THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

A long journey from bioanode to biocathode

Effects of storage, starvation, and potential changes on biological electrodes

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Cover: A schematic of a bioanode and a biocathode. Several photos from different experiments.

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Abstract

Better utilization of renewable sources of energy and recovery of resources from waste streams are important challenges for researchers. Bioelectrochemical systems (BESs) are new technologies which e.g. could be used to produce green energy from waste sources or store renewable electricity as chemical fuels. They rely on microorganisms which can catalyse oxidation/reduction reactions on anodes/cathodes. BESs have a wide range of potential applications such as sensing, bioremediation, recovery of nutrients and metals, valorisation of wastewater organics, and production of energy carriers and other chemicals. However, further research is needed before these applications can be realized.

The goal of this thesis was to understand the effect of three different dynamic conditions and disturbances that bioanodes and biocathodes may encounter namely storage, starvation, and potential change. Storage and starvation are disturbances that can affect biological electrodes in all kinds of systems, and it is important to understand their consequences for performance. Changing electrode potential has been shown as a promising method for start-up of biocathodes from enriched bioanodes, but little is known about the long-term performance and changes in microbial community composition as the biocathode develops.

First, the possibility for storage of acetate-oxidizing bioanodes using refrigeration, glycerol freezing, and acetone dehydration was investigated. It was shown that storage of acetate-oxidizing bioanodes was possible. Bioanodes stored using refrigeration were the only electrodes that showed biological activity right after five weeks of storage. Then, starvation of acetate-and glucose-fed bioanodes was investigated. It was shown that the acetate- and glucose-fed bioanodes can survive 10 days starvation. However, the overall performance of the glucose-fed bioanodes deteriorated more after each starvation phase compared to the acetate-fed bioanodes. The conversion of acetate- and glucose-fed bioanodes to biocathodes was also compared. Immediately after the potential change, the glucose-fed bioanodes showed better cathodic activity but over time the performance converged. Then, we compared the conversion of bioanodes to biocathodes with direct start-up of biocathodes from a wastewater inoculum. Bare electrodes started-up faster compared to pre-enriched bioanodes. In the end, both types of enrichment procedures led to very similar biocathode communities, which were completely different from the bioanode communities. Indeed, for the microbial communities, it was a long journey from bioanode to biocathodes. Hydrogen appeared to be an important intermediate in the biocathode biofilms, therefore, start-up of biocathodes with pre-enriched hydrogenotrophic cultures was investigated. Hydrogenotrophic microorganisms could facilitate start-up of the biocathodes. All the microbial electrolysis cells inoculated by the enrichment cultures started to generate noticeable current directly after inoculation.

In summary, the bioelectrodes in our experiments were robust and could handle storage and starvation periods although the results depended on the experimental conditions, the feed, and the microbial communities. Conversion of bioanodes into biocathodes was less successful and resulted in a complete transition of the microbial community on the electrode. Start-up of biocathodes with hydrogen-oxidizing enrichment cultures was a more successful strategy.

Keywords: Bioelectrochemical system, Microbial fuel cell, Microbial electrolysis cell, Storage, Starvation, Bioanode, Biocathode, Hydrogenotrophic microorganisms, Mixed microbial communities

List of publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I. SAHEB ALAM, S., PERSSON, F., WILEN, B. M., HERMANSSON, M. & MODIN, O. (2015). Effects of storage on mixed-culture biological electrodes. *Scientific Reports,* 5**,** 18433.

II. SAHEB-ALAM, S., PERSSON, F., WILEN, B. M., HERMANSSON, M. & MODIN, O. Biodiversity, community composition and response to dynamic conditions of microbial electrodes enriched on acetate and glucose. (Manuscript)

III. SAHEB-ALAM, S., SINGH, A., HERMANSSON, M., PERSSON, F., SCHNÜRER, A., WILEN, B. M., & MODIN, O. (2018). Effects of start-up strategies and electrode materials on carbon dioxide reduction on biocathodes. *Applied and Environmental Microbiology* 84(4).

IV. SAHEB-ALAM, S., PERSSON, F., WILEN, B. M., HERMANSSON, M. & MODIN, O. A variety of hydrogenotrophic enrichment cultures catalyse cathodic reactions. (Manuscript)

Other publications not included in this thesis

MODIN, O., SAHEB ALAM. S., PERSSON, F. & WILEN, B. M. (2015). Sorption and Release of Organics by Primary, Anaerobic, and Aerobic Activated Sludge Mixed with Raw Municipal Wastewater. *Plos One,* 10.

SAHEB-ALAM, S., PERSSON, F., WILEN, B. M., HERMANSSON, M. & MODIN, O. (2017). Electricity-driven microbial production of polyhydroxybutyrate and soluble organics under feast/famine conditions, *IWA 10th International conference on biofilm reactors, University College Dublin, Ireland,* (Conference abstract)

Contribution report

The author of this thesis has made the following contributions:

Paper I: I participated in planning the study, performed the experimental work, carried out the analysis of the data (except microbial community analysis), and wrote the first draft of the paper and contributed to the further revisions till the final manuscript .

Paper II: I provided a minor contribution to the experimental work, carried out analysis of the reactor data, and wrote the first draft of the paper.

Paper III: I planned the experiment, performed the experimental work (except TRFLP), carried out analysis of the data, and wrote the first draft of the paper and contributed to the further revisions till the final manuscript.

Paper IV: I planned the experiment, performed all the experimental work, carried out analysis of the data, and wrote the first draft of the paper and contributed to the further revisions till the final manuscript.

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Terminology used in this thesis

Agen$_{MEC}$: MEC inoculated by microorganisms from Agen1 enrichment cultures (paper IV).

Agen: Homoacetogens. Acetogens pre-enriched using hydrogen as an only electron donor (paper IV).

Anodic current: Flow of electrical charge into a working electrode as a result of an oxidation on the electrode surface. Anodic current is shown by positive current.

BES: Bioelectrochemical system.

Cathodic current: Flow of electrical charge out of working electrode which is leading to a reduction on the electrode surface. Cathodic current is shown by negative current.

Current density: Electrical current per unit area of electrode surface.

CV: Cyclic Voltammetry.

Electrode potential: Electromotive force between the electrode of interest and a reference electrode (e.g. SHE).

MEC: Microbial electrolysis cell.

MFC: Microbial fuel cell.

MgenA: Acetoclastic methanogens. Methanogens pre-enriched using acetate as an only electron donor (paper IV).

MgenA$_{MEC}$: MEC inoculated by microorganisms from MgenA1 enrichment cultures (paper IV).

MgenH: Hydrogenotrophic methanogens. Methanogens pre-enriched using hydrogen as an only electron donor (paper IV).

MgenH$_{MEC}$: MEC inoculated by microorganisms from MgenH1 enrichment cultures (paper IV).

NR: Hydrogenotrophic nitrate-reducers pre-enriched using hydrogen as an only electron donor (paper IV)

SHE: Standard Hydrogen Electrode. Standard electrode potential for SHE is equal to zero volts.

SR: Hydrogenotrophic sulphate-reducers pre-enriched using hydrogen as an only electron donor (paper IV).

SR$_{MEC}$: MEC inoculated by microorganisms from SR1 enrichment cultures (paper IV).

OTU: Operational taxonomic unit (Paper I, III)

SV: Sequences variant, analogous to OTU (Paper II, IV)

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Chapter 1

1 Introduction

Limited resources of fossil fuels and their negative impact on the Earth has motivated researchers, in both academia and industry, to search for renewable green energies, which contribute less to environmental stresses such as climate change and global warming. In 2014, only 19% of the global energy consumption was provided from renewable energy sources. These sources of energy are mainly providing energy for heating, power generation and transportation. The major part, 9%, was provided from biomass (REN21, 2014). Therefore, developing new technologies for harnessing renewable energy is a great challenge for society. Recently, bioelectrochemical systems (BESs) have received a lot of attention by researchers. BESs are new techniques for storing energy or converting chemical energy present in biomass to more valuable forms such as electricity, fuels and biogas. They are based on the use of living microorganisms as catalysts for electrochemical reactions. The research field has developed recently and the technology is still surrounded by lots of unknowns. Very few large-scale applications of BES for energy storage or energy production exists. However, BESs could potentially play a key role for renewable energy production in the future. Research on BES can help to develop this technique for future generations.

1.1 Electron transferring mechanisms in BESs

BESs are relying on microorganisms and their abilities in electron transferring which is a vital process for sustaining their lives. Microorganisms can gain energy by oxidizing compounds with low reduction potential (electron donors) and reducing compounds with high reduction potential (electron acceptors). Recent studies (Logan et al., 2006, Rabaey et al., 2011) show the capability of microorganisms to use a solid-state electrode as electron donor or electron acceptor. Such an ability of microorganisms can be used in BESs for catalysing different reactions on electrodes. Lithgow et al. (1986) showed that using a chemical mediator could facilitate electron transfer from microorganisms to a solid electrode. However, new research have moved towards mediator-less BESs. In these technologies bacteria have different mechanisms for transferring electrons to or from electrodes. Some bacteria have electrochemically active redox proteins on their outer membrane which can transfer electrons directly to the electrode. Kim et al. (1999) showed that *Shewanella purefaciens* could oxidize lactate and transfer electron to solid electrode in absence of a mediator. Reguera et al. (2005) showed that *Geobacter sulfurreducens* produced conductive pili, nanowires, which could be used for transferring electrons from the cell surface to the surface of Fe(III) oxides. Rabaey et al. (2005) showed that *Pseudomonas aeruginosa* produced soluble redox mediators, electron shuttles, which could be used by themselves or by other bacteria to enhance electron transfer between the cells and solid electrodes.

Oxidation of acetate is an important process on biological anodes and it is readily used by a wide range of microorganisms. Different microorganisms can utilize acetate differently. Many bacteria can assimilate acetate as a carbon source for producing cell materials. Acetate can also be an energy source for microorganisms that can oxidize acetate anaerobically or aerobically. Microorganisms such as *Geobacter sp.* (Bond and Lovley, 2003, Cord-Ruwisch et al., 1998, Nevin et al., 2008) and *Rhodopseudomonas* (Xing et al., 2008) sustain their life while oxidizing acetate anaerobically. Furthermore, methanogenic archaea can produce methane while utilizing

acetate as an electron donor for obtaining energy (Thauer et al., 2008). In the majority of laboratory studies on microbial fuel cells, acetate has been used as feed to the biological anode (Bond et al., 2002). However, also when more complex substrates are fed to the anode, acetate appears to be important for current generation. Freguia et al. (2008) showed that when bioanodes in a MFC were fed with glucose, first, glucose was fermented to acetate and hydrogen. Then, both hydrogen and acetate served as the actual electron donors for electricity-generating microbes.

1.2 Potential applications of bioelectrochemical systems

BESs could be used to generate e.g. electrical power, biochemical compounds and biogas (Rozendal et al., 2008a). Microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) are two types of BES, which could be used for recovering the biochemical energy present in organic wastes (Modin and Gustavsson, 2014). MFCs generate electrical current by degrading organic wastes on a bioanode (Logan et al., 2006). In MFCs, anode respiring microorganisms oxidize organic substrates, such as acetate, and transfer electrons to the anode to obtain energy (Torres et al., 2010). Then, electrons travel through an external circuit, where electrical current is harvested, to a typically aerated cathode (Liu et al., 2005a) (Figure 1A), or a biocathode where microorganisms catalyse reduction reactions such as denitrification (Clauwaert et al., 2007) (Figure 1B).

Recently, research about BESs has shifted from electricity generation to valuable chemical production. In MEC, which can be used for different purposes, microorganisms catalyse reactions on bioanodes (Figure 1C) or biocathodes (Figure 1D) or on both electrodes simultaneously (Figure 1B). Microorganisms oxidize organic compounds and deliver electrons to the anode, then, other microorganisms present on the cathode harvest electrons to reduce soluble compounds. In MECs, the electrons are given an extra energy boost by applying an external input voltage, which can be provided by renewable sources such as solar energy. MECs can produce valuable bio-gases, such as hydrogen (Rozendal et al., 2006, Liu et al., 2005b, Oh and Logan, 2005, Escapa et al., 2016) and methane (Villano et al., 2011, Cheng et al., 2009) as well as valuable chemicals such as ethanol (Steinbusch et al., 2010), acetate (Marshall et al., 2013, Nevin et al., 2010, Marshall et al., 2012), and caproate (Van Eerten-Jansen et al., 2013a).

