THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Influence of electrode material and stochastic factors on the performance and microbial community assembly in microbial electrochemical systems

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Cover:
The experimental setup of MECs investigating the effects of different anode materials.
Photo: Marie Abadikhah

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ABSTRACT

Microbial electrolysis cells (MECs) are systems with microbial communities in the form of biofilms on electrode surfaces. The electrogenic bacteria in the anode biofilm act as catalysts for the oxidization of organic compounds, leading to release of electrons, generation of electrical current, and production of hydrogen and methane at the cathode. In addition to production of energy carriers, MECs can be used for other applications as well; for example, as biosensors to monitor biochemical oxygen demand or toxicity. The performance of MECs is determined by both deterministic and stochastic factors influencing the microbial communities on the electrode surfaces, most of which as still poorly understood. In this thesis, the effects of electrode materials on microbial community assembly and MEC performance was investigated. Two experiments were carried out. In the first, three cathode materials (carbon nanoparticles, titanium, and steel) were compared. In the second, three anode materials (carbon cloth, graphene, and nickel) were compared. The cathode materials had no significant effect on the performance of the MECs, as opposed to the anode materials where carbon cloth MECs had the highest current density and the shortest lag time during startup. The differences seen in lag time of replicate systems at the start of the experiment indicated a stochastic initial attachment of the electrogenic bacteria on the anode. Different microbial communities develop in the biofilms on the anodes and cathodes. Electrogens from the *Desulfobacterota* phylum dominated the anode, while various hydrogenotrophic methanogens, e.g., *Methanobacterium*, were found to dominate on the cathodes. Diversity and null model analysis of the electrode communities highlighted stochasticity and not electrode material as the important factor in the community assembly. Network analysis showed that the cathode communities had fewer negative interactions between taxa in comparison to the anode. Since hydrogen gas generated at the cathode surface can diffuse through the biofilm, all microorganisms on the cathode have access to the substrate, reducing the need for competition between species. In contrast, electrogens require a short distance to the anode to be able to use it as electron acceptor. Limited space on the anode and competition between electrogens shaped the anode communities and explain the higher number of negative interactions observed. Based on the findings in this thesis, it is suggested that stochastic factors have more influence than electrode material on the anode community even though there is a selective pressure for electrogenic bacteria.

Keywords: bioanode, biocathode, biochemical system, microbial community assembly

In memory of my Aunt Pari

PREFACE

The research within this licentiate thesis was carried out at the division of Water and Environmental Technologies, at Chalmers between 2019 and 2022, under the supervision of Oskar Modin, Frank Persson, Britt-Marie Wilén and Anne Farewell with funding from FORMAS (project 2018-00622).

Firstly, I would like to thank my supervisor Oskar Modin for his guidance and helpfulness during this time. I also would like to thank my co-supervisors, Anne Farewell, Frank Persson and Britt-Marie Wilén for their insightful feedback and ideas. Thank you to all my friends and colleagues at the division of Water and Environmental Technologies. I would like to thank Cecilia Burzio for her warm welcome on my first day as well as all the fun discussions and helpful conversations. Thank you to Amir Saeid Mohammadi for all his help in the lab.

Lastly, I would like to thank my mother Behnaz, my father Mahmood, my brother Hossein, and my sister-in-law Nasrin for their unconditional support.

THESIS

The licentiate thesis is based on the following appended manuscripts:

A. Abadikhah M., de Celis Rodriguez M., Persson F., Wilén BM., Farewell A., Modin O., Evidence of competition between electrogens shaping electroactive microbial communities in microbial electrolysis cells, *Manuscript*
B. Abadikhah M., Liu M., Persson F., Wilén BM., Farewell A., Sun J., Modin O., Effect of different anode material on the performance and microbial community in microbial electrolysis cells, *Manuscript*

Contents

1 Introduction

The main aim of wastewater treatment is the production of effluent water which has a sufficient quality for release into the environment. During wastewater treatment, the focus is on removal of nitrogen, phosphorous and organic material (Kadier et al. 2016). Most of these processes require the input of energy and chemicals. However, our wastewater can be considered a resource as well, as it contains a large amount of nutrients, organics, and metals (Modin et al. 2012). To obtain a more sustainable future, the optimization of our wastewater treatment is needed where the resources found in the wastewater can be utilized and recycled for further use. To achieve this, new technologies need to be implemented. Microbial electrochemical technologies (METs) could contribute in various ways.

1.1 Microbial electrochemical technologies

Microbial electrochemical technologies (METs), such as microbial electrolysis cells (MECs) and microbial fuel cells (MFCs), use electrogenic bacteria in the anode biofilm as catalysts in the oxidation of organic material (Fig 1) (Mateo et al. 2018). The oxidation reaction releases electrons that are transferred by the bacteria to the anode, leading to the generation of current (Logan et al. 2015, Shin et al. 2017). The electrons are then subsequently transferred to the cathode where they drive reduction reactions, such as hydrogen generation. METs can be applied to numerous areas within water and wastewater treatment (Modin et al. 2017). It can aide in wastewater treatment. Since it generates current upon consumption of organic substrates, it can reduce the energy input required during this process. METs can be used in the recovery of energy in the form of hydrogen and methane gas and for recovery of resources such as metals and nutrients (Modin et al. 2017). METs can also be used as biosensors, such as those for the measurement of biochemical oxygen demand or toxicity (Kim et al. 2003, Patil et al. 2010).

Certain aspects such as design and operation of the system influence the function and performance. One important factor to take into consideration is the material used for the anode and cathode. Because of the exposure of the material to an aqueous environment, the materials chosen for the anode are typically carbon-based. Conventionally, materials such as carbon cloth and carbon paper are used (He et al. 2012, Baranitharan et al. 2013). Recently graphene, a new carbon-based material, has become the target of research regarding its use as an anode material due to its favourable properties. Graphene has a two-dimensional structure, which has been shown to have a large surface area as well as a high conductivity (Geim et al. 2007, McAllister et al. 2007, Pumera 2009). There are however some conflicting reports regarding graphene and its biocompatibility. Some studies have found graphene to have antimicrobial properties, while other highlight its optimal structure for the attachment of microorganisms and use in technologies such as METs (Park et al. 2010, Das et al. 2011, Ruiz et al. 2011). Based upon the research performed to analyse the toxicity of graphene to microorganism, one likely cause of the antimicrobial properties noted are due to toxic residues remaining from the manufacturing process and not due to any specific antimicrobial properties of graphene itself (Wang et al. 2015, Yu et al. 2016). Other research highlights graphene's structure consisting of vertical flakes as a potential cause for its antimicrobial properties (Lu et al. 2017, Pandit et al. 2018). It has been speculated that initial attachment of microorganisms to the graphene flakes result in cell death due to the sharpness of the flakes (Pandit et al. 2021).

Organic matter left after cell death could allow for new bacteria to attach without being pierced by the sharp flakes.

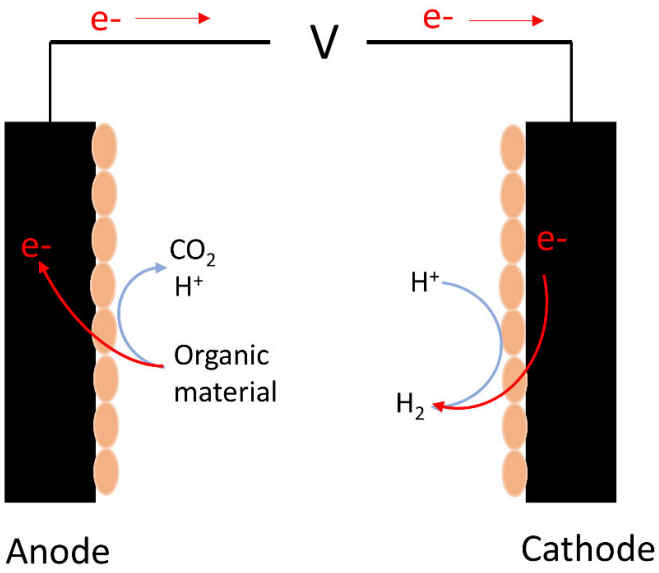

Figure 1. Illustration of the redox reactions that occur on the electrodes in METs.

In MECs, a potential is applied to drive the electrochemical reactions in the system (Rozendal et al. 2006). In dual chamber MECs, the anode and cathode are located in different compartments separated by an ion conductive spacer. This causes a larger space between the two electrodes leading to a higher overpotential (Kadier et al. 2016). Typically, the two compartments are separated by an ion exchange membrane. This membrane results in a pH imbalance which in turn lead to a higher degree of voltage loss. In single chamber MECs, where the anode and cathode are in the same compartment, these issues have been resolved (Call et al. 2008). Since the electrodes share a compartment, both the biofilm communities have access to the hydrogen present in the system. In instances where methane gas production is not the aim, a drawback of single chamber systems is that methanogens tend to consume a large portion of the hydrogen generated at the cathode leading to the production of methane gas (Kadier et al. 2016).

1.2 Microbial communities within METs

The anode biofilm is responsible for the breakdown of organic material leading to the generation of current. These reactions are typically facilitated by electrogenic bacteria, which are capable of extracellular electron transfer (De Vrieze et al. 2018, Logan et al. 2019). The electrogenic bacteria most commonly found in these systems belong to the *Desulfobacterota* phylum, formerly known as *Deltaproteobacteria* (Bond et al. 2003, Waite et al. 2020). Specifically, those from the *Geobacter* genera have been well researched and are commonly found in high abundance (Reguera et al. 2006, Logan et al. 2019). *Shewanella* sp. are another group of electrogenic bacteria that have been well documented with regards to their function and abundance in METs (Kim et al. 2002, Gorby et al. 2006, Marsili et al. 2008, Logan et al. 2019). The biofilm of the cathode consists mainly of methanogens involved in hydrogen evolution and methanogenesis. *Methanobacterium* sp. are commonly found on the cathode

surface (Siegert et al. 2015). Acetogens, e.g., *Acetobacterium* sp., are also commonly found on the cathode where they reduce CO_2 to acetate with the use of the cathode or hydrogen generated at the cathode as electron donor (Balch et al. 1977, Nevin et al. 2011, Wang et al. 2018).

1.3 Microbial community assembly in METs

The performance of the system is dependent on the microbial community residing on the electrode surfaces. *Geobacter* spp. have been highlighted as important players in the function of METs (Yates et al. 2012). Even though the importance of the abundance of electrogenic bacteria is known, much of the assembly process and the different factors influencing the outcome of the microbial community assembly is poorly understood. For instance, Zhou et al. (2013) reported variation in the performance and microbial community composition of replicate systems operated under identical conditions. There are, however, some conflicting reports. For instance, Yates et al. (2012) reported that the development of similar microbial communities over time could be attributed to deterministic and not stochastic factors. To obtain a better understanding of microbial community assembly in METs, the ecological processes that help shape the communities need to be taken into account. The ecological processes can be divided into four categories: dispersal, diversification, drift and selection (Nemergut et al. 2013). The one most studied is selection, which describes the fitness of the different species under specific conditions. In the case of METs, this refers to the ability of the bacteria to transfer electrons and facilitate the generation of current. Deterministic factors controlled by the operation of the MECs such as substrate composition and material influence the selection as well (Koch et al. 2019, Saheb-Alam et al. 2019). Diversification pertains to changes brought on by mutations and horizontal gene transfer which aid in acquiring new characteristics. Dispersal is the random attachment, detachment and movement that occurs in the system over time. The random death and replication of organisms is referred to as drift (Nemergut et al. 2013).

