T. Lee, "Electricity generation and microbial community in a submerged-exchangeable microbial fuel cell system for low-strength domestic wastewater treatment," *Bioresource Technology*, vol. 117, pp. 172–179, 2012.

[193] C. Koch, K. J. Huber, B. Bunk, J. Overmann, and F. Harnisch, "Trophic networks improve the performance of microbial anodes treating wastewater," *npj Biofilms and Microbiomes*, vol. 5, dec 2019.

[194] M. Hasany, M. M. Mardanpour, and S. Yaghmaei, "Biocatalysts in microbial electrolysis cells: A review," jan 2016.

[195] J. F. Heidelberg, I. T. Paulsen, K. E. Nelson, E. J. Gaidos, W. C. Nelson, T. D. Read, J. A. Eisen, R. Seshadri, N. Ward, B. Methe, R. A. Clayton, T. Meyer, A. Tsapin,

J. Scott, M. Beanan, L. Brinkac, S. Daugherty, R. T. DeBoy, R. J. Dodson, A. S. Durkin, D. H. Haft, J. F. Kolonay, R. Madupu, J. D. Peterson, L. A. Umayam, O. White, A. M. Wolf, J. Vamathevan, J. Weidman, M. Impraim, K. Lee, K. Berry, C. Lee, J. Mueller, H. Khouri, J. Gill, T. R. Utterback, L. A. McDonald, T. V. Feldblyum, H. O. Smith, J. C. Venter, K. H. Nealson, and C. M. Fraser, "Genome sequence of the dissimilatory metal ion-reducing bacterium Shewanella oneidensis," *Nature Biotechnology*, vol. 20, no. 11, pp. 1118–1123, 2002.

[196] F. Rezaei, D. Xing, R. Wagner, J. M. Regan, T. L. Richard, and B. E. Logan, "Simultaneous cellulose degradation and electricity production by Enterobacter cloacae in a microbial fuel cell," *Applied and Environmental Microbiology*, vol. 75, no. 11, pp. 3673–3678, 2009.

[197] A. K. Manohar and F. Mansfeld, "The internal resistance of a microbial fuel cell and its dependence on cell design and operating conditions," *Electrochimica Acta*, vol. 54, no. 6, pp. 1664–1670, 2009.

[198] Y. Mohan, S. Manoj Muthu Kumar, and D. Das, "Electricity generation using microbial fuel cells," *International Journal of Hydrogen Energy*, vol. 33, no. 1, pp. 423–426, 2008.

[199] J. Niessen, U. Schröder, and F. Scholz, "Exploiting complex carbohydrates for microbial electricity generation - A bacterial fuel cell operating on starch," *Electrochemistry Communications*, vol. 6, no. 9, pp. 955–958, 2004.

[200] C. Dumas, R. Basseguy, and A. Bergel, "Microbial electrocatalysis with Geobacter sulfurreducens biofilm on stainless steel cathodes," *Electrochimica Acta*, vol. 53, pp. 2494–2500, jan 2008.

[201] S. Ishii, K. Watanabe, S. Yabuki, B. E. Logan, and Y. Sekiguchi, "Comparison of electrode reduction activities of Geobacter sulfurreducens and an enriched consortium in an air-cathode microbial fuel cell," *Applied and Environmental Microbiology*, vol. 74, pp. 7348–7355, dec 2008.

[202] V. Watson, "Shewanella oneidensis MR-1 compared to mixed cultures for electricity production in four different microbial fuel cell configurations," no. May, p. 53, 2009.

[203] M. Behera and M. M. Ghangrekar, "Performance of microbial fuel cell in response to change in sludge loading rate at different anodic feed pH," *Bioresour Technol*, vol. 100, no. 21, pp. 5114–5121, 2009.

[204] K. P. Nevin, H. Richter, S. F. Covalla, J. P. Johnson, T. L. Woodard, A. L. Orloff, H. Jia, M. Zhang, and D. R. Lovley, "Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells," *Environmental Microbiology*, vol. 10, pp. 2505–2514, oct 2008.

[205] E. S. Heidrich, T. P. Curtis, S. Woodcock, and J. Dolfing, “Quantification of effective exoelectrogens by most probable number (MPN) in a microbial fuel cell,” *Bioresource Technology*, vol. 218, pp. 27–30, oct 2016.

[206] P. Chatterjee, P. Dessì, M. Kokko, A. M. Lakaniemi, and P. Lens, “Selective enrichment of biocatalysts for bioelectrochemical systems: A critical review,” jul 2019.

[207] V. G. Gude, B. Kokabian, and V. Gadhamshetty, “Beneficial bioelectrochemical systems for energy, water, and biomass production,” *Journal of Microbial and Biochemical Technology*, vol. 5, no. SPECIALISSUE.2, p. 6, 2013.

[208] A. Stubbs, S. G. Ball, and British Entomological and Natural History Society., *British hoverflies : an illustrated identification guide.* 2002.

[209] Bristol Post, “Rat-tailed maggots crawling around Glastonbury Festival toilets captured on video - Bristol Live,” 2017.