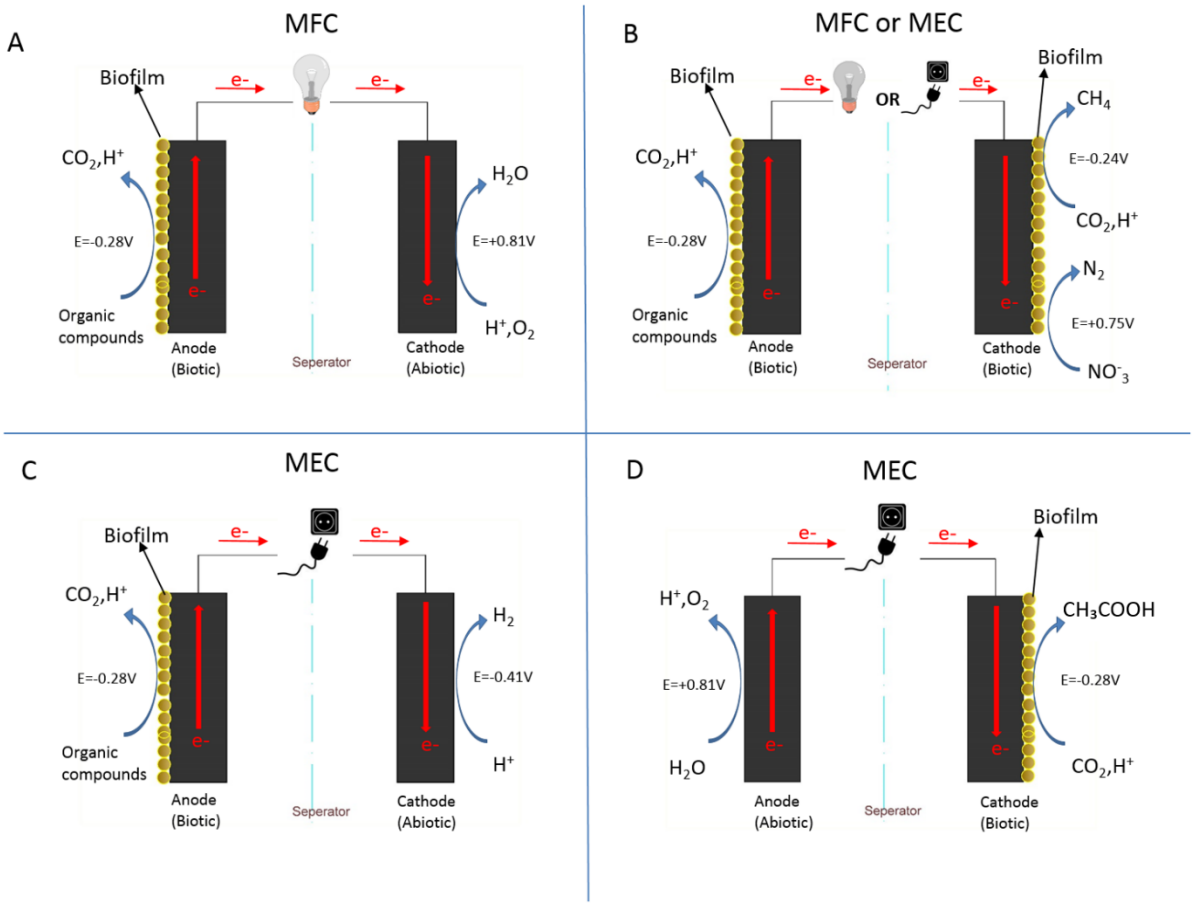

Figure 1. Schematic figure of different possible set-ups of microbial fuel cells (MFC) and microbial electrolysis cells (MEC) in which microorganisms can grow either on the anode or the cathode or both. (A) MFC with bioanode and abiotic O_2-reducing cathode, (B) MFC with denitrifying cathode or MEC with CH_4-producing cathode, (C) MEC with bioanode and abiotic H_2-producing cathode, and (D) MEC with abiotic anode and acetate-producing biocathode. The standard reduction potentials (E) at pH 7 for the electrochemical reactions are shown.

1.3 Effect of dynamic conditions on bioanodes and biocathodes

BES potentially can be used for different purposes and the concept is beneficial since the processes can be sustained with a very low energy input. However, one challenge for designing such a system in larger scale is to provide favourable conditions (e.g. pH, temperature, anaerobic conditions, feed, and applied potential) constantly for a biofilm that is growing on the electrode. During operation of bioanodes and biocathodes there are different dynamic conditions and disturbances that biological electrodes may encounter. These disturbances can affect biological electrodes in all kinds of systems, and it is important to understand their consequences for performance. Storage and starvation are common disturbances that bioelectrodes may encounter during operation. Moreover, changing electrode potential can affect the performance and microbial community composition of biological electrodes. However, it has also been shown as a promising method for start-up of biocathodes from enriched bioanodes.

1.3.1 Storage

Storage of bioelectrodes is challenging and potentially cause negative impact on the electrocatalytic activity of the electroactive biofilm. Understanding the consequences of storage becomes important when the BES is out of use for a specific period. The storage of pure cultures using cryopreservation and drying methods have been studied before (Prakash et al., 2013, Morgan et al., 2006, Suslow and Schroth, 1981). During the prolonged storage, a

large fraction of microbial cells will die. However, having a high initial concentration (>10^7 cell mL^{-1}) some cell will survive and maintain the culture for future use (Morgan et al., 2006). This is an ideal situation for pure culture, however, for mixed culture the change in relative abundance of different members of the microbial communities is expected. Very few have studied the effect of storage on anaerobic mixed cultures. Bae et al. (1995) showed that the anaerobic sludge can be stored in room temperature for 10 months. Castro et al. (2002) showed the re-activation process for anaerobic sludge stored in room temperature or refrigerated conditions (4°C) is faster than freezing (-20°C) or freeze-drying. However, there is a lack of knowledge about the storage of electrochemically active biofilms.

1.3.2 Starvation

In all environmental biotechnologies, the microorganisms may from time to time be exposed to periods of starvation. Sometimes MFCs need to be inactive e.g. between experimental run, because of technical problem or maintenance. For example, a MFC used as a BOD-sensor may be taken out of service temporarily or it may be exposed to feed water with very low concentrations of biodegradable organics. During such periods, electrogenic biofilms could die due to lack of substrate and it could lose its electrical contact with the electrode. Therefore, it is essential that the viability of the microorganisms is preserved in order to maintain the performance of the BESs. It was previously shown that the acetate-oxidizing MFC can survive a starvation period for only 5 days when it was operated in open-circuit while closed-circuit conditions improved the resilience up to 11 days (Ruiz et al., 2015). However, there is a lack of knowledge about the ability of different types of electroactive biofilms to recover after starvation and the general performance of BESs after starvation periods.

1.3.3 Conversion of a bioanode to a biocathode

Variation in potential of a working electrode can happen either due to malfunctioning (e.g. of the reference electrode) or as part of experimental design. This disturbance affects the catalytic activity of the biofilm and consequently the relative abundance of electroactive microorganisms. The technique of changing the potential of a bioanode was previously shown as a promising method for start-up of biocathodes (Pisciotta et al., 2012, Rozendal et al., 2008b) since it is more difficult to enrich biocathodes than to enrich bioanodes (Jeremiasse et al., 2010) and the process usually needs several months. Converting a bioanode into a biocathode is challenging because it requires specific types of microorganisms that can catalyse both anodic and cathodic reactions. Rozendal et al. (2008b) showed that hydrogen-oxidizing bioanodes can catalyse hydrogen production when the polarity of the electrodes was reversed. Liang et al. (2014) showed that lowering the cathode potential from -0.9 V to -1.3 V versus a saturated calomel electrode (SCE) and increasing bicarbonate concentration from 0.05 mol/d to 0.5 mol/d enhanced the cathodic reactions on the biocathodes pre-enriched as the hydrogen-oxidizing bioanodes. van Eerten-Jansen et al. (2015) showed that methanogens mainly used hydrogen and acetate produced by the biocathode, instead of the cathode itself, to reduce carbon dioxide to methane. However, we need more information about the change in microbial community composition when a bioanode is converted into a biocathode, and the strategy should be compared to other strategies for biocathode enrichment.

Hydrogenotrophic microorganisms appear to play a key role on biocathodes (Rozendal et al., 2008b, Liang et al., 2014). Therefore, another strategy to facilitate start-up is to pre-enrich a suitable microbial community and use as inoculum instead of using active anodic biofilm. Hydrogenotrophic microorganisms may catalyse the reactions taking place on the cathode because they contain hydrogenases that can catalyse the reversible reaction of $2H^+ + 2e^- \leftrightarrow H_2$. It was previously shown that purified hydrogenases can enhance the hydrogen production on a carbon electrode (Vignais et al., 2001, Lojou and Bianco, 2004, Vincent et al., 2007). However, the enzymes are very unstable and usually lose their catalytic activity over the time. Therefore, using whole cells can help to improve the stability of the system and enhance the cathodic reactions. In several previous studies, detailed information about the microbial community composition in the inoculum and studies on which microorganisms in the inoculum are retained on the biocathode are lacking. Moreover, many different groups of hydrogenotrophic microorganisms exist and there is a lack of knowledge about how widespread the ability to catalyse biocathode reactions is.

In summary, MECs rely on the electrocatalytic ability of microorganisms. The electroactive biofilm is usually sensitive to disturbances such as storage, starvation, and potential variations. In this thesis, I investigated the effect of storage and starvation on bioanodes as well as the possibility of converting bioanodes to biocathodes by changing the potential. Additionally, different hydrogenotrophic enrichment cultures were used to test the possibility of enhancing start-up of biocathodes.

Chapter 2

2 Goals of the thesis

The main goal of this thesis was to understand the effects of different disturbances including storage, starvation and potential changes on microbial bioelectrodes. The specific objectives were to:

- Assess the effect of storage on microbial community composition and performance of acetate-fed bioanodes (paper I).
- Assess the effect of starvation on the performance of acetate- and glucose-fed bioanodes (paper II).
- Test the hypothesis that start-up of biocathodes is facilitated by pre-enriched bioanodes (paper III & II).
- Evaluate the start-up of biocathodes using hydrogenotrophic enrichment cultures (paper IV).

In paper I, we investigated the possibility of storing bioelectrodes. We tried to find a suitable method for storing acetate-fed bioanodes for a period of time while the MFC/MEC is out of use e.g. between experimental run, because of technical problem or maintenance. Previous studies (Castro et al., 2002, Lv et al., 2013) have shown the possibility of storing different mixed microbial communities. However, this study was the first to investigate storage of biological electrodes.

In paper II, we investigated the effect of starvation on acetate- and glucose-fed bioanodes. MFCs/MECs may experience starvation periods when out of use or when exposed to feed water with very low concentrations of organic substrates. During such periods, the electrogenic biofilm could die due to lack of substrate and it could lose its electrical contact with the anode. In this study we compared the ability of acetate- and glucose-fed MFC to withstand 10-d starvation periods under both open- and closed-circuit conditions.

In papers II-III, we investigated the effect of changing the bioanode potential for start-up of biocathodes. Recent studies showed that it is more difficult to produce microorganisms on cathodes compared to obtain microorganisms on anodes. Thus, pre-enrichment of bioanodes is one possible solution (Rozendal et al., 2008b). However, very little is known about the performance and efficiency of the mentioned method. Also, very little is generally known about the community structure of the microorganisms on the biocathode. The strategy was to enrich bioanodes and then switch to a lower potential to make the electrodes function as biocathodes. In paper III, we compared this strategy to another strategy which was to directly enrich biocathodes on bare electrodes from a wastewater inoculum. We also tried to find a suitable electrode material that can help to enhance the performance of biocathodes. Three different electrode materials were examined: graphite foil, carbon felt and graphite rod.

In paper IV, we investigated the ability of four hydrogenotrophic cultures, enriched using different electron acceptors, to catalyse cathode reactions. One acetate-oxidizing methanogenic culture was also tested. The experiments in paper IV were designed based on the finding in paper III, that H_2 was likely playing an important role as an intermediate on the biocathodes. The strategy was to pre-enrich a hydrogenotrophic microbial community and use as inoculum

for start-up of MECs. Other researchers have also suggested that hydrogenotrophic microorganisms are important on biocathodes and some studies have used pre-enriched H_2-oxidizing bioanodes (Rozendal et al., 2008b, Liang et al., 2014) and hydrogenotrophic methanogens (Villano et al., 2010) with promising results. However, detailed information about the microbial community composition in the inoculum and studies on which microorganisms in the inoculum are retained on the biocathode are lacking. Many different groups of hydrogenotrophic microorganisms exist and there is a lack of knowledge about how widespread the ability to catalyse biocathode reactions is.

2.1 Structure of the thesis

The thesis explains four different and connected projects that I have carried out during the five years of my doctoral studies. Papers I-II are demonstrating the effect of storage, starvation and feed on the performance of bioanodes in MECs/MFCs. The effects of changing the potential of bioanodes and converting them to biocathodes is explained in paper II & III. Paper IV is investigating the ability of hydrogenotrophic microorganisms for catalysing the cathodic reactions and facilitating start-up of biocathodes.