In single-chamber MECs, at least two distinct habitats exist with different functions. Due to this there is a selection pressure based on location within the systems. For instance, the microbial community on the anode need to have the ability to transfer electrons to the anode surface (Logan et al. 2019), therefore there is a selection for microorganisms that are electrogenic. In contrast, the cathode microbial community is involved in the hydrogen and methane generation (Li et al. 2019). This puts a selection pressure for microorganism that are hydrogen oxidizers. Other factors that may influence the selection pressure in MECs are the deterministic factors implemented. The choice in electrode material, the substrate composition, the operation, and the design of the system will all have an impact on the selection pressure in the different habitats within MECs. For example, the substrate composition can consist of a single carbon source or a mixture of different carbon sources. In the case where you only have acetate, selection occurs for those able to use acetate as an energy source. While in the case of a more complex substrate composition, such as the mixture of acetate, propionate and butyrate, there is a selection for organisms that can use any of these as an energy source. The movement of the microorganisms is explained by dispersal. In contrast to dual-chamber MECs where the electrodes are placed in different compartments, single-chamber MECs have all the different locations within one compartment. As the liquid circulates in the system, all microorganisms have equal access to everything within the system. This allows for dispersal to

occurs between different habitats within the system. Dispersal also refers to the attachment and detachment of the electrode surfaces, since the anode and cathode are located in the same space this allows for dispersal between locations to occur.

2 Research motivation and scope of thesis

METs are systems that have many different real-life applications, such as resource and energy recovery. These systems rely on the microbial communities residing on the electrode surfaces for their function. However, the underlying factors and ecological processes influencing the microbial communities are still poorly understood. Previous research has mainly focused on the performance of the METs and how different deterministic factors, such as design, may optimize the systems, without taking into consideration the influence of the ecological processes on the microbial community assembly. Therefore, it is of importance to study both how deterministic factors as well as stochastic factors influence the performance, stability, and microbial community assembly over time in METs.

The goal of this thesis was to study the effect of electrode material (a deterministic factor) and stochastic factors on the performance and microbial community assembly in single-chamber MECs. Different anode and cathode materials were investigated to examine the effect on the current generation and the microbial community composition within single-chamber MECs. A comparison of the different ecological processes such as selection, dispersal and drift were studied by operating replicate systems of each material. For the anode, the conventional material carbon cloth was compared to graphene and nickel. During the manufacturing of the graphene electrodes, nickel was used as a base which the graphene flakes were placed on. Therefore, nickel was included as a comparison to the effect of the graphene. It was hypothesized that based on the large surface area, conductivity, and biocompatibility of graphene; a higher current generation could be obtained in comparison to more conventional carbon materials. The cathode materials investigated were carbon nanoparticles, titanium, and steel. For this experiment, it was hypothesized that the cathode materials would have an influence on the microbial communities developing on both the cathode and the anode, and that this would impact the current generation in the systems. The only differing selection pressure in both experiments were the electrode materials being investigated. Therefore, it was speculated that differences seen between MECs having different electrode materials could be attributed to the materials while differences seen between replicate MECs having the same material could be attributed to stochastic factors.

3 Materials and Methods

Two experiments were performed, which are included in this thesis. The focus of the first experiment (paper A) was on the effect of different cathode materials on the performance of the MECs. This experiment will be referred to as the cathode experiment henceforth. The second experiment (Paper B) focused on the effect of different anode materials on the generation of current and will be referred to as the anode experiment.

3.1 Experimental design and operation

To investigate the effects of different electrode materials on the performance of the MECs, single-chamber reactors were constructed. These systems were fed with nutrient broth (NB) containing acetate, propionate, and butyrate, at regular intervals (Paper A; Paper B). Samples were also taken of the effluent for further analysis. The reactors were inoculated with 5mL of anaerobic mesophilic digester sludge. To be able to determine the consistency in the results obtained, the set-up consisted of 3-4 replicate reactors of each material.

Reactor design for different cathode materials
The cathode reactors consisted of one of three cathode materials: steel, titanium, or carbon nanoparticles (CNP) (Fig 2). There were triplicates of each material giving a total of nine reactors. To establish the effect of the cathode material on the performance and microbial community assembly all reactors had a carbon cloth anode. They were operated for 106 days, with an applied potential of 1 V between the anode and cathode and a total system volume of 70 mL. The reactors were fed at an interval of 2-3 days.

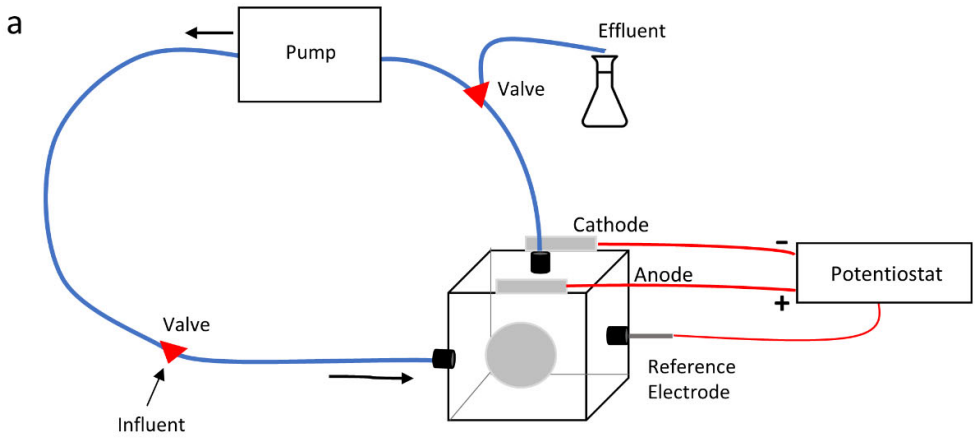

Figure 2. Schematic illustration of the setup of the cathode experiment.

Reactor design for different anode materials
The conventional anode material carbon cloth was compared to graphene and nickel in a setup consisting of twelve reactors, consisting of four replicates for each material. Four systems were constructed consisting of three reactors, one of each material, in a loop (Fig 3). All reactors within the system had a steel cathode to eliminate any influences of the cathode material on the anode. The total volume of a loop with three reactors was 225 mL. The reactors were operated for 56 days, under an applied potential of 1 V between the anode and cathode and fed at intervals of 4-5 days.

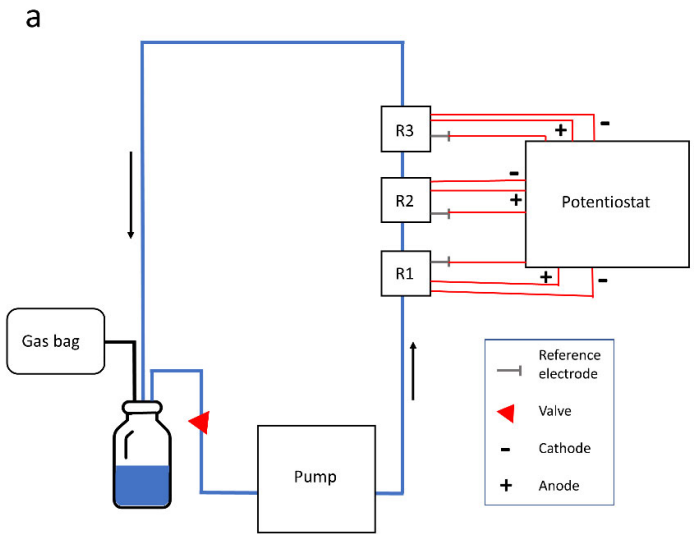

Figure 3. Schematic illustration of the system set up in the anode experiment.

3.2 Analytical methods for characterization of systems

A high-performance liquid chromatograph (HPLC) with a UV-detector (Shimadzu) and an Aminex HPX-87H Ion exclusion column (BioRad) was used to establish the changes in carbon source concentrations within the systems during the experimental run. The concentration of carbon sources in the liquid of the system was sampled before each feeding. During specific periods after a stable current production had been established, the system was sampled at the interval of 0, 24 and 72 hours after feeding to track the consumption patterns. Cyclic voltammetry (CV) was performed against a reference electrode (Ag/AgCl) to establish the changes in bioelectrochemical activity between the start and end of the experiment. Cyclic voltammetry characterizes the electrochemical reactions of the system, specifically the electrode of interest (Elgrishi et al. 2018). A cyclic voltammetry is performed by applying a potential with a linear sweep to the working electrode against a reference electrode with a constant potential (Elgrishi et al. 2018). While the potential is applied to the working electrode, the resulting current generation is measured. This way, the bioelectrochemical activity of the working electrode can be evaluated and its capacity to catalyse oxidation and reduction reactions can be determined (Elgrishi et al. 2018). Cyclic voltammetry can be used to determine the changes in the electrodes ability to initiate redox reactions. It also can be used to identify the generation of hydrogen when the potential reaches negative values. In the case of MECs, it can be used to establish the changes in the material, which indicate the growth of a biofilm on the surface of the electrodes involved in the catalysis of redox reactions within the system. The current generation was measured using a potentiostat in accordance with methods described in Paper A.

3.3 Microbial community analysis

At the end of the experimental runs, the inoculum, biofilms from the electrode surfaces as well as the biomass suspended in the liquid were sampled for further analysis and identification. The DNA extraction was performed using the FastDNA spin kit for Soil (MP Biomedicals),

subsequent methods used for preparation and sequencing of the DNA samples can be found in the appended paper A and B.

Two sequencing methods were employed in the identification of the taxa present in the microbial community composition. For the experiment investigating the cathode, amplicon sequencing was used, while the microbial community of the anode experiment was investigated using shotgun metagenomic sequencing. Amplicon sequencing is a target specific sequencing method, designed to sequence short fragments of a specific gene or area of interest. With regards to taxonomical identification of prokaryotes, the v4 region of the 16S rRNA gene is targeted, due to it being highly conserved (Woo et al. 2008). The sequencing is done by first PCR-amplifying the target region, creating a high concentration of amplicon copies. This is followed by a purification step. Illumina sequencing was used to sequence the v4 region of the 16S rRNA gene. Illumina next generation sequencing (NGS) uses adaptors to attach fragments of DNA onto the flow cell, where they undergo clonal amplification with bridge PCR (Ambardar et al. 2016). The sequencing is a paired-end, meaning that the reaction occurs on both ends of the DNA fragment, thereby allowing for more accuracy and higher coverage (Ambardar et al. 2016). The sequencing occurs by addition of the reversible terminator (RT) nucleotides each with a specific fluorescent marker, in addition to a modified DNA polymerase. Once the nucleotide is attached the signal is read, and the colour of the fluorescent marker registered. The sequencing data is then error-corrected and amplicon sequence variants (ASVs) are determined. The ASVs can be identified taxonomically by comparison to databases with known taxa.