Chapter 3 explains the material and methods that we used in all experiments and reasons for selecting them. The bulk of this thesis, chapter 4, consists of results and discussions about the experimental measurements. Chapter 5 highlights the main conclusions of the thesis. Chapter 6 gives some suggestions for future research. Finally, chapter 7 discusses four potential practical applications of MECs/MFCs.

Chapter 3

3 Methods

3.1 Experimental set-ups

In this section, the experimental set-up and methodology used in each individual study is described briefly. More detailed information can be found in the materials and methods section of each paper. A schematic of the different MECs/MFCs described in this thesis is shown in Figure 2.

3.1.1 Acetate-oxidizing microbial electrolysis cell (paper I)

A plexiglas single-chamber microbial electrolysis cell (MEC) containing 16 graphite rod electrodes (8 anodes and 8 cathodes) was set-up to investigate storage of anaerobic acetate-oxidizing bioanodes (Figure 3). The reactor was first operated for 20 days until steady-state bioelectrochemical activity was achieved. Then the electrodes were stored for five weeks using different methods. After that, the electrodes were again inserted into the reactor, which was operated for another 15 days to evaluate the effects of storage. Three different storage methods were investigated (Figure 3): (1) storage by submerging the electrodes in nutrient medium in a refrigerator (+4°C), (2) storage by submerging the electrodes in 10% glycerol solution followed by freezing at -70°C, and (3) acetone dehydration followed by storage at room temperature according to (Lv et al., 2013). Each storage method was evaluated using duplicate anodes. Two anodes that had been harvested for microscopy and microbial community analysis at the time of storage were replaced by two new graphite rod anodes inserted into the reactor. Further details about experimental set-up are explained in paper I (Saheb Alam et al., 2015).

One single chamber MEC was used in this study in order to provide conditions that made it possible to produce replicate electrodes; all being exposed to the same environmental conditions and microbial inoculum in the bulk liquid in the reactor. Producing replicate electrodes before storage was important because the goal was to compare the performance of different storage methods and to evaluate the microbial communities on the electrodes before storage and after re-start of the reactor. A drawback of using a single-chamber reactor is that hydrogen produced on the cathode electrode can be oxidized on the anode, which could affect current generation and microbial community on the anodes. Moreover, microorganisms from communities developed on one type of electrode in the single-chamber reactor could act as inoculum for other types of electrodes (e.g. anodes vs cathodes, or electrodes exposed to different storage methods). This could have an effect on the microbial community composition.

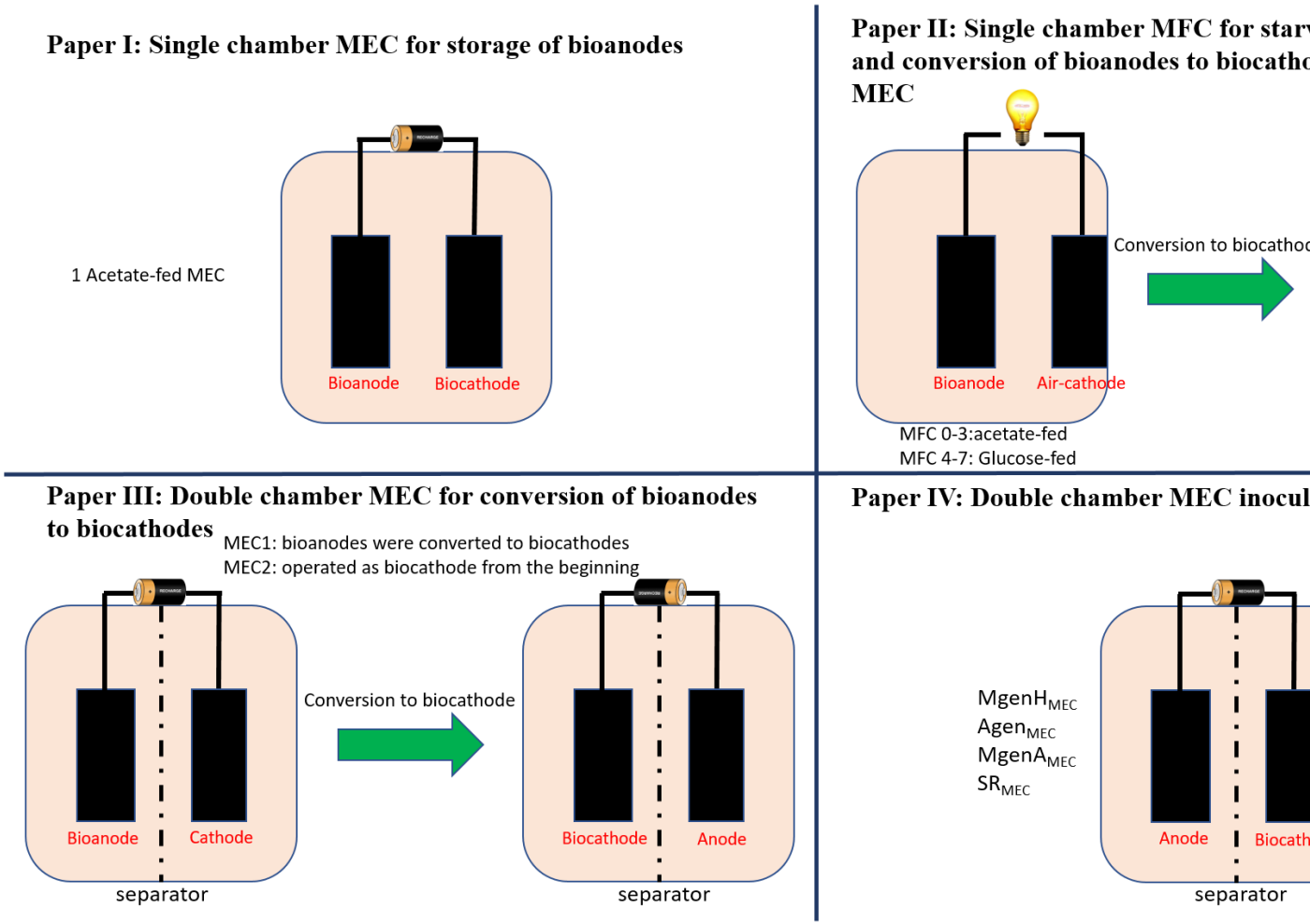

Figure 2. Schematic of MECs/MFCs operated in different experiments in this thesis.

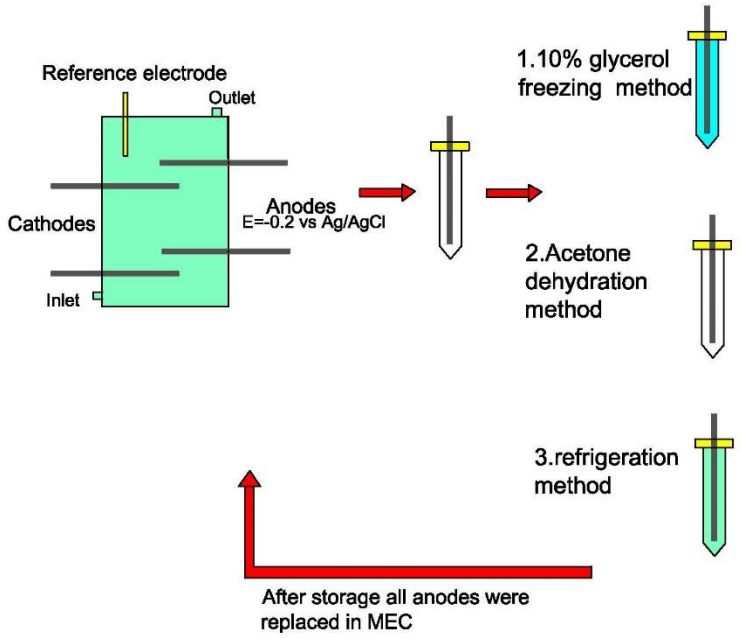

Figure 3. Different methods for storing bioanodes in MEC (paper I). Totally 16 graphite rod electrodes were used, 8 as anode and 8 as cathode, in 4 different rows inside the MEC. In the figure, each electrode represents one row which consisted of 4 electrodes.

3.1.2 Acetate- and glucose-oxidizing microbial fuel cells (paper II)

Eight single-chamber air-cathode MFCs were constructed. Each MFC consisted of a cylindrical compartment with a length of 5 cm and a diameter of 4 cm. The anode was a 4-cm diameter carbon cloth (AvCarb 1071 HCB, Fuelcellearth.com) placed on one side of the cylindrical compartment. The carbon cloth was pressed again a sheet of graphite foil and a stainless-steel mesh functioning as current collector. The cathode was a 4-cm diameter gas-diffusion electrode placed on the other side of the cylindrical compartment. A glass fiber filter (Munktell) pressed against the liquid-side of the cathode served as a separator between the cathode catalyst layer and the bulk liquid. A schematic of the MFC design is shown in Figure 4.

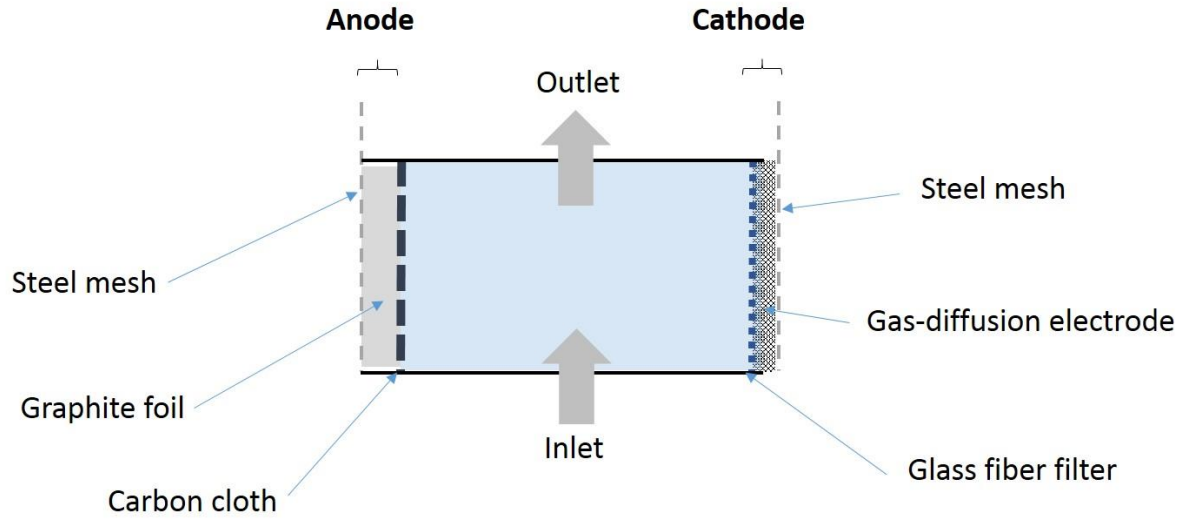

Figure 4. Schematic of the MFCs set-up.

Eight MFCs (numbered from 0 to 7) were placed in four hydraulic loops (A-D). Thus, each hydraulic loop consisted of two MFCs (Figure 5). The total liquid volume of one hydraulic loop (including tubing and two MFCs) was 160 mL. The reactors were fed in a semi-continuous mode. Every day, each loop was fed with approximately 46 mL nutrient medium. During feeding, liquid medium was simultaneously wasted from the loop. Acetate was added to the nutrient medium fed to loops A-B while loops C-D were fed with glucose. Unless otherwise specified, the carbon source concentration was 1.28 g/L chemical oxygen demand (COD) (1.64 g/L CH_3COONa or 1.2 g/L $C_6H_{12}O_6$). Each loop was inoculated with 15 mL of activated sludge collected from a municipal wastewater treatment plant. A 1000 ohm resistor was placed between the anode and the cathode for each MFC and the voltage across the resistor was recorded every 30 s. After 15 days of operation, 100 ohm resistors were used in order to increase current generation. Further details about electrodes, loops set-up, and systematic losses in the MFCs are explained in paper II.