Metagenomic sequencing on the other hand sequences all DNA present in the sample, thereby giving a larger overview of the microbial composition (Quince et al. 2017). Before sequencing can occur, the DNA samples are prepared by first undergoing DNA fragmentation to obtain smaller pieces of DNA. The sequencing method employed is similar to the amplicon sequencing (Quince et al. 2017). Once the sequencing data has been obtained, the metagenomic data needs to be analysed. It is possible to use an assembly-based approach where the sequences are combined into longer contigs, which can be further grouped into metagenome-assembled genomes. It is also possible to use a read-based approach where the short sequence reads are compared to databases. In this thesis, SingleM (https://github.com/wwood/singlem) was used to generate operational taxonomic units (OTUs) from the short sequence reads. SingleM searches the dataset for reads that encode single copy marker genes and provides information about the number of reads that map to each OTU and the taxonomic affiliation of the OTUs.

Amplicon sequencing and shotgun metagenomic sequencing both have their advantages and limitations. In the case of amplicons sequencing, it is a more cost-efficient method for identifying microorganisms present in the microbial community (Liu et al. 2021). It also is much quicker to analyse, and low amounts of DNA is needed for the sequencing (Rausch et al. 2019). However due to it targeting a specific conserved rRNA region of the microorganisms, a PCR procedure is needed to amplify the region of interest. The target gene and region also differ between prokaryotes and eukaryotes, and both cannot be amplified at once (Jo et al. 2016). Due to different copy numbers of the 16S rRNA in different species there also is a risk for biased results, where those with a higher copy number seem to be found in higher abundance (Jo et al. 2016, Rausch et al. 2019). Shotgun metagenomics on the other hand is

considered to be more expensive to perform, it also has a more time-consuming and complicated analysis procedure, therefore being more difficult to work with (Liu et al. 2021). However, since all the DNA in the sample is sequenced without amplification, an overview of all the different microorganisms, such as prokaryotes as well as eukaryotes can be analysed in the same sample (Jo et al. 2016, Liu et al. 2021). This also eliminates the biases for genes with multiple copy numbers in the final abundance of each identified species. Moreover, shotgun metagenomic sequencing also has a higher taxonomical resolution in comparison to amplicon sequencing as well as the ability to assign potential functional properties for the identified species (Liu et al. 2012).

For the cathode experiment amplicon sequencing of the v4 region of the 16S rRNA gene was done to generate amplicon sequence variants (ASVs), which were used to identify the taxonomical profile of the samples. In the anode experiment shotgun metagenomic sequencing was used followed by SingleM to obtain the abundance of operational taxonomic units (OTUs) based on highly conserved single copy marker genes.

Community diversity analysis

The microbial community diversity was established using alpha- and beta diversity. Alpha diversity refers to the diversity within a sample. A high alpha diversity indicates there are a high number of species present in the sample, while a low alpha diversity indicates the opposite (Whittaker 1960). Beta diversity on the other hand evaluates the similarities or dissimilarities between two samples. The higher the beta diversity, the more dissimilar the two sample communities are (Whittaker 1960, Whittaker 1972). The Hill-based framework uses Hill-number as a way to obtain quantitative measures of the alpha- and beta diversity (Modin et al. 2020). Hill numbers uses a diversity order to give weight to the relative abundance of different taxa in a community. Depending on the diversity order used to quantify the biodiversity of the samples, there will be a difference in importance of the abundance distribution. For instance, a diversity order of 0 applies no importance to the relative abundance of the ASVs/OTUs within the sample, it only quantifies the diversity based on the ASVs/OTUs present. A diversity order of 1, on the other hand, places importance on the relative abundance. This means that the weight of the ASVs/OTUs is determined by their relative abundance. On the other hand, if the diversity order is above 1, even more importance is given to those with a higher relative abundance (Modin et al. 2020). The Python package qdiv (Modin et al. 2020) was used to calculate the alpha- and beta diversity of the microbial communities with a Hill-based framework. A one-way single ANOVA followed by a post hoc (Tukey) test was performed on the anode alpha diversity using Pinguoin (Vallat 2018). A principal component analysis (PCoA) was used to depict the beta diversity. To establish whether the differences observed in community composition between different samples were due to random chance, a Raup-Crick null model was used (Raup et al. 1979, Modin et al. 2020). The difference in community composition and its correlation with system performance was evaluated using a Mantel test (Mantel 1967).

A network analysis, described in Paper A, was performed to establish potential negative and positive interactions within the microbial community in a specific habitat. of the analysis included 146, 113 and 229 unique ASVs on the anode, cathode, and suspension, respectively.

4 Results and Discussion

4.1 Performance of MECs

Correlation between start-up time and the electrode material

The lag time corresponds with the time from the start of the experiment until a sufficient current generation has been obtained for the system to be considered actively generating current. The threshold for this was set at 1 A/m^2. There was a variation in start-up for all reactors with the exception of those with carbon cloth as the anode material (Fig 4b; Fig 5b). MECs with a carbon cloth anode (C1-C4) had a lag time of 8-10 days, while graphene (G1-G5) and nickel (N1-N4) had a lag time varying from 18-38 days. Similarly, the MECs with different cathode materials all showed variation of a lag time from 5-17 days. A large variation in the average peak and total charge for the first three weeks could be observed, this can be attributed to the variation in the start-up time for the different reactors (Fig 4c; Fig 5c). Previous reports have indicated the potential antimicrobial properties of graphene materials due to toxic residues that remain from the manufacturing process (Yu et al. 2016). Considering graphene's reported high conductivity and large surface area (Geim et al. 2007, McAllister et al. 2007) it is possible that one factor contributing to the lag time is toxic residues that may reduce in concentration over time allowing for the bacteria to colonize the surface. The structure of the graphene also influences the bacteria's ability to colonize it. Since the graphene consist of sharp flakes, as the bacteria attach onto the surface, the flakes pierce them leading to cell death (Pandit et al. 2018). Once a layer of organic matter coats the flakes, the bacteria can then safely attach and colonize the anode surface. A more likely explanation for the variation in current generation for the replicate reactors is the stochastic properties involved in the initial colonization of the electrode surface. Previous research has shown the importance of stochastic factors on the development of the anammox biofilm (Niederdorfer et al. 2021). Similarly, research done studying the microbial community in floating and settled granules indicated the importance of stochastic factors for the community assembly (Trego et al. 2021).

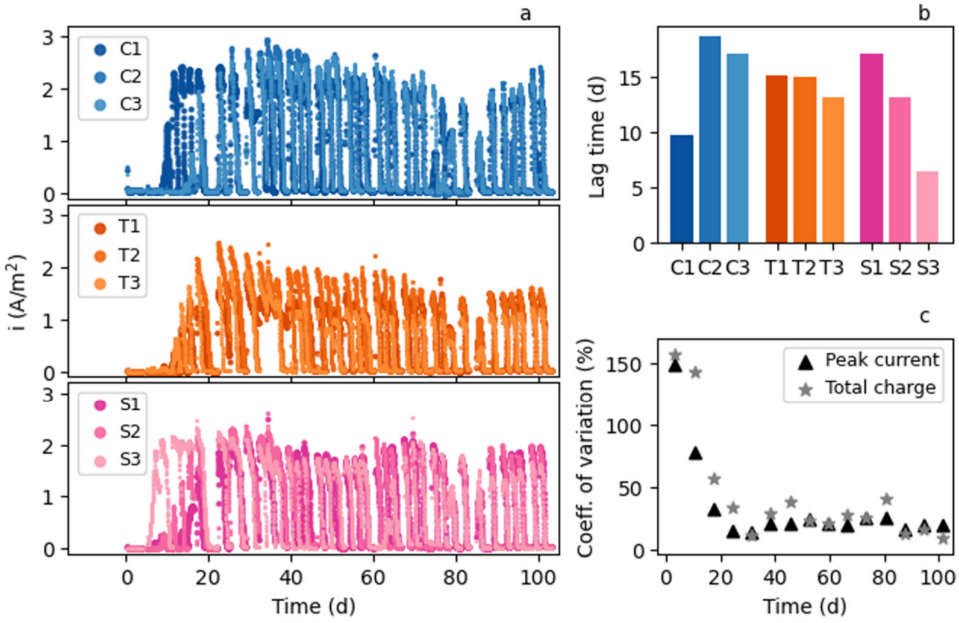

Figure 4. a) Chronoamperometry measurements of the current generation for the 9 MECs with different cathode materials. b) Bar graph of the start-up time before each reactor started generating current. c) The variation in average peak current and total charge over time for all nine reactors combined.

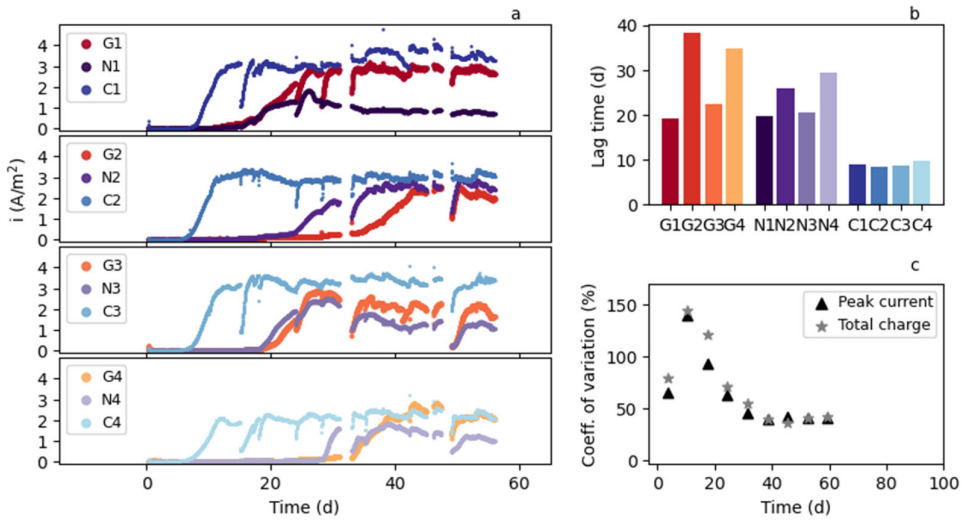

Figure 5. a) Chronoamperometry measurements of the current generation for the 12 MECs with different anode materials. b) Bar graph of the start-up time before each reactor started generating current. c) The variation in average peak current and total charge over time for all twelve reactors combined.

Comparison of current generation of the different electrode materials

Even with a difference in lag time between both replicates and materials, no difference in current generation was observed once the systems reached a stable level in the cathode experiment (Fig 4a). For the anode experiment, the carbon cloth cathodes had a higher current generation in comparison to both the graphene and nickel (Fig. 5a). Comparing the graphene and nickel there was some variation, but in general the graphene had a slightly higher current generation (Fig 5a). When comparing the current generated in the two experiments (Fig 4a; Fig 5a), continuous periods of current being generated followed by a dip in the current can be observed for the cathode experiment. This is related to the access to carbon sources during operation. During the cathode experiment, the organic material used as an energy source for the microbial organisms were depleted at the end of each batch cycle, leading to the dip in current generation. In contrast, the systems in the anode experiment always had access to organic material and therefore no dip in current generation was observed.