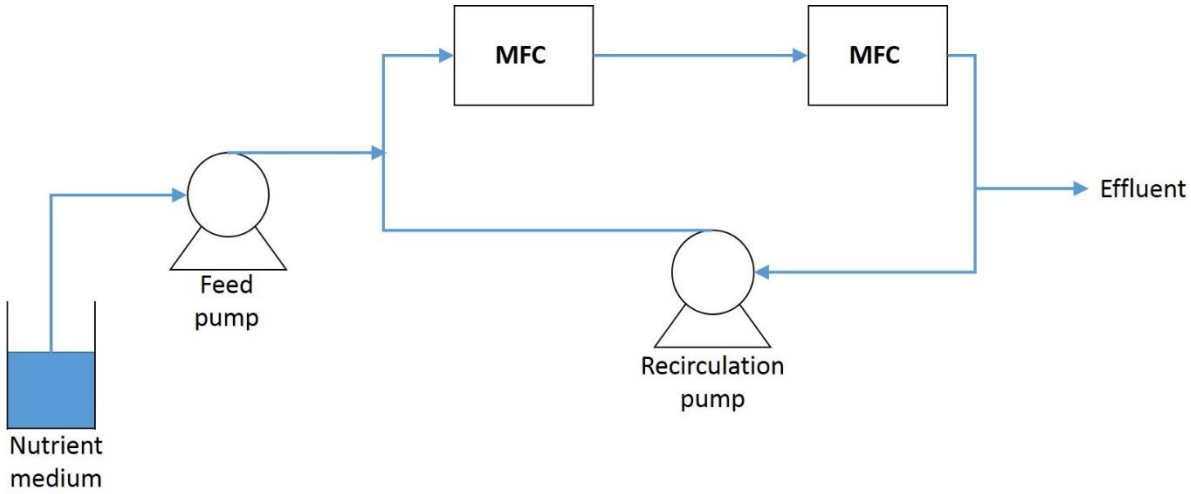

Figure 5 . Schematic of a hydraulic loop containing two microbial fuel cells (MFC).

Three starvation tests were carried out during the experiment. During a starvation test, organic carbon was excluded from the nutrient medium for 10 consecutive days. The MFCs were

operated with either open-circuit conditions or with a 100 ohm resistor placed between the anode and cathode (Table 1).

Table 1. Overview of the position and operational conditions for the eight MFCs.

MFC	Loop	Carbon source	Starvation 1	Starvation 2	Starvation 3
			Day 25-34	Day 45-54	Day 74-83
0[a]	A	Acetate	100 Ω	Open-circuit	Open-circuit
1[a,b]	A	Acetate	100 Ω	Open-circuit	Open-circuit
2[a]	B	Acetate	Open-circuit	100 Ω	100 Ω
3[c]	B	Acetate	Open-circuit	100 Ω	100 Ω
4[a]	C	Glucose	100 Ω	Open-circuit	Open-circuit
5[a,b]	C	Glucose	100 Ω	Open-circuit	Open-circuit
6[a]	D	Glucose	Open-circuit	100 Ω	100 Ω
7[c]	D	Glucose	Open-circuit	100 Ω	100 Ω

[a]These MFCs were harvested for microbial community analysis.
[b]These MFCs were tested for alternative carbon sources in the end of the experiment.
[c]The anodes in these MFC were converted to biocathodes in the end of the experiment.

In two of the MFCs (3 and 7), the possibility to convert the bioanodes into biocathodes was tested. After 127 days of operation as MFCs, the reactors were converted. The gas-diffusion cathode was replaced with another cylindrical compartment harbouring a 4-cm long, 3-mm diameter platinum-coated titanium wire (Magneto Special Anodes Bv). The two compartments were separated by a glass-fibre filter and both were filled with nutrient medium. A silver/silver chloride reference electrode, fabricated as described previously (Modin et al., 2017), was placed in the compartment hosting the biological electrode. Nutrient medium was also circulated through this compartment at a flow rate of 40 mL/min. The biological electrode was controlled at a potential of -0.65 V vs SHE in order to convert it into a biological cathode. After 128 days of operation, the potential was lowered to -0.8 V vs SHE. The reactors were operated in biocathode mode for a total of 203 days.

3.1.3 Microbial electrolysis cell for converting bioanodes to biocathodes (paper III)

Two plexiglas double-chamber MECs were operated as batch reactors to investigate the effect of reversing the electrode potential on start-up of the biocathodes. In this experiment, a double-chamber reactor configuration was used to prevent the chemicals that are produced at the biological cathodes from being oxidized at the anodes. A cation exchange membrane was installed between two chambers to prevent ions (e.g. acetate) to travel through the membrane to the anode compartment (Figure 6). Gas bags were installed at the top of the cathode compartments for collecting the produced biogas. At the start of the experiment, the MECs were inoculated with 20 mL of a mixture of raw municipal wastewater and anaerobic digester sludge of a ratio of 9:1 and then filled up to 750 mL with a nutrient medium. At the end of the experiment, 10 mM 2-bromoethanesulfonate, which inhibits methanogens, was added to the nutrient medium. Further details about the experimental set-up are provided in paper III.

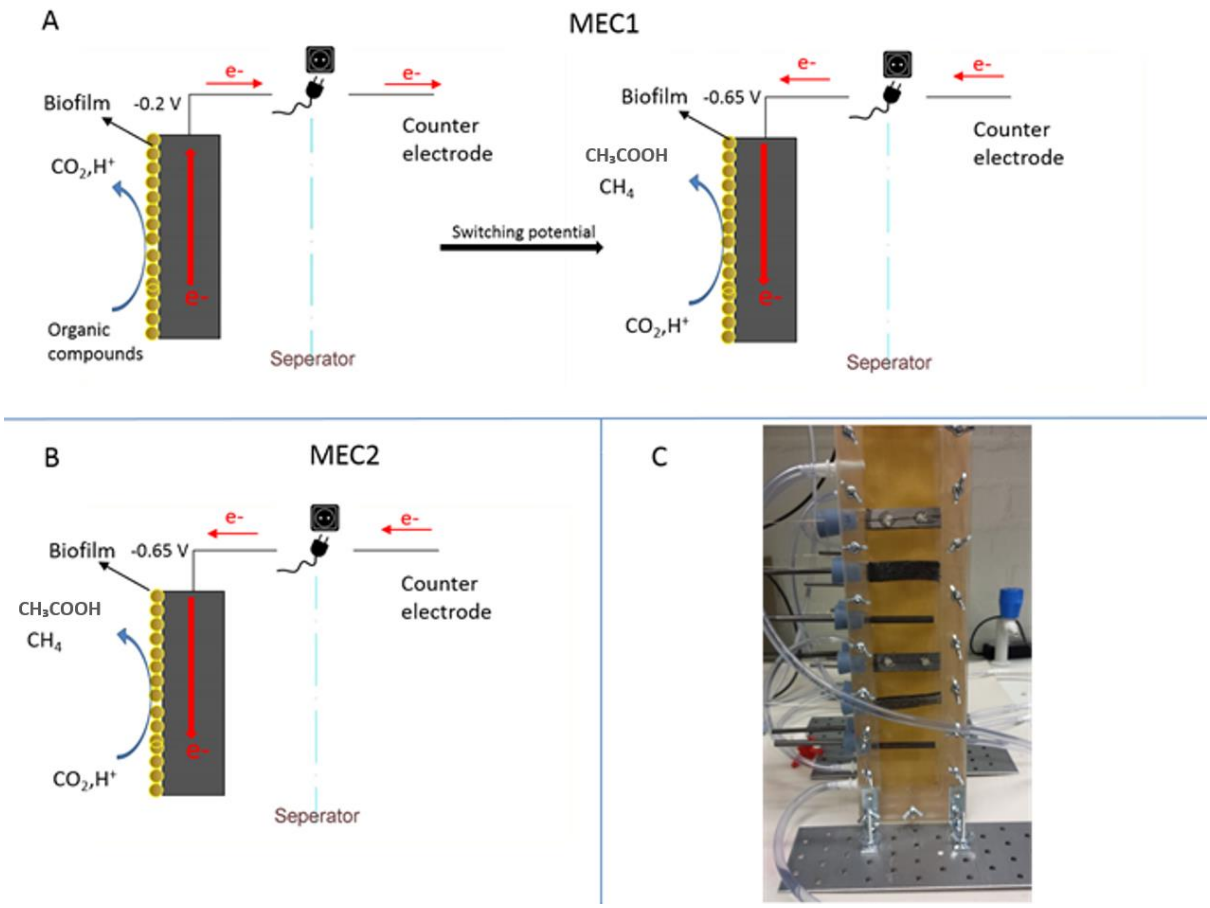

Figure 6. Schematic figure of different strategies that were used in paper III. (A) MEC1 and (B) MEC2. (C) MECs photo from a side which illustrates the different electrode materials used in the MECs. The orange sheet is the cation exchange membrane. The counter electrode is not shown in the schematic.

The MECs were operated with two different start-up strategies (Figure 6). The purpose was to investigate the capability of an acetate-oxidizing biofilm to catalyse cathodic reactions after reversing the electrode potential and to facilitate the start-up of biocathodes compared to conventional start-up of biocathodes. The two strategies were: (1) electrodes in MEC1 were enriched as acetate-oxidizing bioanodes by controlling the potential at -0.2 V vs SHE for the first 71 days. Then, acetate was removed from nutrient medium and the potential was decreased to -0.65 V vs SHE, where the electrodes worked as cathodes during the rest of the experiment. (2) MEC2 was operated by controlling the cathode potential at -0.65 V vs SHE from the start of the experiment.

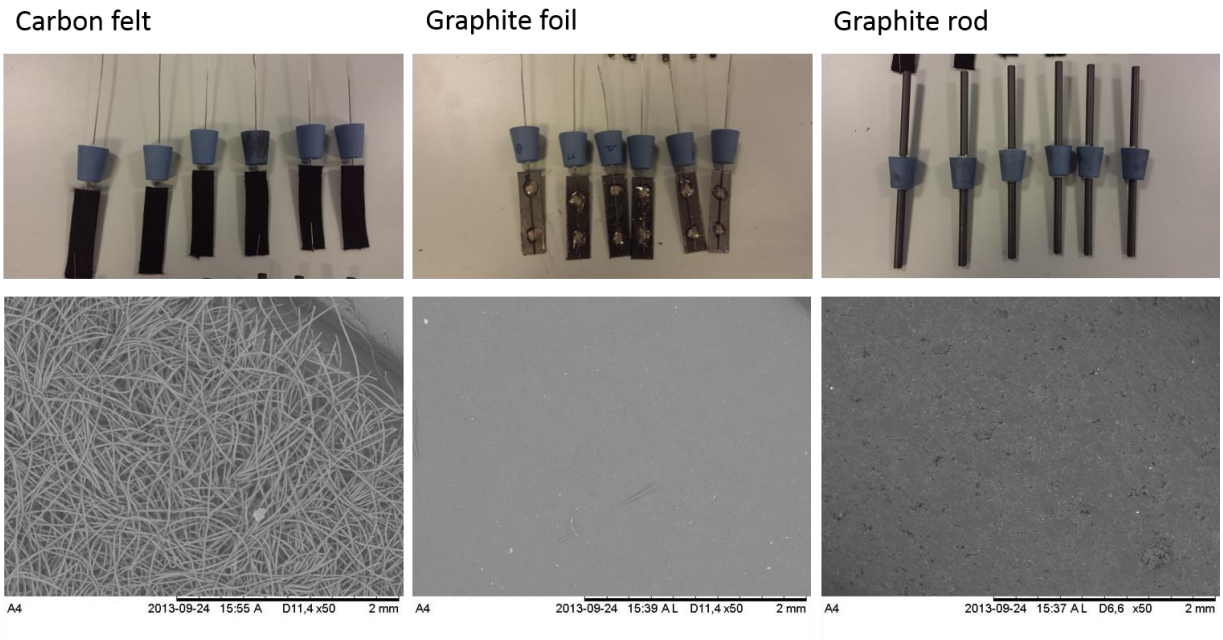

Figure 7. Different electrode materials that were investigated in MECs, paper III. The SEM images show the differences in material structure. Carbon felt consists a lot of carbon fibres while graphite rod and graphite foil has smoother surface.

Three different electrode materials were used in this study: graphite foil, carbon felt and graphite rod (Figure 7). Figure 8 shows the time plan with different experimental actions that took place in both MECs.