In both experimental set-ups, the peak current generation was achieved during the period around day 40 of the experimental run (Fig. 6; Fig. 7). At this point, a slight reduction in current generation could be observed, followed by a stable current production for the cathode experiment. The anode experiment also had a slight decrease in current generation once the peak current was reached, but when compared to the cathode experiment it was not as prominent (Fig. 6; Fig, 7). One likely explanation for this could be that the duration of the experiment differed in the two experiments. The cathode experiment was run for 104 days while the anode experiment was operated for 56 days. In the anode experiment, there is a downward trend in the total charge per week as well as for the peak current (Fig. 7). If the experiment had been prolonged to a similar timeframe as that of the cathode experiment, a similar progression in current generation would have most likely been observed. In the case of the anode this most likely indicates that once the initial colonization of the electrode surfaces has occurred and a biofilm has been formed, the electrogenic bacteria present have equal ability and conditions to oxidise organic substrate, transfer electrons, and generating current. In the early stages, when the current generation is reaching its peak, the biofilm on the anode surface consists of thin layer of microorganisms. This allows the electrogenic bacteria attached to the anode surface to access both the nutrients in the liquid as well as the anode itself for maximum electron transfer ability. As the biofilm becomes thicker, fewer bacteria have access to both the anode surface and the nutrients, reducing their ability to generate current. Some electrogenic bacteria, such as those from the *Geobacter* sp. have mechanisms to overcome this limited access. They use structures such as nanowires and mediators to aide their electron transfer ability (Reguera et al. 2005, Reguera et al. 2006). This allows electrogenic bacteria that are further away from the anode surface to contribute to the current generation in the form of electron transfer into the biofilm. Other than electrogenic bacteria, there are many species of fermenters present in the anode biofilm. Bacteria from *Anaerolineaceae family, Clostridiales* order and *Spirochaetaceae* family have been seen in the microbial community of the anode biofilm. These have a fermentative metabolism, where they can utilize sugars, such as glucose in their fermentative process leading to the production of hydrogen and acetate (Menes et al. 2002, Maune et al. 2012, McIlroy et al. 2017). The presence of fermenters in the anode biofilm could lead to synergistic interactions where the transfer of electrons or nutrients could occur. Another aspect of importance when considering these systems, is the concentration of the carbon sources in the environment. Since the carbon sources are present

in high concentrations, the diffusion of these substrate through the biofilm is also a likely reason as to why the reduction in current is only to a small degree.

Coulombic efficiency is the ratio between the number of electrons transferred by the electrogenic bacteria to the anode from the consumption of the carbon substrates and the theoretical maximum electrons that can be transferred based on substrate concentration (Escapa et al. 2009). The coulombic efficiency for all systems with differing cathode material had a similar pattern to the peak current density. A peak in the coulombic efficiency of approximately 33 % to 56 % was reached around day 40 (Fig 6). Once the peak coulombic efficiency had been reached, all systems had a drastic reduction of their coulombic efficiency to approximately 20%. Charge generated per week for the carbon cloth reactors (anode experiment) reached a peak during the period between day 20 and day 30, apart from C1 which had a peak i during the period between day 40 and day 50 (Fig 7). For graphene and nickel MECs, the peak charge per week was reached during the period between day 30 and day 40. After the peak in charge was reached, the systems stabilized at a lower level and in most systems a slight reduction can be observed towards the period between day 40 and day 60 in most MECs.

The drastic reduction in coulombic efficiency and generated electric charge that occurs after the initial peak is most likely is an indication of changes in the pathways utilizing the carbon sources in the systems as the microbial communities develop over time. Methanogens are commonly found on the cathode biofilm in MECs (Siegert et al. 2015). However, methanogens can also compete with electrogens for substrate. The development of methanogens in the system could be a likely reason for the shift to a lower coulombic efficiency, as methanogenesis pathway increase over time when the methanogens increase in abundance.

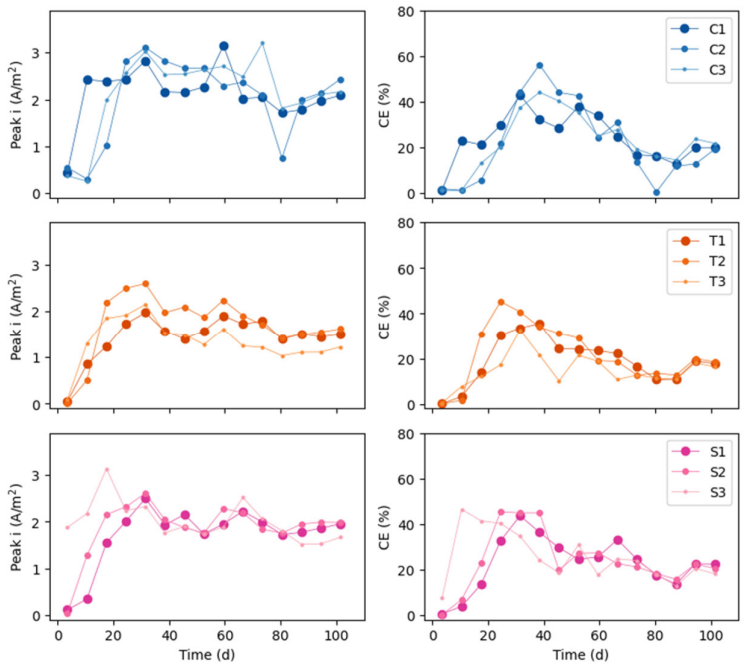

Figure 6. Peak current density (i) and coulombic efficiency (CE) during the anode experiment. Weekly values are shown.

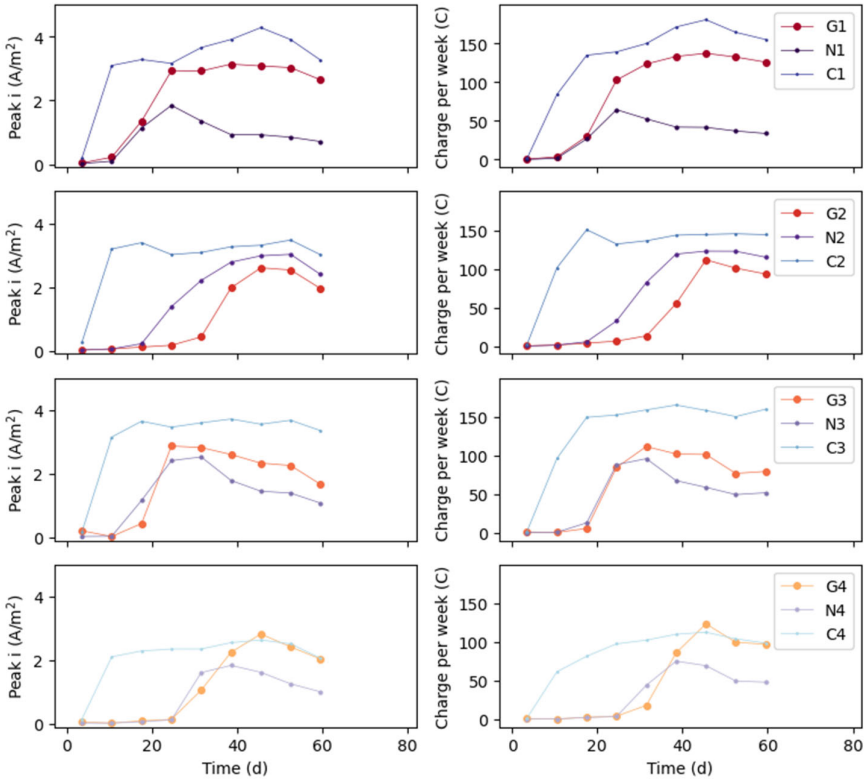

Figure 7. Peak current density (i) and charge per week (C) during the cathode experiment. Weekly values are shown.

Changes in bioelectrochemical activity of the electrodes during experimental run

The bioelectrochemical activity of the anode and cathode was measured at the beginning and end of the experimental run to characterize the differences and changes against a Ag/AgCl reference electrode (Fig 8; Fig 9). An improvement in the anodes bioelectrochemical catalysis was seen for all material. This indicates that the bacterial growth on the electrode surfaces resulted in a better performance for all reactors. The graphene and nickel reactors had a lower ability to catalyse the oxidation of the organic substrates compared to the carbon cloth (Fig 9). During a cyclic voltammetry measurement, the capacity of the electrode to generate hydrogen can be evaluated. This can be observed at the negative potential where there is an exponential increase in negative current (Fernández-Valverde et al. 2010, Elgrishi et al. 2018). The bioelectrochemical activity measurements of the cathode of the MECs with different cathode materials indicated an improvement in the hydrogen generation for the steel and titanium at the end of the experiment compared to the beginning (Fig 8c-d). In the case of the carbon nanoparticles MECs, the bioelectrochemical activity of the cathode remained relatively unchanged from start to end of the experimental run with regards to hydrogen evolution (Fig 8c-d). Since the carbon nanoparticles have a large surface area for bioelectrochemical reactions to occur, the attachment of microorganisms on its surface does not have a large impact on its abilities. The voltammograms for the cathode also indicated a reduction peak around -0.5 V (Fig 8c-d)., which may be associated with redox active compound in the biofilm residing on the cathode surface.