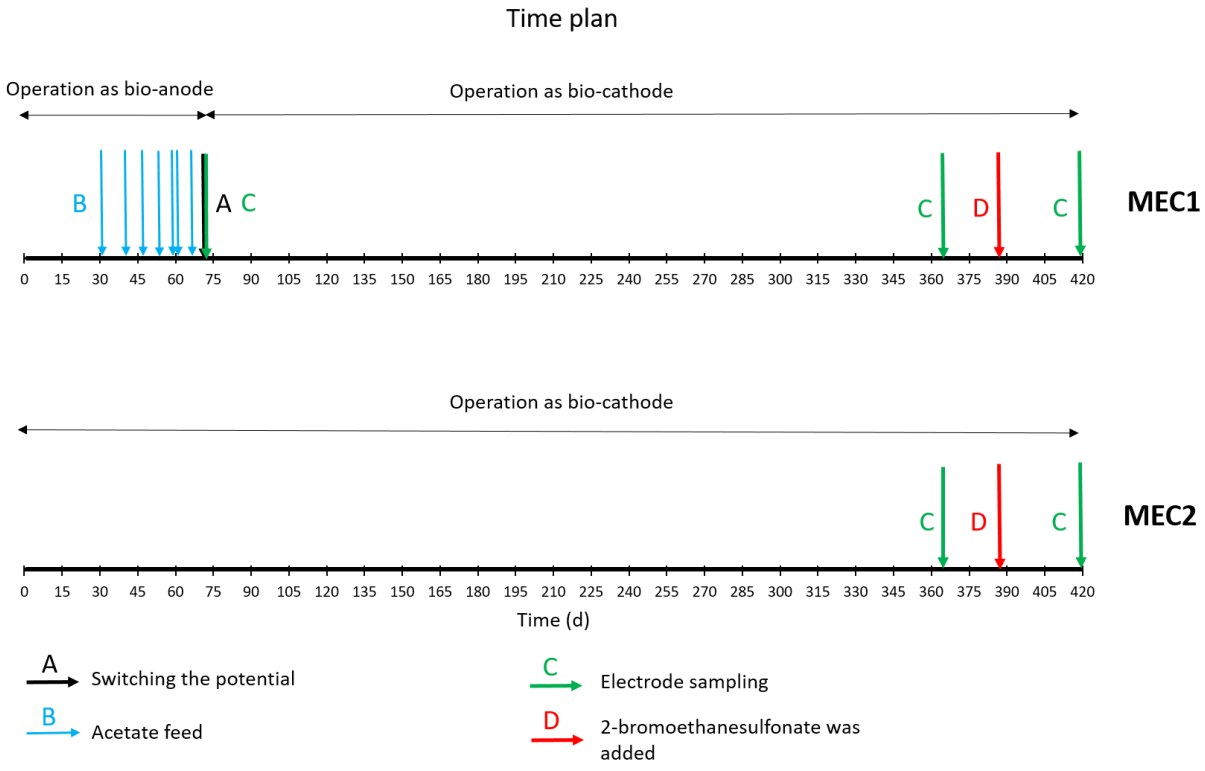

Figure 8. Time-plan for MECs, paper III. Arrows represent different actions that took place during the experiment.

3.1.4 Hydrogenotrophic enrichment cultures used for inoculating biocathodes in MECs (paper IV)

Duplicate glass bottles (325 mL total volume each) were used for enriching five different cultures. Activated sludge (1 mL) was added to the bottles as inoculum. The bottles were filled up to 250 mL with a nutrient medium. The goal was to enrich hydrogenotrophic cultures performing methanogenesis, acetogenesis, sulphate reduction, and nitrate reduction, as well as an acetate-oxidizing methanogenic culture. To accomplish this, the nutrient medium was amended with different electron acceptors and in some cases 2-bromoethanesulfonate to inhibit methanogens, as described in Table 2. Medium compositions that were used to cultivate different type of microorganisms in different bottles. The bottles were sealed with rubber caps and the head space (70 mL) was sparged with Ar/CO_2 gas (85%/15%) to remove oxygen. Then, the head space of the hydrogenotrophic bottles were filled with pure hydrogen gas at an overpressure of 160-180 kPa. Further details about the enrichment of cultures are provided in paper IV.

Table 2. Medium compositions that were used to cultivate different type of microorganisms in different bottles. *The medium in the bottles marked with an asterisk (*) also contained 10 mM 2-bromoethanesulfonate to inhibit methanogens.

Enrichment culture	Electron donor	Electron acceptor
Hydrogenotrophic methanogens (MgenH)	H_2	CO_2/HCO_{3-}
Homoacetogens (Agen)*	H_2	CO_2/HCO_{3-}
Hydrogenotrophic sulphate-reducers (SR)*	H_2	20 mM $NaSO_4$
Hydrogenotrophic nitrate reducers (NR)	H_2	20 mM $NaNO_3$
Acetoclastic methanogens (MgenA)	Acetate	CO_2/HCO_{3-}

Later, 140 mL liquid from hydrogenotrophic cultures performing methanogenesis, acetogenesis, sulphate reduction, and the acetate-oxidizing methanogenic culture was extracted to test the ability of the enrichment cultures to colonize a cathode in a glass double-chamber MEC, with a total volume of 340 mL in each chamber. A graphite foil cathode (Alpha Aesar, 43083-1 mm thick, 39.2 cm^2) was installed as working- and counter- electrode in each chamber. A Ag/AgCl reference electrode with an offset of 0.197 V versus SHE was installed in working-chamber. The two chambers were separated by a cation exchange membrane (CMI-7000, Membranes International Inc.). The working electrode potential was initially controlled at -0.65 V and later at -0.8 V versus SHE using Wenking M lab potentiostats and the current was recorded by MlabSci470c sequencer multichannel potentiostat software (version 4.7.0).

3.2 Nutrient medium composition

In paper I, the nutrient medium consisted of 2.925 g of NaCl, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of NH_4Cl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 1.65 g of $NaC_2H_3O_2$, 2.75 g of KH_2PO_4, 5.2 g of K_2HPO_4 and 0.05 g of yeast extract. 1 mL L^{-1} of trace element solution was added to the medium. The trace element solution contained (per litre): 0.05 g of H_3BO_3, 0.05 g of $ZnCl_2$, 0.03 g of $CuSO_4$, 0.5 g of $MnCl_2 \cdot 4H_2O$, 0.05 g of $(NH_4)\ Mo_7O_{24}$, 0.05 g of $AlCl_3$, 0.05 g of $CoCl_2 \cdot 6H_2O$, 0.05 g of $NiCl_2$, 0.1 g of Na_2SeO_3, and 0.05 g of $Na_2WO_4 \cdot 2H_2O$. In paper II, III, and IV, liquid nutrient medium consisted of 0.1 g/L of KCl, 0.6 g/L of KH_2PO_4, 0.25 g/L of NH_4Cl, 3 g/L of $NaHCO_3$,

0.1 g/L of MgCl, and 0.03 g/L of CaCl mixed by Trace element and vitamin solutions, as described by Marshall et al. (2012),were used.

3.3 Analytical methods

Organic acids and glucose were analysed using high performance liquid chromatography (HPLC). The gas phase was analysed by gas chromatography (micro-GC Agilent). The pH was measured with a pH sensor (WTW multi 350i). In most of the studies (except MFC operation in paper II), the anode and/or cathode potentials were controlled using a potentiostat. The potentiostat controlled the potential of working electrodes against the Ag/AgCl reference electrode, which has an offset of 0.197 V vs SHE. Cell growth (paper IV) was measured spectrophotometrically at a wavelength of 600 nm using a Shimadzu spectrophotometer. All analytical methods are further described in paper I, II, III, and IV.

3.4 Electrochemical test: Cyclic voltammetry

Cyclic voltammetry was used in all of the studies to assess redox process at the electrodes. Cyclic voltammetry is a potentiodynamic electrochemical measurement. Potential, between working electrode and reference electrode, is ramped linearly over time during the CV test. Current generation is measured between working electrode and counter electrode and it is plotted versus potential. Figure 9 (A) shows a typical CV test for a reversible system when the redox active component is oxidized in the forward scan and then reduced in the reverse scan. The CV starts at (a) and continues to (c) where the scan is reversed. In this region potential is scanned positively to cause an oxidation. The peak that appears at (b) is called anodic peak and it is formed when the redox active component is oxidized on the surface of the electrode. After reversing the scan at (c), the potential is scanned negatively to cause a reduction, which is shown by peak (d). The mean value of anodic peak potential and cathodic peak potential represents the formal redox potential (E°') of the redox active component, which depends on the specific conditions that are applied to the cell. It is possible to evaluate the efficiency of the redox reactions that takes place on an electrode by comparing formal redox potential to standard redox potential. Figure 9 (B) shows a CV test when an electrode is supplied by substrate continuously. In such a system, anodic/cathodic peaks are different from the peaks which were shown in Figure 9 (A) because substrate is always available and it is oxidized and reduced in a constant rate after the current reaches to its maximum. Figure 9 (B) is an example of a biological anode supplied with acetate. CV experiments can be done with different scan rates (V/s). Lower scan rates provide more accurate diagrams since the number of measurements are more compared to higher scan rate. Moreover, the non-Faradaic current, which is a current that is not related to chemical reactions of liquid components but is caused by changes in electrode potential, is higher at higher scan rates. CV tests, in BESs, help to identify the oxidation/reduction reactions that are catalysed by microorganisms on working electrodes. Increase in current generation at a specific potential represents the biological activity (oxidation/reduction) on the surface of the electrodes. Additionally, it is possible to compare performance of different electrodes by performing CV tests individually for each electrode. In order to investigate the biological activity of MECs, CV tests have been done occasionally in all studies. Further details about the CV tests are provided in the methods section of paper I, II, III, and IV.

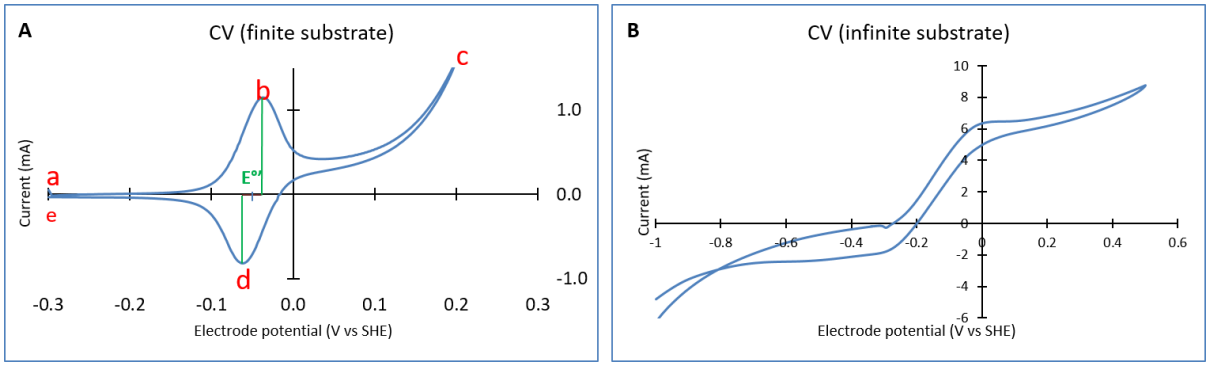

Figure 9. Two different typical cyclic voltammetry tests. (A) represents a CV in absence of substrate. (B) represents a CV while substrate is supplied all the time in a reactor.

3.5 Microbial community analysis

The microbial community analysis was carried out in each study. Samples were collected from the reactors and stored at -20 ºC prior to DNA extraction. DNA was extracted using the FastDNA spin kit for soil (MP biomedicals). The DNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) or Qubit Flurometer. PCR was carried out using the primers 515F and 806R (Caporaso et al., 2011) (paper I) and 515'F and 806R (Hugerth et al., 2014) (paper II-IV) to amplify partial V4 region sequences of the bacterial and archaeal 16S rRNA gene. Dual index labelling for primers was done according to the approach described by Kozich et al. (2013). The products were purified (Agencourt AMPure system, Beckman Coulter), normalized per concentration and pooled prior to sequencing. Sequencing was performed on an Illumina MiSeq using the MiSeq Reagent Kit v2 (paper I and III) and v3 (paper II and IV). The obtained sequences were processed either in Mothur (Schloss et al., 2009) (paper I) or Usearch using the Unoise (Edgar, 2016) (paper II and IV) or UPARSE (Edgar, 2013) (paper III) algorithms before analysis with the ampvis package in R (Albertsen et al., 2015) or an in-house written Python module. More technical details about the microbial community analysis are explained in the method section of each paper. Illumina MiSeq was chosen due to the fact that it can provide high sequencing depth compared to the conventional cloning and sequencing method.

Chapter 4

4 Results and Discussion

This thesis consists of four studies. The first, paper I, investigated the storage of acetate-fed bioanodes. The second study, paper II, investigated starvation of bioanodes as well as conversion to biocathodes by reversing the potential. The third study, paper III, compared start-up of the biocathodes by reversing the potential of pre-enriched acetate-oxidizing bioanodes to direct start-up from a sewage/anaerobic sludge inoculum. The last study, paper IV, investigated the ability of hydrogenotrophic enrichments (hydrogen-oxidizing enrichment cultures) for colonizing the biocathodes in MECs. This chapter starts with a summary of the main results, followed by an in-depth discussion, which explains the effects of dynamic conditions on electroactive biofilms grown on bioanodes and biocathodes.

4.1 Storage of bioanodes

Current generation. Figure 10 shows the total current generated by all the eight anodes controlled at 0 V vs SHE in the MEC (paper I). After 100 hours, current generation started. Then, it increased up to 35 mA (3.7 A/m^2) after 300 hours of operation and then reached a stable level of around 28 mA (3.01 A/m^2) for the rest of the experiment before storage of the electrodes. Directly after 5 weeks of storage, the current increased from 3 mA to around 8 mA. 330 hours after storage, current generation reached about 85% of the values generated before storage. The increase in current after storage showed that at least some of the stored bioanodes were capable of acetate oxidation.