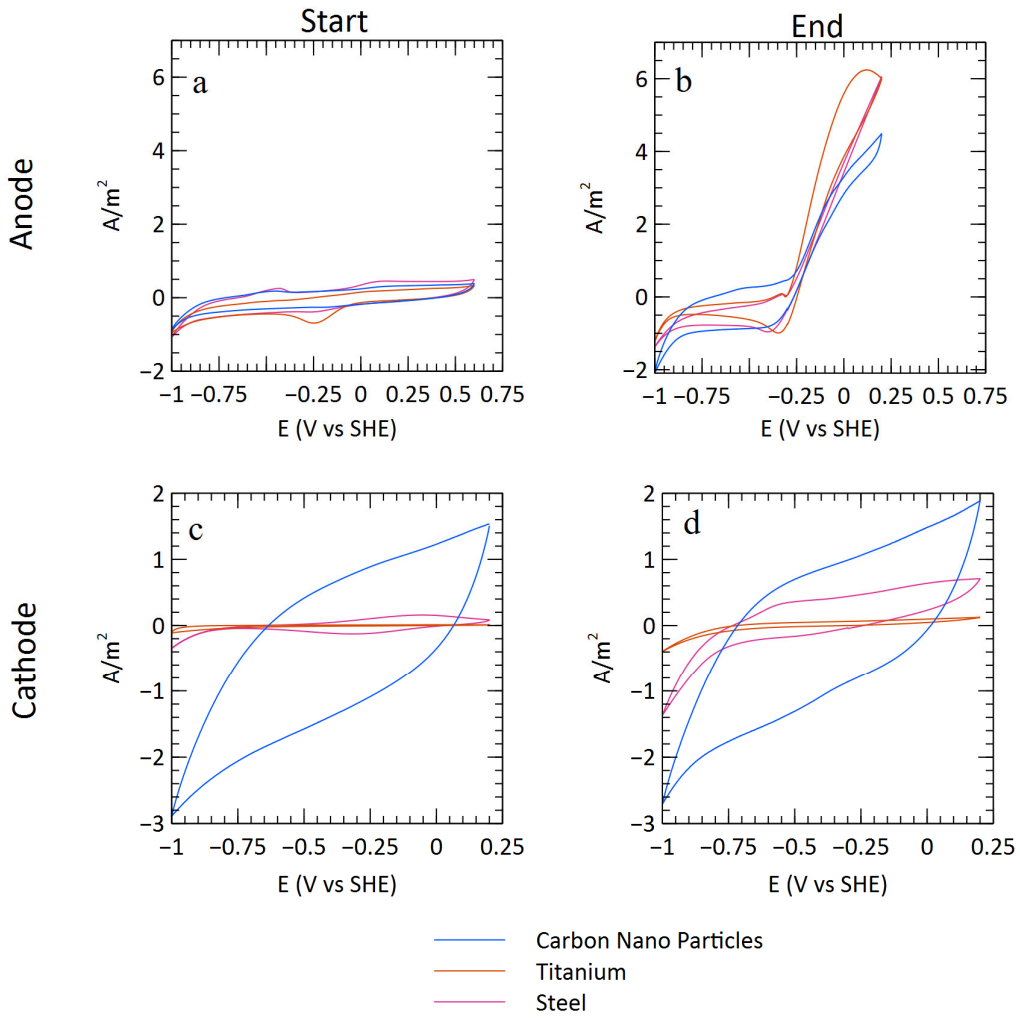

Figure 8. Cyclic voltammetry measurements from the start and end of the cathode experiment. the graph depicts a representative MEC for each material a) Anode vs reference electrode at start of the experimental run. b) Anode vs reference electrode at end of the experimental run. c) Cathode vs reference electrode at start of the experimental run. d) Cathode vs reference electrode at end of the experimental run.

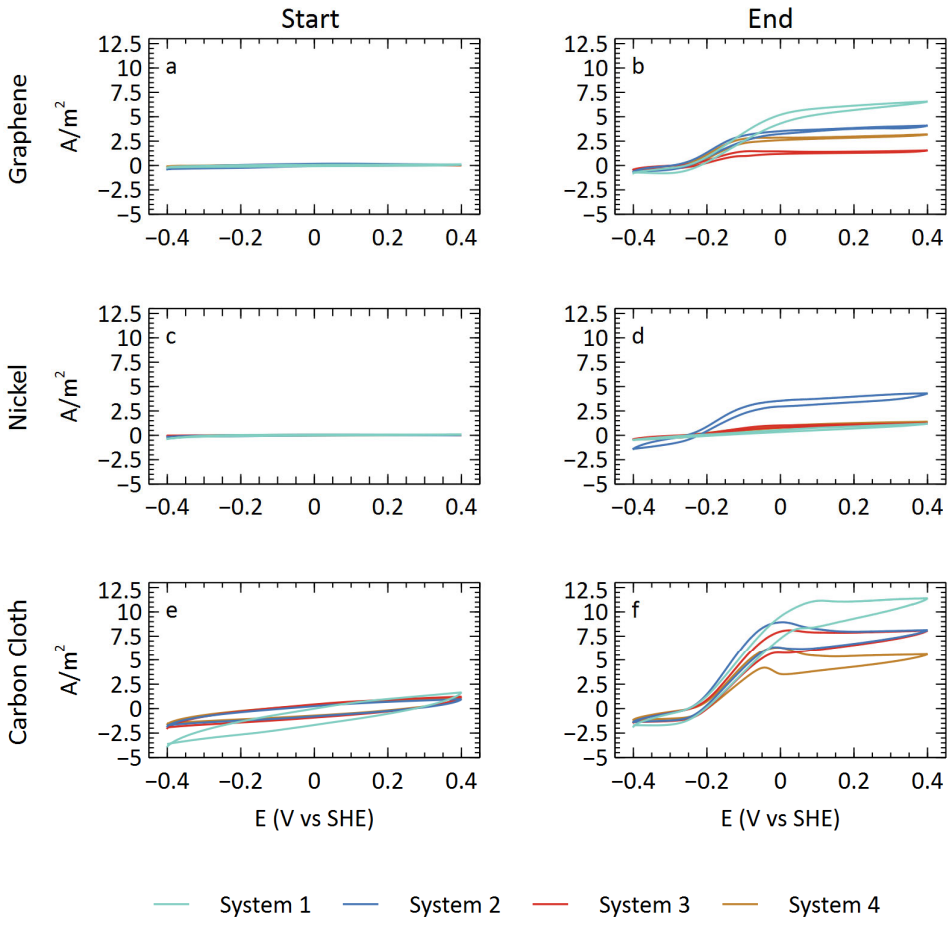

Figure 9. Cyclic voltammetry measurements of the anode from the start and end of the anode experiment. Each plot depicts the replicates from all four systems for each material. a) Graphene anodes, for the four systems, vs reference electrode at start of the experimental run. b) Graphene anodes vs reference electrode at end of the experimental run. c) Nickel anodes vs reference electrode at start of the experimental run. d) Nickel anodes vs reference electrode at end of the experimental run. e) Carbon cloth anodes vs reference electrode at start of the experimental run. f) Carbon cloth anodes vs reference electrode at end of the experimental run.

4.2 Microbial community analysis

Microbial diversity of the different habitats within the MECs

The alpha diversity gives information about the diversity within each sample. The Hill number with a diversity order of 1 (Fig 10a; Fig 11a), showed a significant variation between different habitats ($p<0.05$, ANOVA). The suspension had a significantly higher alpha diversity in comparison to either electrode in the cathode experiment ($p<0.05$, ANOVA). In both experimental set-ups, the anode had the lowest diversity of all habitats. From the comparison of the alpha diversity of the anode or cathode MECs with different materials, it was concluded

that neither the anode nor the cathode material had a significant impact on the alpha diversity of the electrode microbial communities ($p>0.05$, ANOVA).
The anode has been shown to be dominated by electrogenic bacteria (Logan et al. 2019). Since these bacteria need direct contact with the anode surface to be able to transfer electrons, there is a competition during colonization. Due to this, it is very likely that the microbial community diversity becomes limited, in comparison to the suspension where different bacteria can coexist without the need to compete for space and nutrients. Similarly in the cathode there is a lower community diversity in comparison to the suspension. However, in comparison to the anode there is a slightly higher degree of diversity. Since hydrogen produced at the cathode can diffuse through the biofilm, more of the biofilm as well as the bacteria near the cathode surface have access to it. This reduces the degree of competition present in the habitat, allowing for more species to coexist.

Beta diversity describes the dissimilarity or similarity of the microbial communities between samples, which can be illustrated using a principal coordinate analysis. From the principal coordinate analysis (PCoA), a clear separation of the microbial communities based on location could be observed (Fig 10b; Fig 11b). This highlights the difference in microbial niches at different habitats within the MECs. To determine whether the dissimilarity seen between the same location in different MECs could be attributed to random chance, a null model analysis was performed (Paper A: Table S1, Supplementary material; Paper B: Table S1, Supplementary material). The null model analysis randomly redistributes the ASVs present in the communities being compared while keeping the number of ASVs in a sample intact. It then recalculates the dissimilarity for each pair of communities and compares the dissimilarity between the randomly generated communities with the dissimilarity between actual communities. This establishes whether the community dissimilarity seen is due to random chance or not. In almost all cases the null model analysis indicated that the dissimilarity observed was due to stochasticity and not the difference in material.

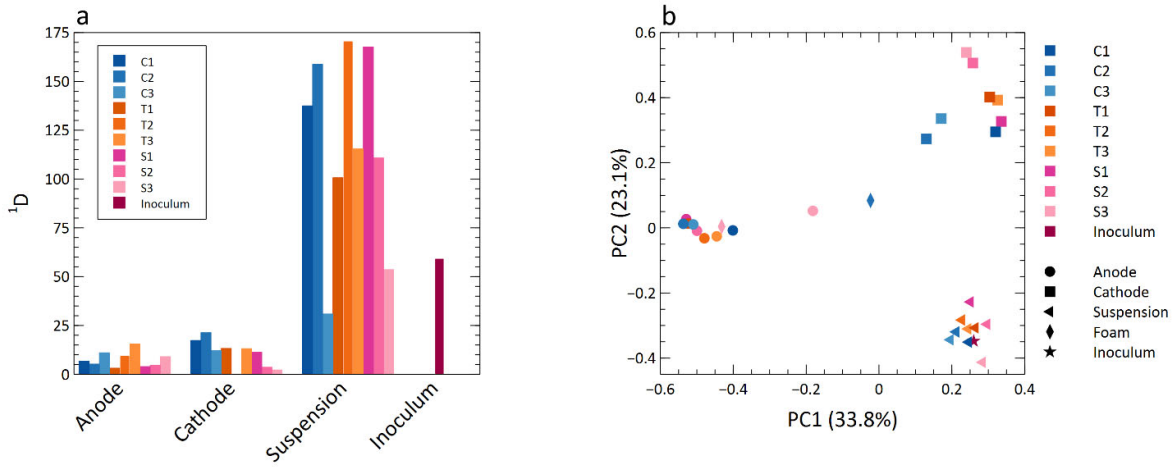

Figure 10. a) Bar graph of the alpha diversity for all samples with a Hill number order of 1, for the cathode experiment. b) Principal coordinate analysis from the dissimilarity matrix with a Hill number order of 1, for the cathode experiment.

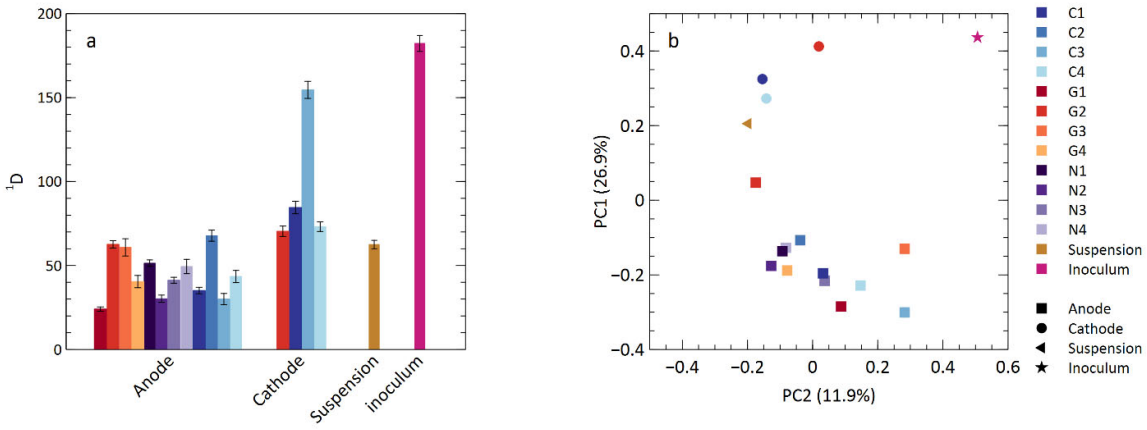

Figure 11. a) Bar graph of the alpha diversity for all samples with a Hill number order of 1, for the anode experiment. b) Principal coordinate analysis from the dissimilarity matrix with a Hill number order of 1, for the anode experiment.