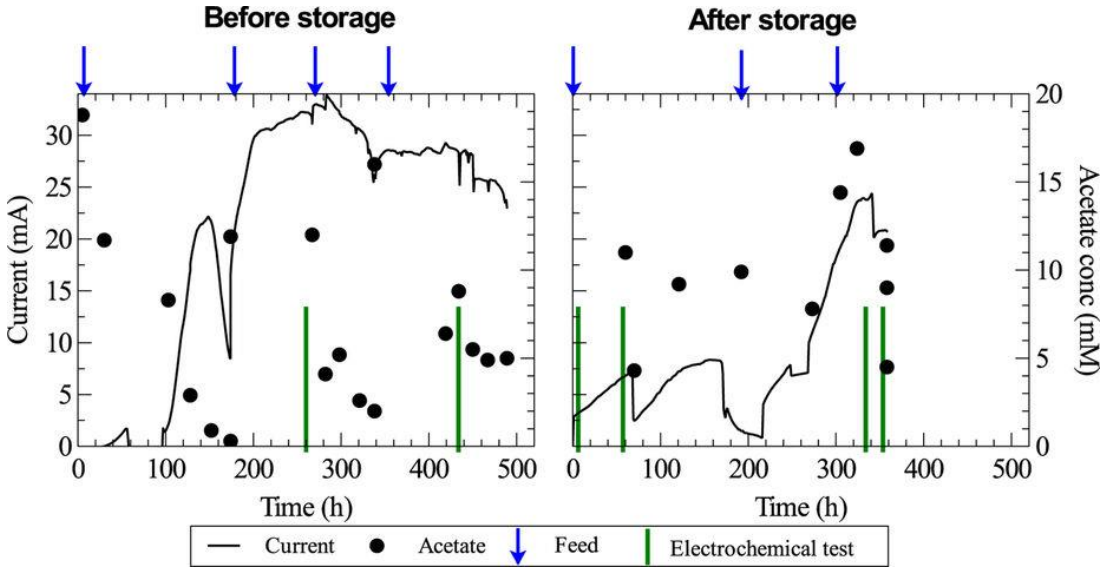

Figure 10. Current generation with time in AO-MEC when the anodes where controlled at 0 V vs SHE. Green lines show times when the CV tests were performed.

Electrochemical test. Differences between anodes stored for five weeks using different methods was assessed with CV tests (Figure 11). The steep rise in current at a potential close to -0.17 V indicates biological oxidation of acetate. Marsili et al. (2008) also observed similar CVs, with a steep rise in current at around -0.17 V vs SHE for thin *Geobacter sulfurreducens* biofilms growing on acetate. Right after storage, anodes stored by refrigeration showed biological activity. For the other stored anodes as well as the newly inserted anodes, no activity

was observed until after 334 hours when also the anodes stored by glycerol-freezing showed bioelectrochemical response. After 358 hours, the results showed a similar pattern in CV curves for the anodes stored by refrigeration, glycerol-freezing, and the new anodes, with noticeable rises in current at −0.17 V vs SHE. However, the anodes stored using the acetone dehydration method did not show any bioelectrochemical activity during the whole re-activation period.

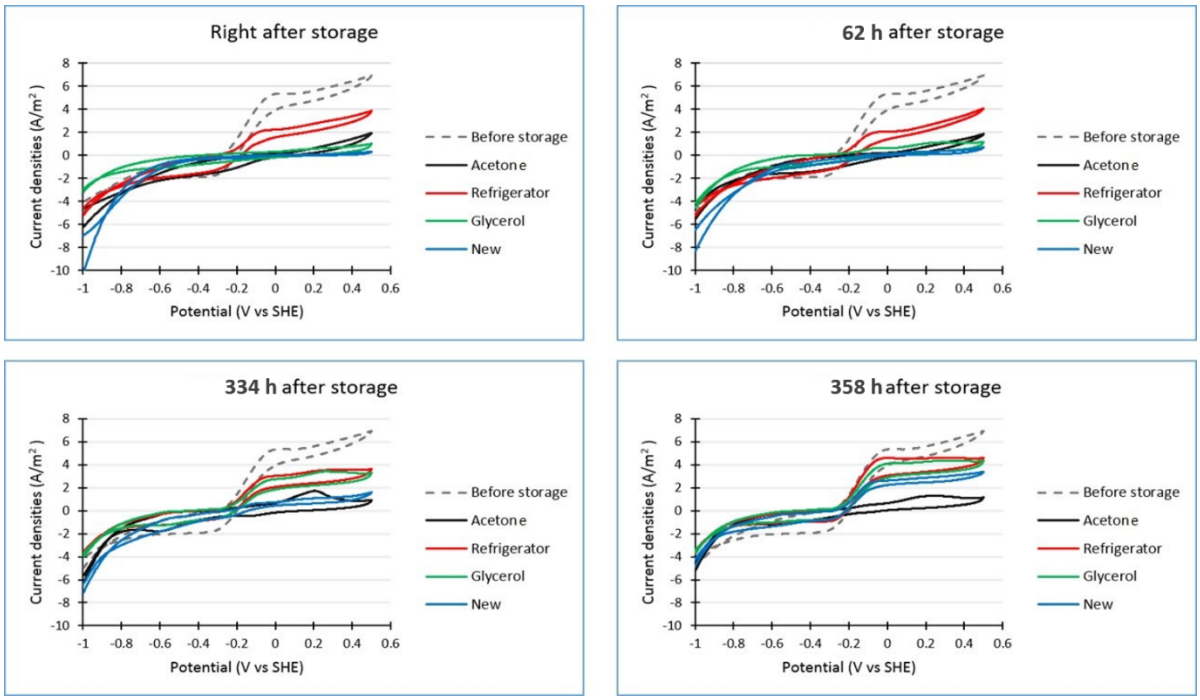

Figure 11. Cyclic voltammograms of anodes in the MEC. The dashed lines show the average cyclic voltammograms for all eight anodes in the reactor obtained during the second test before storage. The solid lines show the average cyclic voltammograms for duplicate electrodes exposed to the three storage methods: acetone dehydration, refrigeration, and freezing in 10% glycerol solution. New refers to new graphite rod anodes placed in the reactor when the system was restarted after storage.

Microbial community analysis. In the MEC (paper I), the bacterial community on the anodes prior to storage was dominated by *Geobacter* sp. (Figure 12) which can generate electricity by transferring electrons directly to a solid electrode, in the absence of an electron shuttle (Bond et al., 2002), via pili (Lovley et al., 2011, Reguera et al., 2005). Predominance of *Geobacter* sp. has previously been observed in anode biofilms of acetate-fed MFCs (Chae et al., 2009, Yates et al., 2012) and in MECs with mixed culture inoculum operated at anode potentials in the range of -0.15 to 0.02 V vs SHE (Torres et al., 2009). The enrichment of *Geobacter* sp. on the anodes in this study further confirms that this type of bacteria are highly selected for at anode potentials of around 0 V vs SHE and lower. The microbial analysis results showed that the microbial communities that developed on the stored anodes after 15 days of re-activation were distinctly different from the microbial community on the anodes prior to storage. In particular, the relative abundance of *Geobacter* sp. decreased and more diverse communities developed with higher relative abundance of sequences belonging to the phyla *Bacteroidetes*, *Synergistetes*, and *Spirochaetes* as well as *Desulfovibrio* sp. within *Proteobacteria* and *Clostridiales* within *Firmicutes*. The produced current density appeared to be related with the relative abundance of *Geobacter* sp. on the bioanodes which was changed from 71% relative

abundance before storage to 12%-35% relative abundance after storage. Among the stored anodes, the highest activity was recorded for the ones stored in the refrigerator and the lowest activity of the ones stored using acetone dehydration. This is also reflected in the *Geobacter* sp. relative abundances of 33% and 12%, respectively, for these two anodes.

In summary, after storage, results from the CV tests and the other electrochemical tests (see paper I) showed that the electrodes stored using refrigeration could revive faster than the electrodes stored using other methods. Indeed, immediately after storage, the refrigerator-stored electrodes showed bioelectrochemical response. It should be noted that the refrigerator-stored anodes were exposed to aerobic condition during storage; however, bioelectrochemical activity could still be maintained, which simplifies handling of the electrodes during storage. Even after storage by refrigeration and by glycerol-freezing, electrodes showed a higher electrochemical activity than new electrodes that were installed in the reactor after storage. This shows that the surfaces of the electrodes stored by refrigeration and glycerol-freezing both had some viable biofilms which could be revived after storage. This biofilm probably served as inoculum for the new electrodes placed in the reactor after storage. The acetone dehydration method has been shown to be suitable for storage of aerobic granular sludge (Lv et al., 2013); however, it appears to be unsuitable for storage of biological anodes since the bioelectrochemical activity seems to have been destroyed. The bioelectrochemical activity of the anodes stored by acetone dehydration could not be revived during the 15 days of operation after storage. The reason could be a dead layer of biofilm on the surface of the electrodes, which would prevent a new biofilm from establishing electrical contact with the electrode surface.

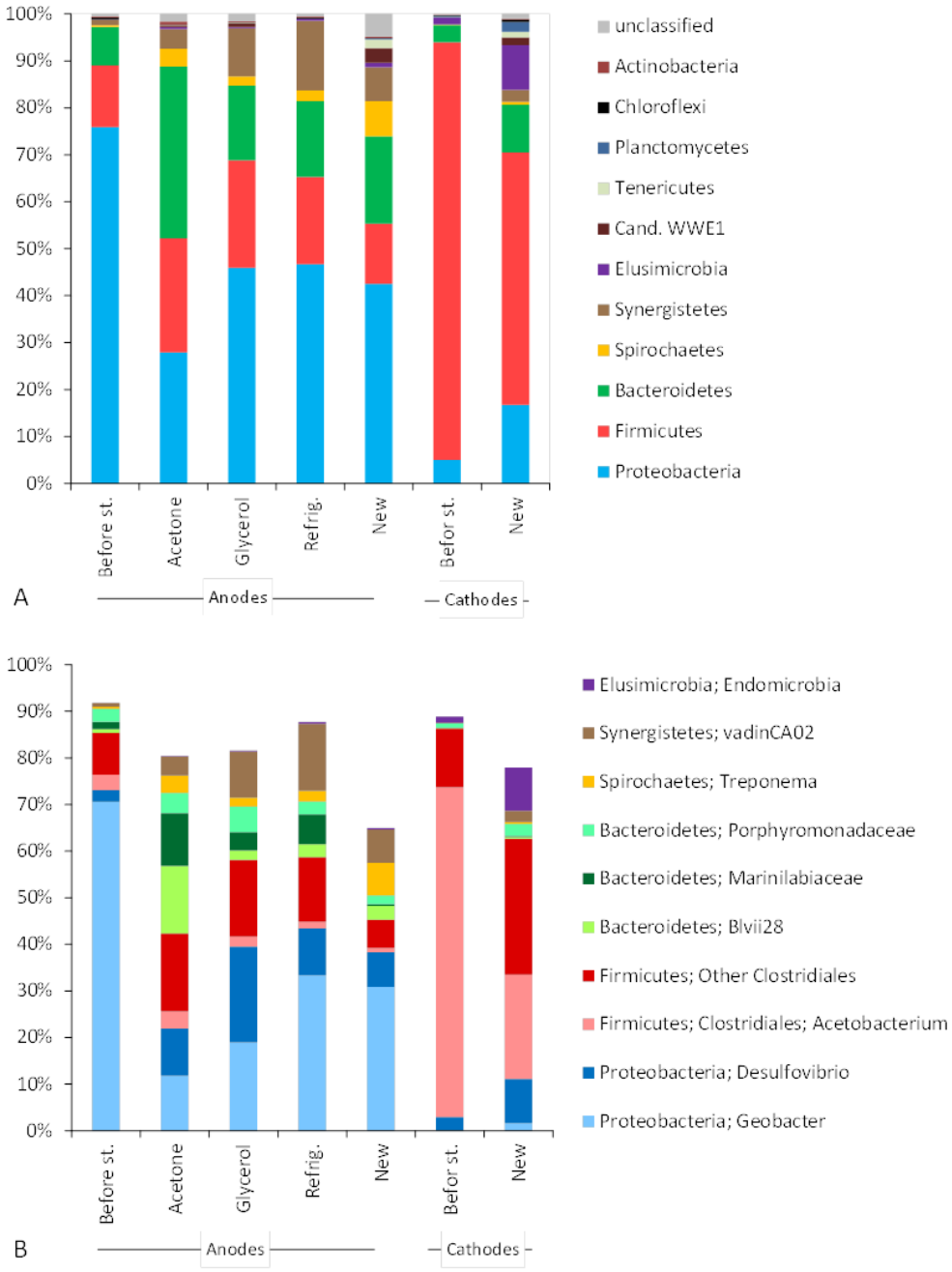

Figure 12. Bacterial community in the MEC. (A) Distribution of phyla with relative abundance >0.5% in any of the samples. (B) Distribution of major taxa (relative abundance >5% in any of the samples) at highest possible taxonomic determination using the Greengenes taxonomy.

4.2 Starvation of bioanodes

Current generation. Figure 13A shows the current generation during start-up of the MFCs (paper II). MFC 4-7, which were fed with glucose (Table 1), started to produce current after 4 days. MFC 0-3, which were fed with acetate (Table 1), started to produce current at the end of day 5. Around 8 days after start-up, all MFCs produced more than 0.15 A/m^2 except for MFC6 and MFC7, which started up much slower. After 15 days, the current generation had reached relatively stable values in all MFCs. At this time, the daily current peaks were between 0.21-0.29 A/m^2 and the resistors were changed to 100 ohm. With this external resistance, a stable peak current of about 1.1-1.3 A/m^2 developed for the acetate-fed MFCs and 0.9-1.0 A/m^2 in the glucose-fed MFCs (Figure 13B).

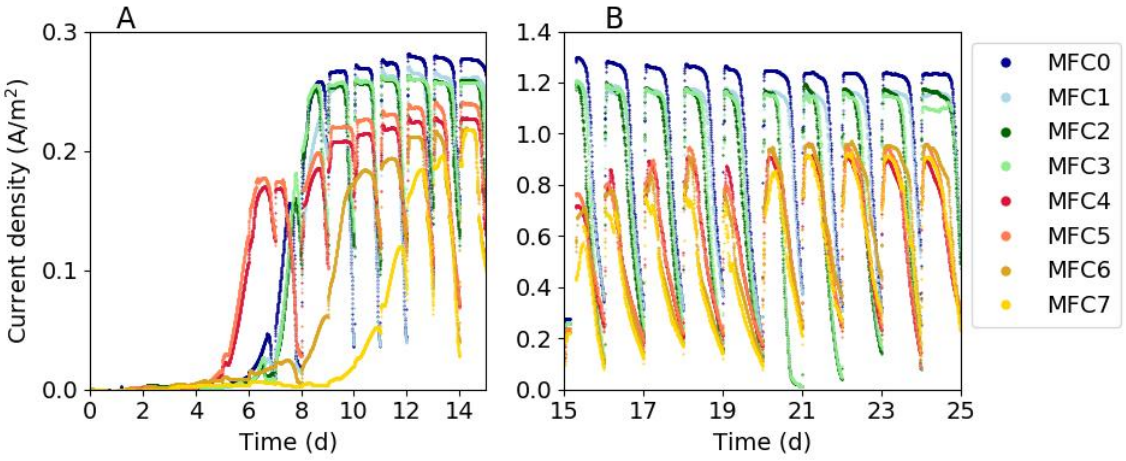

Figure 13. Current density generated by the eight MFCs during (A) the initial 15 days with 1000 ohm resistors, and (B) day 15-25 with 100 ohm resistors.

The current generation before, during, and after the three starvation periods is shown in Figure 14. After each starvation period, the current quickly recovered to a stable value. In the acetate-fed MFCs the recovery was immediate whereas the glucose-fed MFCs required 1-2 days to return to a stable peak current. In the MFCs operated with closed-circuit during starvation, a slight increase in current was observed directly after the fresh medium solution was added, even though it did not contain carbon source. The reason could be that low levels of oxygen in the feed led to partial oxidation of biomass in the reactor, which could have served as electron donors for electrogenic bacteria. Vitamins in the nutrient medium may also have contributed to the current generation. In the MFCs operated with open-circuit during starvation, a large current peak was sometimes observed right after organic feeding was resumed and the circuit was closed. This could be because of an accumulation of reduced molecules in the microbial cells when they were no longer able to use the anode as electron acceptor during the starvation phase. Once the circuit was closed, these reduced molecules were rapidly oxidized, resulting in a burst of current.

The maximum current generation recorded during the starvation phase was between 0.1 and 0.2 A/m^2 which was 7-13 % of the peak current produced during the normal operation.

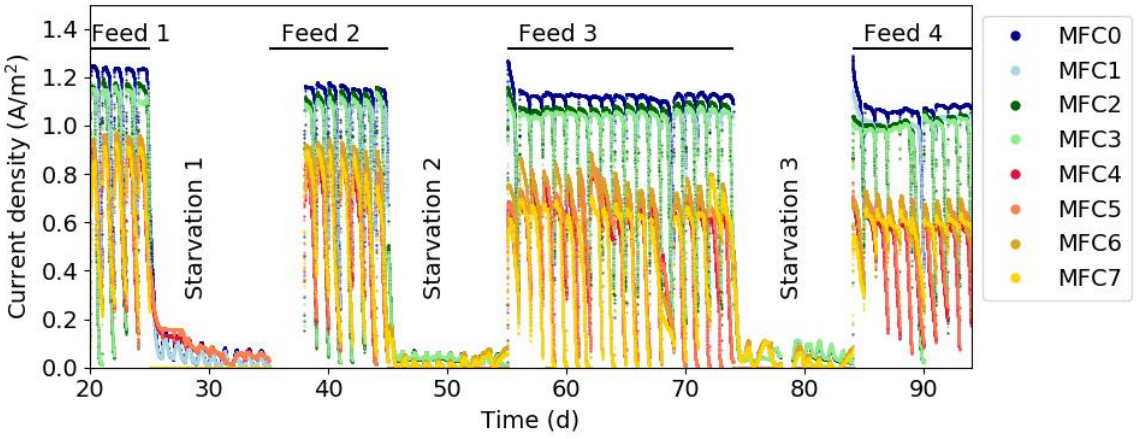

Figure 14. Current density generated in the MFCs during the feed- and starvation periods.

In general, the peak current decreased slightly after each starvation period (Figure 15). When comparing the period before the first starvation phase to the period after the last starvation phase, the peak current in the acetate-fed MFCs had dropped by 11.4±0.6% while it had dropped by 28.6±2.8% in the glucose-fed MFCs. In the acetate-fed MFC, there was a small (4.1±1.4%) and statistically significant drop ($p < 0.001$ except for MFC0-1 in starvation period 3) over each starvation period. No reduction of peak current was observed during the feeding periods. This could mean that each starvation led to some decay (death) of the electrogenic biomass (4.1±1.4%) per period. If the dead biomass remained on the anode it could have prevented new bacteria from colonization the surface, thereby preventing the peak current from reaching its pre-starvation value. In the glucose-fed MFCs, the drop in peak current was 10.4±6.8% per period. However, the glucose-fed MFCs also showed a decreasing current during the feeding periods. Current generation from glucose seemed to require cooperation of different taxa which starvation can have negative effect on the relative abundance of them. Results from columbic charge showed different pattern compared to peak current. There was no clear decreasing trend and the changes were generally not statistically significant ($p>0.05$). For MFC 0-3, the columbic charge observed before the 3rd starvation period had the highest value compared to other measurements (Figure 15).

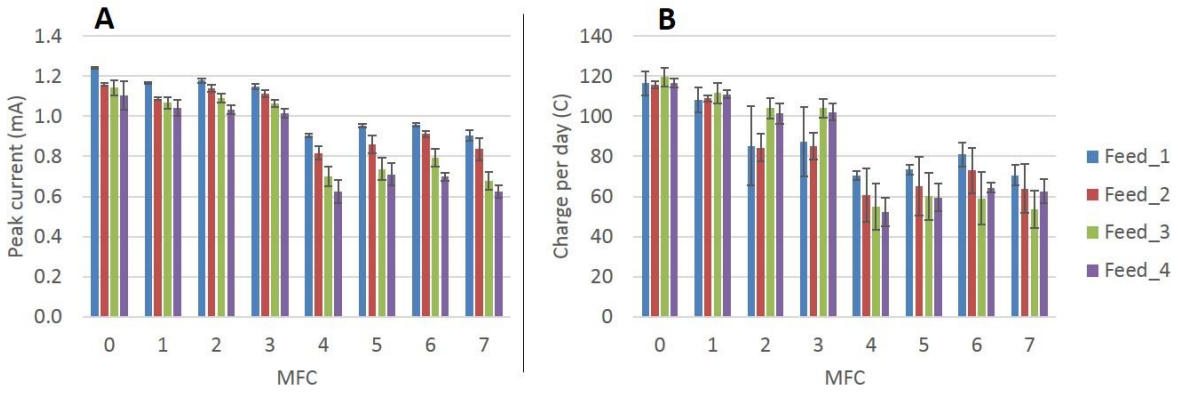

Figure 15. Average peak current density (A) and total charge generated per day (B) during the feed periods before and after each starvation period.

Microbial community analysis. Heatmaps showing the microbial community composition of the inoculum and the bioanodes (paper II) at class and genus taxonomic levels are shown in

Figure 16. The anodes in both acetate- and glucose-fed MFCs were dominated by *Deltaproteobacteria* (16-79%). The glucose-fed anodes also had a high proportion *Bacilli* (13-34%). On the genus level, the acetate-fed anodes were dominated by *Desulfuromonas* (62-77%). *Desulfuromonadales* spp. are often associated with anodes in MFCs (Ishii et al., 2013). For example, Tender et al. (2002) found microorganisms related to *Desulfuromonas acetoxidans* associated with the anode in a sediment microbial fuel cell. Members of the *Desulfuromonadales* order also include *Geobacter*, which was dominating the anodes in the first study on storage (paper I). Several species in *Desulfuromonadales* are capable of respiration with solid electron acceptors such as sulphur and Fe(III) (Greene, 2014). Therefore, we propose that the *Desulfuromonas* sp. present on the acetate-fed anode was directly converting acetate into electrical current. There were two taxa in the glucose-fed anodes with a high relative abundance: *Trichococcus* (13-34%) and a bacterium that was unclassified at the genus-level but belonging to the *Desulfuromonadales* order (6.8-42%), which is the same order as the dominant taxa on the acetate-fed anodes. *Trichococcus* spp. are aerotolerant fermenters known to produce acetate and lactate from glucose (Rainey, 2015). Current generation from glucose seemed to require cooperation of at least these two taxa (likely *Trichococcus* and the *Desulfuromonadales* sp.) where the *Trichococcus* sp. fermented glucose to acetate, which was utilized by the bacterium classified within *Desulfuromonadales*. The balance between these two taxa could have changed during the experiment due to starvation and other disturbances. Therefore, in glucose-fed MFCs, the drop in current after each starvation period was higher (10.4±6.8%) compared to acetate-fed MFCs. The fermentation products generated by *Trichococcus* may also have been scavenged by other microorganisms during the run, e.g. methanogens and aerobes. The more complex microbial community needed to convert glucose to current may also explain why these anodes needed 1-2 days to reach a stable current value after each starvation period.

In summary, both acetate- and glucose-fed bioanodes could survive 10 days of starvation. However, it seems that acetate-fed bioanodes lost its functional ability less compared to glucose-fed bioanodes which their performance deteriorated during both starvation and feeding phase. We speculate that the balance between the communities grown on glucose were affected more compared to those grown on acetate and this led to less current generation and lower overall performance after each starvation period.