Microbial community composition of the cathode

From the heatmaps of the most abundant taxa within each DNA sample, the community composition could be determined (Fig 13; Paper B: Fig 7-8). The anodes mainly consisted of different species of methanogens. Species from the *Methanobacteriaceae* and *Methanoregulaceae* family were some of the methanogens found in high abundance. Another group of bacteria dominating the cathode were acetogens. Based on the microbial community profile identified for the cathode, it can be concluded that hydrogen generation was an important aspect driving the microbial community assembly. Species from the *Methanoregulaceae* family have been reported to use hydrogen, carbon dioxide as well as acetate in their hydrogen production (Imachi et al. 2008, Sakai et al. 2012). Likewise, the *Methanothrix* has also been shown to use acetate as an energy source (Patel et al. 1990). From the network analysis of the cathode experiment (Fig 14b; Paper A: Table 1) it could be established that the cathode had fewer negative interactions (7.6 %) in comparison to the anode (30.0 %). As mentioned previously, the hydrogen generated at the cathode surface can diffuse through the biofilm. This eliminates the competition for access to the hydrogen allowing for more species with similar functions to reside on the cathode.

Microbial community composition of the anode

The anode community composition was dominated by bacteria involved in electrogenesis (Fig 13; Paper B: Fig 7). Some of the most commonly found bacteria were *Desulfobacterota*, which are known electrogens present in electrochemical systems (Bond et al. 2003, Summers et al. 2010, Logan et al. 2019). Even though the anode experiment had a more varied microbial community composition between samples, the dominating species on almost all anodes were *Desulfobacterota* (Paper B: Fig 7). From the network analysis the negative interactions between the electrogens could be established (Fig 14a). This further highlights the competitive nature of the colonization of the anode surface. Electroactive microorganisms, need access to the surface of the anode to transfer electrons (Costa et al. 2018). Due to the limited space on the anode surface, there is a higher degree of competition for initial colonization.

The correlation between ASV1 and ASV7+ASV8, illustrates the competitive nature of the colonization on the anode surface. The network analysis showed that when there is a high abundance of ASV1, ASV7+ASV8 was found in low abundance (Fig 12).

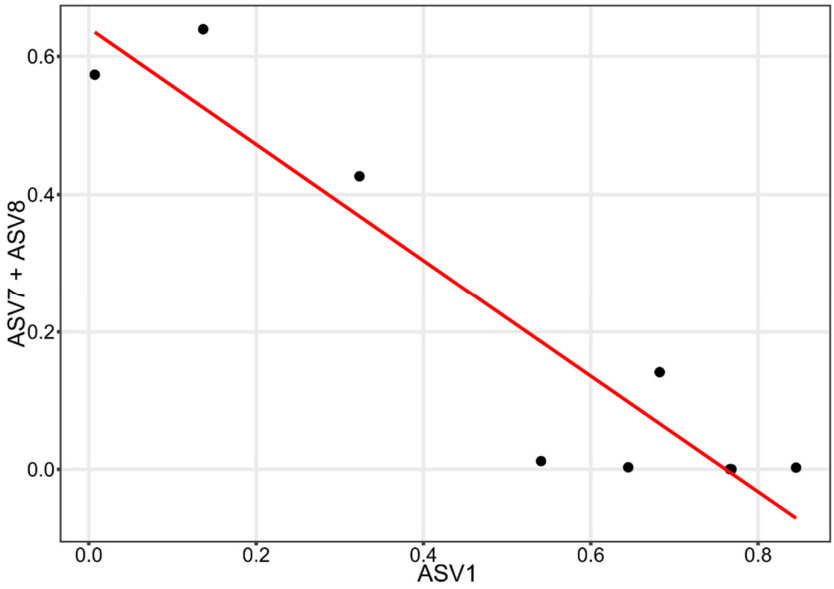

Figure 12. the correlation between the combined abundance of ASV7 and ASV8 with the abundance of ASV1 in the anode DNA samples for the 9 cathode reactors.

Besides electrogens there were many genera present who were involved in fermentative process resulting in the production of acetate, butyrate, and hydrogen. Genera from the *Synergistacea* family such as *Acetomicrobium,* as well as those from the *Anaerolineaceae, Spirochaetaceae* and *Dysgonomonadaceae* family are some of the species found in abundance on the anode surfaces, known to use a fermentative process in their energy harvesting process (Rees et al. 1997, Maune et al. 2012). Leading to the production of biproducts such as acetate and hydrogen (Tomazetto et al. 2018).

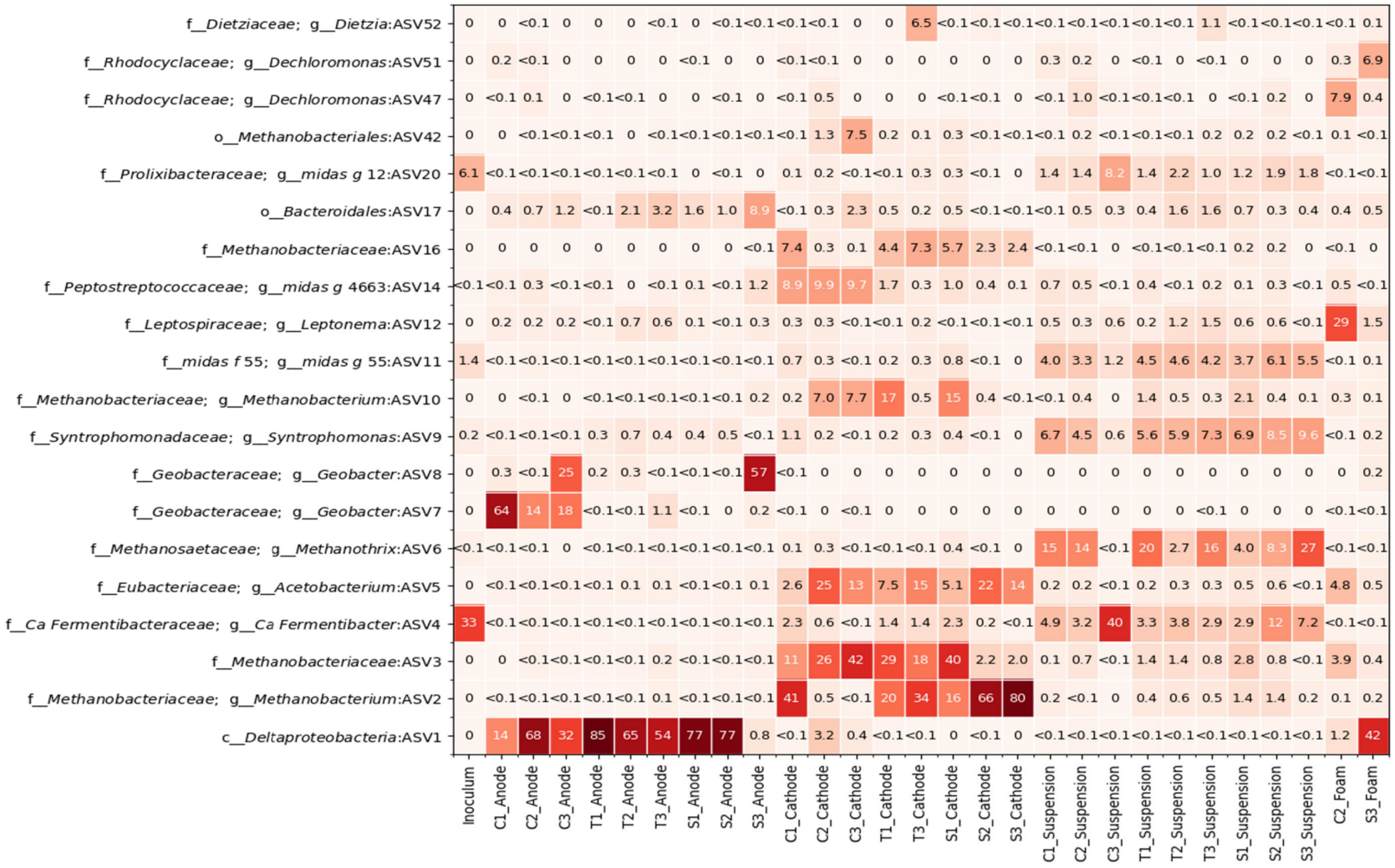

Figure 13. Heatmap depicting the relative abundance of the top 20 most abundant taxa present in the 9 cathode reactors, the inoculum as well as the foam samples from C2 and S3. R5 cathode was excluded due to low number of reads. The y-axis shows the phylum and the ASV tag for each taxon.

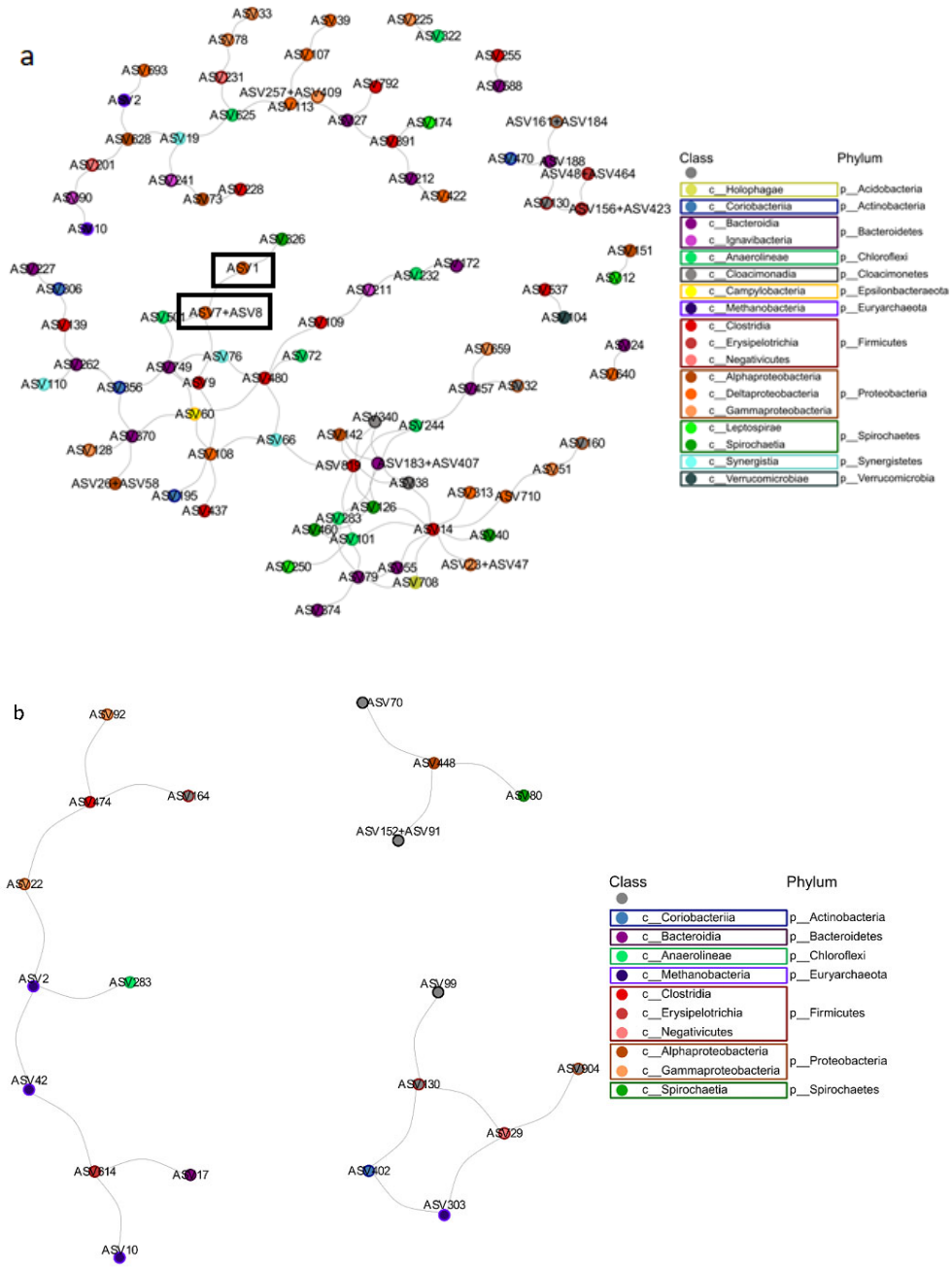

Figure 14. Microbial correlation network illustrating the negative interactions of the cathode experiment. a) The anode microbial community. The interaction between ASV1 and the combination of ASV7 and ASV8 is highlighted. b) The cathode microbial community. Colours depict taxonomy of each ASV at the class level.

5 Conclusions and future research

The choice of electrode material did not seem to cause differences in current generation in the cathode experiment MECs once the system had stabilized and reached a steady state. In the anode experiment, the carbon cloth MECs generated a higher current compared to that of the graphene and nickel MECs. The microbial community composition was also not affected by the difference in material. Instead, most of the differences seen in the MECs could be attributed to stochastic factors. There was a variation in the lag time of 5-38 days, which then reached a peak in current production around day 40 at which point the systems current generation reached a steady state at a slightly lower level. The variation in lag time could be contributed to stochasticity influencing the initial colonization of the electrogens on the anode surface. The variation in the profile of the electrogenic bacteria on different anode samples could be explained by a stochastic initial colonization between competing taxa. In comparison to other habitats, the anode showed a higher degree of competitive interactions between the different taxa within the microbial community.

Based on these findings, the stochastic factors have an important role in how the microbial community on the electrodes in MECs develop. This, in turn, affects the performance and function of the MECs. To be able to optimize these systems, it is of interest to further research the microbial community assembly and the intra- and inter-community interactions between microorganisms present in the system. Most of the research done in the field of METs has been on the prokaryotic community. Very little focus has been given to interactions between prokaryotes with potential predators. Therefore, it is of interest to study the biotic interactions between prokaryotes and eukaryotes or phages with regards to microbial electrochemical systems. This could shed some light on stochastic factors that may influence the development of the microbial communities over time.

6 References

Ambardar, S., R. Gupta, D. Trakroo, R. Lal and J. Vakhlu (2016). "High Throughput Sequencing: An Overview of Sequencing Chemistry." Indian J Microbiol **56**(4): 394-404.

Balch, W. E., S. Schoberth, R. S. Tanner and R. S. Wolfe (1977). "Acetobacterium, a New Genus of Hydrogen-Oxidizing, Carbon Dioxide-Reducing, Anaerobic Bacteria." International Journal of Systematic Bacteriology **27**(4): 355-361.

Baranitharan, E., M. R. Khan, D. M. R. Prasad and J. B. Salihon (2013). "Bioelectricity Generation from Palm Oil Mill Effluent in Microbial Fuel Cell Using Polacrylonitrile Carbon Felt as Electrode." Water, Air, & Soil Pollution **224**(5): 1533.

Bond, D. R. and D. R. Lovley (2003). "Electricity Production by Geobacter sulfurreducens Attached to Electrodes." Applied and Environmental Microbiology **69**(3): 1548-1555.

Call, D. and B. E. Logan (2008). "Hydrogen Production in a Single Chamber Microbial Electrolysis Cell Lacking a Membrane." Environmental Science & Technology **42**(9): 3401-3406.

Costa, N. L., T. A. Clarke, L. A. Philipp, J. Gescher, R. O. Louro and C. M. Paquete (2018). "Electron transfer process in microbial electrochemical technologies: The role of cell-surface exposed conductive proteins." Bioresour Technol **255**: 308-317.

Das, M. R., R. K. Sarma, R. Saikia, V. S. Kale, M. V. Shelke and P. Sengupta (2011). "Synthesis of silver nanoparticles in an aqueous suspension of graphene oxide sheets and its antimicrobial activity." Colloids Surf B Biointerfaces **83**(1): 16-22.

De Vrieze, J., J. B. A. Arends, K. Verbeeck, S. Gildemyn and K. Rabaey (2018). "Interfacing anaerobic digestion with (bio)electrochemical systems: Potentials and challenges." Water Res **146**: 244-255.

Elgrishi, N., K. J. Rountree, B. D. McCarthy, E. S. Rountree, T. T. Eisenhart and J. L. Dempsey (2018). "A Practical Beginner's Guide to Cyclic Voltammetry." Journal of Chemical Education **95**(2): 197-206.

Escapa, A., M. F. Manuel, A. Morán, X. Gómez, S. R. Guiot and B. Tartakovsky (2009). "Hydrogen Production from Glycerol in a Membraneless Microbial Electrolysis Cell." Energy & Fuels **23**(9): 4612-4618.

Fernández-Valverde, S., E. Ordonez-Regil, J. Cabanas-Moreno and O. Solorza-Feria (2010). "Electrochemical Behavior of Ni-Mo Electrocatalyst for Water Electrolysis." Journal of the Mexican Chemical Society **54**: 169-174.

Geim, A. K. and K. S. Novoselov (2007). "The rise of graphene." Nature Materials **6**(3): 183-191.

Gorby, Y. A., S. Yanina, J. S. McLean, K. M. Rosso, D. Moyles, A. Dohnalkova, T. J. Beveridge, I. S. Chang, B. H. Kim, K. S. Kim, D. E. Culley, S. B. Reed, M. F. Romine, D. A. Saffarini, E. A. Hill, L. Shi, D. A. Elias, D. W. Kennedy, G. Pinchuk, K. Watanabe, S. i. Ishii, B. Logan, K. H. Nealson and J. K. Fredrickson (2006). "Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms." Proceedings of the National Academy of Sciences **103**(30): 11358-11363.

He, Y. R., X. Xiao, W. W. Li, G. P. Sheng, F. F. Yan, H. Q. Yu, H. Yuan and L. J. Wu (2012). "Enhanced electricity production from microbial fuel cells with plasma-modified carbon paper anode." Phys Chem Chem Phys **14**(28): 9966-9971.

Imachi, H., S. Sakai, Y. Sekiguchi, S. Hanada, Y. Kamagata, A. Ohashi and H. Harada (2008). "Methanolinea tarda gen. nov., sp. nov., a methane-producing archaeon isolated from a methanogenic digester sludge." Int J Syst Evol Microbiol **58**(Pt 1): 294-301.

Jo, J. H., E. A. Kennedy and H. H. Kong (2016). "Research Techniques Made Simple: Bacterial 16S Ribosomal RNA Gene Sequencing in Cutaneous Research." J Invest Dermatol **136**(3): e23-e27.
Kadier, A., Y. Simayi, P. Abdeshahian, N. F. Azman, K. Chandrasekhar and M. S. Kalil (2016). "A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production." Alexandria Engineering Journal **55**(1): 427-443.
Kim, B. H., I. S. Chang, G. Cheol Gil, H. S. Park and H. J. Kim (2003). "Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell." Biotechnology Letters **25**(7): 541-545.
Kim, H. J., H. S. Park, M. S. Hyun, I. S. Chang, M. Kim and B. H. Kim (2002). "A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens." Enzyme and Microbial Technology **30**(2): 145-152.
Koch, C., K. J. Huber, B. Bunk, J. Overmann and F. Harnisch (2019). "Trophic networks improve the performance of microbial anodes treating wastewater." npj Biofilms and Microbiomes **5**(1): 27.
Li, X., C. Zeng, Y. Lu, G. Liu, H. Luo and R. Zhang (2019). "Development of methanogens within cathodic biofilm in the single-chamber microbial electrolysis cell." Bioresource Technology **274**: 403-409.
Liu, J., Y. Qiao, C. X. Guo, S. Lim, H. Song and C. M. Li (2012). "Graphene/carbon cloth anode for high-performance mediatorless microbial fuel cells." Bioresour Technol **114**: 275-280.
Liu, Y.-X., Y. Qin, T. Chen, M. Lu, X. Qian, X. Guo and Y. Bai (2021). "A practical guide to amplicon and metagenomic analysis of microbiome data." Protein & Cell **12**(5): 315-330.
Logan, B. E., R. Rossi, A. a. Ragab and P. E. Saikaly (2019). "Electroactive microorganisms in bioelectrochemical systems." Nature Reviews Microbiology **17**(5): 307-319.
Logan, B. E., M. J. Wallack, K.-Y. Kim, W. He, Y. Feng and P. E. Saikaly (2015). "Assessment of Microbial Fuel Cell Configurations and Power Densities." Environmental Science & Technology Letters **2**(8): 206-214.
Lu, X., X. Feng, J. R. Werber, C. Chu, I. Zucker, J. H. Kim, C. O. Osuji and M. Elimelech (2017). "Enhanced antibacterial activity through the controlled alignment of graphene oxide nanosheets." Proc Natl Acad Sci U S A **114**(46): E9793-e9801.
Mantel, N. (1967). "The Detection of Disease Clustering and a Generalized Regression Approach." Cancer Research **27**(2_Part_1): 209-220.
Marsili, E., D. B. Baron, I. D. Shikhare, D. Coursolle, J. A. Gralnick and D. R. Bond (2008). "Shewanella secretes flavins that mediate extracellular electron transfer." Proceedings of the National Academy of Sciences **105**(10): 3968-3973.
Mateo, S., P. Cañizares, F. J. Fernandez-Morales and M. A. Rodrigo (2018). "A Critical View of Microbial Fuel Cells: What Is the Next Stage?" ChemSusChem **11**(24): 4183-4192.
Maune, M. W. and R. S. Tanner (2012). "Description of Anaerobaculum hydrogeniformans sp. nov., an anaerobe that produces hydrogen from glucose, and emended description of the genus Anaerobaculum." Int J Syst Evol Microbiol **62**(Pt 4): 832-838.
McAllister, M. J., J.-L. Li, D. H. Adamson, H. C. Schniepp, A. A. Abdala, J. Liu, M. Herrera-Alonso, D. L. Milius, R. Car, R. K. Prud'homme and I. A. Aksay (2007). "Single Sheet Functionalized Graphene by Oxidation and Thermal Expansion of Graphite." Chemistry of Materials **19**(18): 4396-4404.
McIlroy, S. J., R. H. Kirkegaard, M. S. Dueholm, E. Fernando, S. M. Karst, M. Albertsen and P. H. Nielsen (2017). "Culture-Independent Analyses Reveal Novel Anaerolineaceae as Abundant Primary Fermenters in Anaerobic Digesters Treating Waste Activated Sludge." Front Microbiol **8**: 1134.
Menes, R. J. and L. Muxí (2002). "Anaerobaculum mobile sp. nov., a novel anaerobic, moderately thermophilic, peptide-fermenting bacterium that uses crotonate as an

electron acceptor, and emended description of the genus Anaerobaculum." Int J Syst Evol Microbiol **52**(Pt 1): 157-164.
Modin, O. and F. Aulenta (2017). "Three promising applications of microbial electrochemistry for the water sector." Environmental Science: Water Research & Technology **3**(3): 391-402.
Modin, O., R. Liébana, S. Saheb-Alam, B.-M. Wilén, C. Suarez, M. Hermansson and F. Persson (2020). "Hill-based dissimilarity indices and null models for analysis of microbial community assembly." Microbiome **8**(1): 132-132.
Modin, O., X. Wang, X. Wu, S. Rauch and K. K. Fedje (2012). "Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute solutions." Journal of Hazardous Materials **235-236**: 291-297.
Nemergut, D. R., S. K. Schmidt, T. Fukami, S. P. O'Neill, T. M. Bilinski, L. F. Stanish, J. E. Knelman, J. L. Darcy, R. C. Lynch, P. Wickey and S. Ferrenberg (2013). "Patterns and processes of microbial community assembly." Microbiol Mol Biol Rev **77**(3): 342-356.
Nevin, K. P., S. A. Hensley, A. E. Franks, Z. M. Summers, J. Ou, T. L. Woodard, O. L. Snoeyenbos-West and D. R. Lovley (2011). "Electrosynthesis of Organic Compounds from Carbon Dioxide Is Catalyzed by a Diversity of Acetogenic Microorganisms." Applied and Environmental Microbiology **77**(9): 2882-2886.
Niederdorfer, R., L. Fragner, L. Yuan, D. Hausherr, J. Wei, P. Magyar, A. Joss, M. F. Lehmann, F. Ju and H. Bürgmann (2021). "Distinct growth stages controlled by the interplay of deterministic and stochastic processes in functional anammox biofilms." Water Research **200**: 117225.
Pandit, S., Z. Cao, V. R. S. S. Mokkapati, E. Celauro, A. Yurgens, M. Lovmar, F. Westerlund, J. Sun and I. Mijakovic (2018). "Vertically Aligned Graphene Coating is Bactericidal and Prevents the Formation of Bacterial Biofilms." Advanced Materials Interfaces **5**(7): 1701331.
Pandit, S., K. Gaska, R. Kádár and I. Mijakovic (2021). "Graphene-Based Antimicrobial Biomedical Surfaces." Chemphyschem **22**(3): 250-263.
Park, S., N. Mohanty, J. W. Suk, A. Nagaraja, J. An, R. D. Piner, W. Cai, D. R. Dreyer, V. Berry and R. S. Ruoff (2010). "Biocompatible, robust free-standing paper composed of a TWEEN/graphene composite." Adv Mater **22**(15): 1736-1740.
Patel, G. B. and G. D. Sprott (1990). "Methanosaeta concilii gen. nov., sp. nov. ("Methanothrix concilii") and Methanosaeta thermoacetophila nom. rev., comb. nov.†." International Journal of Systematic and Evolutionary Microbiology **40**(1): 79-82.
Patil, S., F. Harnisch and U. Schröder (2010). "Toxicity Response of Electroactive Microbial Biofilms—A Decisive Feature for Potential Biosensor and Power Source Applications." ChemPhysChem **11**(13): 2834-2837.
Pumera, M. (2009). "Electrochemistry of graphene: new horizons for sensing and energy storage." The Chemical Record **9**(4): 211-223.
Quince, C., A. W. Walker, J. T. Simpson, N. J. Loman and N. Segata (2017). "Shotgun metagenomics, from sampling to analysis." Nature Biotechnology **35**(9): 833-844.
Raup, D. M. and R. E. Crick (1979). "Measurement of Faunal Similarity in Paleontology." Journal of Paleontology **53**(5): 1213-1227.
Rausch, P., M. Rühlemann, B. M. Hermes, S. Doms, T. Dagan, K. Dierking, H. Domin, S. Fraune, J. von Frieling, U. Hentschel, F.-A. Heinsen, M. Höppner, M. T. Jahn, C. Jaspers, K. A. B. Kissoyan, D. Langfeldt, A. Rehman, T. B. H. Reusch, T. Roeder, R. A. Schmitz, H. Schulenburg, R. Soluch, F. Sommer, E. Stukenbrock, N. Weiland-Bräuer, P. Rosenstiel, A. Franke, T. Bosch and J. F. Baines (2019). "Comparative analysis of amplicon and metagenomic sequencing methods reveals key features in the evolution of animal metaorganisms." Microbiome **7**(1): 133.
Rees, G. N., B. K. Patel, G. S. Grassia and A. J. Sheehy (1997). "Anaerobaculum thermoterrenum gen. nov., sp. nov., a novel, thermophilic bacterium which ferments citrate." Int J Syst Bacteriol **47**(1): 150-154.

Reguera, G., K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen and D. R. Lovley (2005). "Extracellular electron transfer via microbial nanowires." Nature **435**(7045): 1098-1101.
Reguera, G., K. P. Nevin, J. S. Nicoll, S. F. Covalla, T. L. Woodard and D. R. Lovley (2006). "Biofilm and nanowire production leads to increased current in Geobacter sulfurreducens fuel cells." Applied and environmental microbiology **72**(11): 7345-7348.
Rozendal, R. A., H. V. M. Hamelers, G. J. W. Euverink, S. J. Metz and C. J. N. Buisman (2006). "Principle and perspectives of hydrogen production through biocatalyzed electrolysis." International Journal of Hydrogen Energy **31**(12): 1632-1640.
Ruiz, O. N., K. A. Fernando, B. Wang, N. A. Brown, P. G. Luo, N. D. McNamara, M. Vangsness, Y. P. Sun and C. E. Bunker (2011). "Graphene oxide: a nonspecific enhancer of cellular growth." ACS Nano **5**(10): 8100-8107.
Saheb-Alam, S., F. Persson, B.-M. Wilén, M. Hermansson and O. Modin (2019). "Response to starvation and microbial community composition in microbial fuel cells enriched on different electron donors." Microbial Biotechnology **12**(5): 962-975.
Sakai, S., M. Ehara, I. C. Tseng, T. Yamaguchi, S. L. Bräuer, H. Cadillo-Quiroz, S. H. Zinder and H. Imachi (2012). "Methanolinea mesophila sp. nov., a hydrogenotrophic methanogen isolated from rice field soil, and proposal of the archaeal family Methanoregulaceae fam. nov. within the order Methanomicrobiales." Int J Syst Evol Microbiol **62**(Pt 6): 1389-1395.
Shin, H. J., K. A. Jung, C. W. Nam and J. M. Park (2017). "A genetic approach for microbial electrosynthesis system as biocommodities production platform." Bioresour Technol **245**(Pt B): 1421-1429.
Siegert, M., M. D. Yates, A. M. Spormann and B. E. Logan (2015). "Methanobacterium Dominates Biocathodic Archaeal Communities in Methanogenic Microbial Electrolysis Cells." ACS Sustainable Chemistry & Engineering **3**(7): 1668-1676.
Summers, Z. M., H. E. Fogarty, C. Leang, A. E. Franks, N. S. Malvankar and D. R. Lovley (2010). "Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria." Science **330**(6009): 1413-1415.
Tomazetto, G., S. Hahnke, D. Wibberg, A. Pühler, M. Klocke and A. Schlüter (2018). "Proteiniphilum saccharofermentans str. M3/6(T) isolated from a laboratory biogas reactor is versatile in polysaccharide and oligopeptide utilization as deduced from genome-based metabolic reconstructions." Biotechnol Rep (Amst) **18**: e00254.
Trego, A. C., P. G. McAteer, C. Nzeteu, T. Mahony, F. Abram, U. Z. Ijaz and V. O'Flaherty (2021). "Combined Stochastic and Deterministic Processes Drive Community Assembly of Anaerobic Microbiomes During Granule Flotation." Front Microbiol **12**: 666584.
Vallat, R. (2018). "Pingouin: statistics in Python." Journal of Open Source Software **3**: 1026.
Waite, D. W., M. Chuvochina, C. Pelikan, D. H. Parks, P. Yilmaz, M. Wagner, A. Loy, T. Naganuma, R. Nakai, W. B. Whitman, M. W. Hahn, J. Kuever and P. Hugenholtz (2020). "Proposal to reclassify the proteobacterial classes Deltaproteobacteria and Oligoflexia, and the phylum Thermodesulfobacteria into four phyla reflecting major functional capabilities." Int J Syst Evol Microbiol **70**(11): 5972-6016.
Wang, L., L. Singh and H. Liu (2018). "Revealing the impact of hydrogen production-consumption loop against efficient hydrogen recovery in single chamber microbial electrolysis cells (MECs)." International Journal of Hydrogen Energy **43**(29): 13064-13071.
Wang, Y.-Q., H.-X. Huang, B. Li and W.-S. Li (2015). "Novelly developed three-dimensional carbon scaffold anodes from polyacrylonitrile for microbial fuel cells." Journal of Materials Chemistry A **3**(9): 5110-5118.
Whittaker, R. H. (1960). "Vegetation of the Siskiyou Mountains, Oregon and California." Ecological Monographs **30**(3): 279-338.
Whittaker, R. H. (1972). "EVOLUTION AND MEASUREMENT OF SPECIES DIVERSITY." TAXON **21**(2-3): 213-251.

Woo, P. C., S. K. Lau, J. L. Teng, H. Tse and K. Y. Yuen (2008). "Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories." Clin Microbiol Infect **14**(10): 908-934.

Yates, M. D., P. D. Kiely, D. F. Call, H. Rismani-Yazdi, K. Bibby, J. Peccia, J. M. Regan and B. E. Logan (2012). "Convergent development of anodic bacterial communities in microbial fuel cells." The ISME Journal **6**(11): 2002-2013.

Yu, F., C. Wang and J. Ma (2016). "Applications of Graphene-Modified Electrodes in Microbial Fuel Cells." Materials (Basel) **9**(10).

Zhou, J., W. Liu, Y. Deng, Y.-H. Jiang, K. Xue, Z. He, J. D. V. Nostrand, L. Wu, Y. Yang, A. Wang and J. Handelsman (2013). "Stochastic Assembly Leads to Alternative Communities with Distinct Functions in a Bioreactor Microbial Community." mBio **4**(2): e00584-00512.