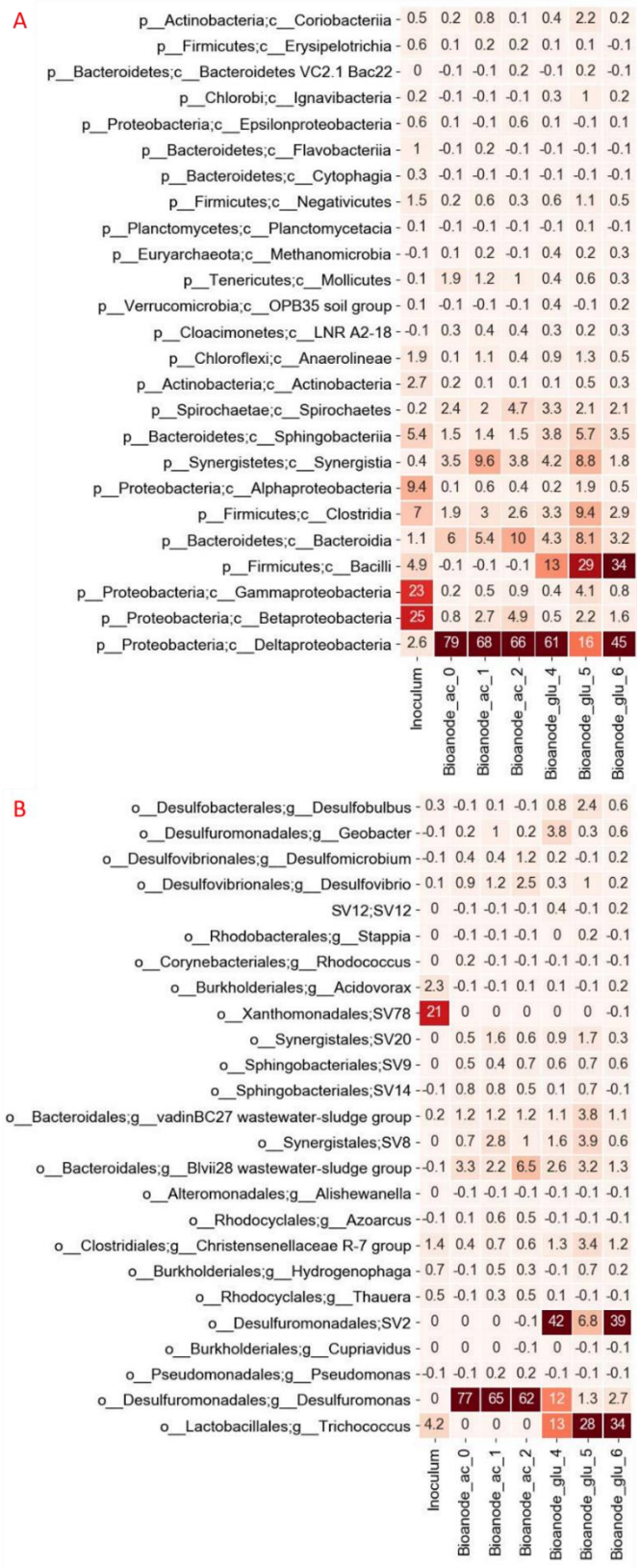

Figure 16. Heatmaps showing the most abundant taxa. The abbreviation *ac* refers to acetate-feed, *glu* refers to glucose-feed, and the numbers refers to the MFC number. A: class-level grouping. B: genus-level grouping. SV followed by a number means that the sequence could not be classified to a known genus. -0.1 means that the relative abundance was less than 0.1% but higher than 0.

4.3 Start-up of biocathodes: a smooth transition to a new function?

In this thesis, the possibility of starting up CO_2-reducing biocathodes from different types of enrichment cultures was investigated. We focused specially on pre-enriched bioanodes as inoculum for biocathodes, since this has been shown to be a promising strategy in previous studies. Acetate-oxidizing bioanodes (paper II & III) and glucose-oxidizing bioanodes (paper II) were tested. I also tested various types of hydrogenotrophic enrichment cultures (paper IV).

Current generation. Figure 17 shows the current generated by acetate and glucose fed bioanodes (paper II) after the potential was lowered to -0.65 V vs SHE. Reactor 3 (paper II), which had been enriched on acetate, initially generated a cathodic current of about 0.3 A/m^2. After about 7 days, the current stabilized at 0.1 A/m^2. Reactor 7 (paper II), which had been enriched on glucose, initially generated a cathodic current of 0.5 to 0.8 A/m^2; however, after 14 days it was about 0.2 A/m^2. The current then eventually reached about 0.1 A/m^2 in both reactors. Once the cathode potential was decreased to -0.8 V vs SHE on day 128, the current in reactor 3 stabilized at 0.15 A/m^2 whereas the cathodic current in reactor 7 showed an increasing trend going from about 0.1 to 0.2 A/m^2.

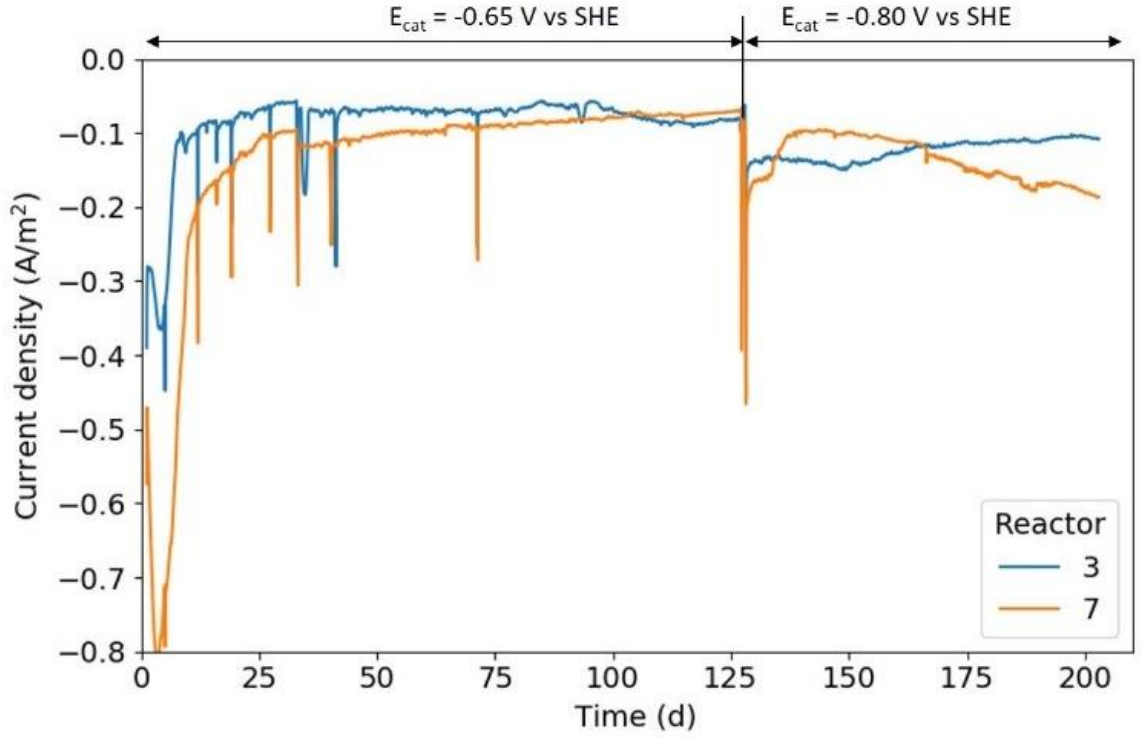

Figure 17. Current density for microbial electrodes reactors 3 and 7 converted to biocathodes.

Figure 18 shows the total current produced by the two MECs in paper III. In MEC1, at the potential of -0.2 V vs SHE, the electrodes were working as anodes and a positive current was generated. The increase in current in MEC1 after 6 days shows the biological activity on the surface of the anodes. Microorganisms began to oxidize acetate and deliver electrons to the electrodes. The current reached around 2.5 A/m^2 before the potential was switched to -0.65 V vs SHE in MEC1. After lowering the potential, the current dropped to 0.016±0.007 A/m^2 for approximately the next 170 days. This showed that the microorganisms dominating on bioanodes were not capable of operating as biocathodes when the potential was switched. About 170 days after the potential switch, the cathodic current increased up to around 0.6 A/m^2

and for the rest of the experiment it fluctuated between 0.6 A/m^2 and 3.6 A/m^2 which showed that biocathodes had been enriched. In MEC2, the cathodic current was initially 0.0078±0.0077 A/m^2. Bioelectrochemical activity was observed to increase after approximately 83 days when the cathodic current reached around 0.1 A/m^2. For the next 120 days, the current increased up to approximately 0.6 A/m^2 and fluctuated between 0.6 A/m^2 and 3.3 A/m^2 until the end of the experiment.

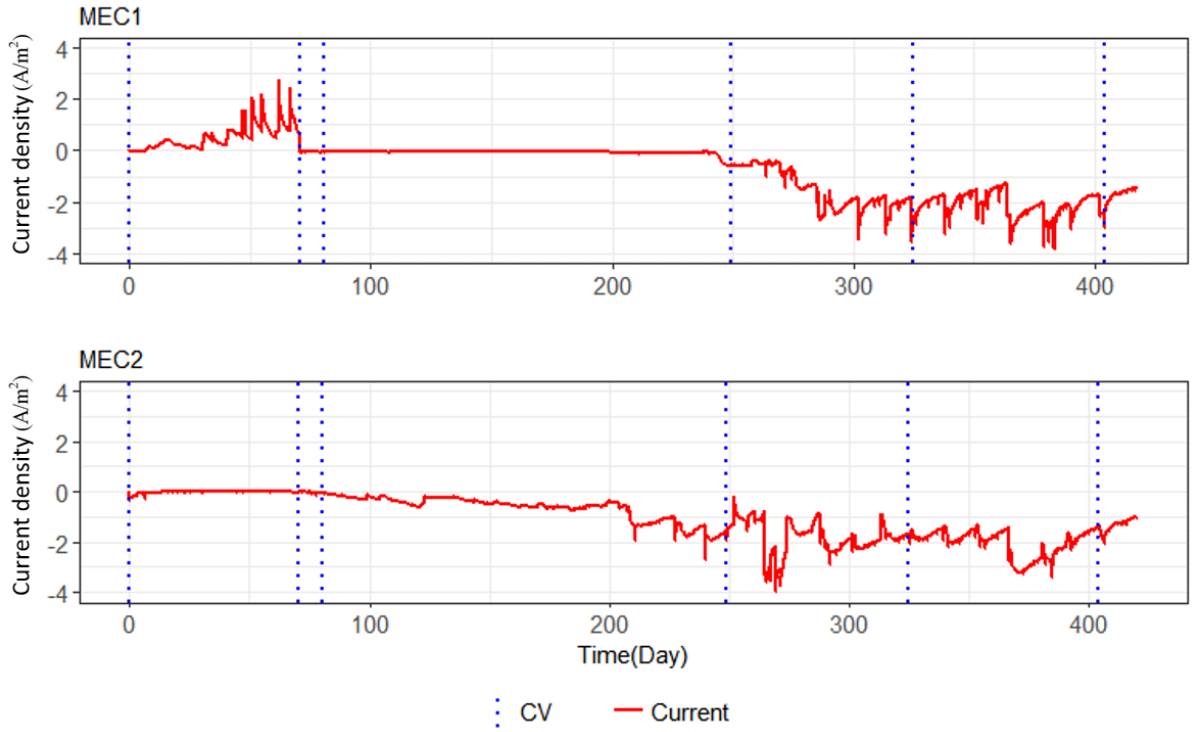

Figure 18.Current generation with time in MEC1. The positive current represents the current when the anodes were controlled at -0.2 V vs SHE. The negative current represents the current when cathodes were controlled at -0.65 V vs SHE. Dash lines indicate when normal operation was stopped and CV tests were carried out.

Different materials tested as biocathode in this study produced different magnitude of current at the beginning of the expeiment. However, at the end of the experiment, all electrode materials produced similar current densities. Therefore, among the materials tested in this experiment, none had clear advantages for generating higher current in long term operation. Comparing the electrode materials, carbon felt electrodes had significantly higher actual surface area compared to graphite foil and graphite rod electrodes. That is because carbon felt material consists of a lot of carbon fibres (Figure 7). Therefore, it would be reasonable to assume that carbon felt would produce higher current than the other electrode materials. However, the results suggest that not all of the available surface area of the carbon felt cathodes could be utilized for electrochemical reactions. Biofilm likely attached on the outer part of the felt limiting diffusion of substrates to the inner part.

In paper IV, four different MECs, inoculated with hydrogenotrophic methanogens, hydrogenotrophic acetogens, hydrogenotrophic sulphate-reducers, and acetate-utilizing methanogens (Table 2), were operated over 8 weeks in order to investigate the catalytic ability of selected enrichments on a cathode over a longer time period. Figure 19 shows the current that was generated during the operation. The enrichments generated current densities of about 0.1-1 A/m^2 directly after inoculation. MgenH enrichments generated current densities of about approximately 0.6 A/m^2 before the potential was switched from -0.65 V to -0.8 V versus SHE

at day 49. After lowering the potential, the current increased up to 0.8 A/m^2. In the MEC inoculated with the hydrogenotrophic acetogens, the current was generated directly after inoculation and increased up to around 0.8 A/m^2 before changing the potential from -0.65 V to -0.8 V versus SHE. After lowering the potential, the current increased up to around 1 A/m^2. In MEC inoculated by the acetate-utilizing methanogens, current was generated up to 0.3 A/m^2 after 4 days followed by a decrease to around 0.02 A/m^2 for next 4 days. The current started to increase gradually and was varied between 0.8 to 0.9 A/m^2 until the potential was switched from -0.65V to -0.8 V versus SHE. After switching the potential, current generation increased to 1 A/m^2 and varied between 0.8 to 1 A/m^2 till the end of the experiment. In SR_{MEC}, the current varied between 0.2-1 A/m^2 for the first 30 days. However, it stabilized at around 0.7±0.05 A/m^2 from day 30 until the end of the experiment. Decreasing the potential from -0.65 V to -0.8 V versus SHE did not have a noticeable effect on current generation in SR_{MEC} compared to the other MECs that was investigated in paper IV.

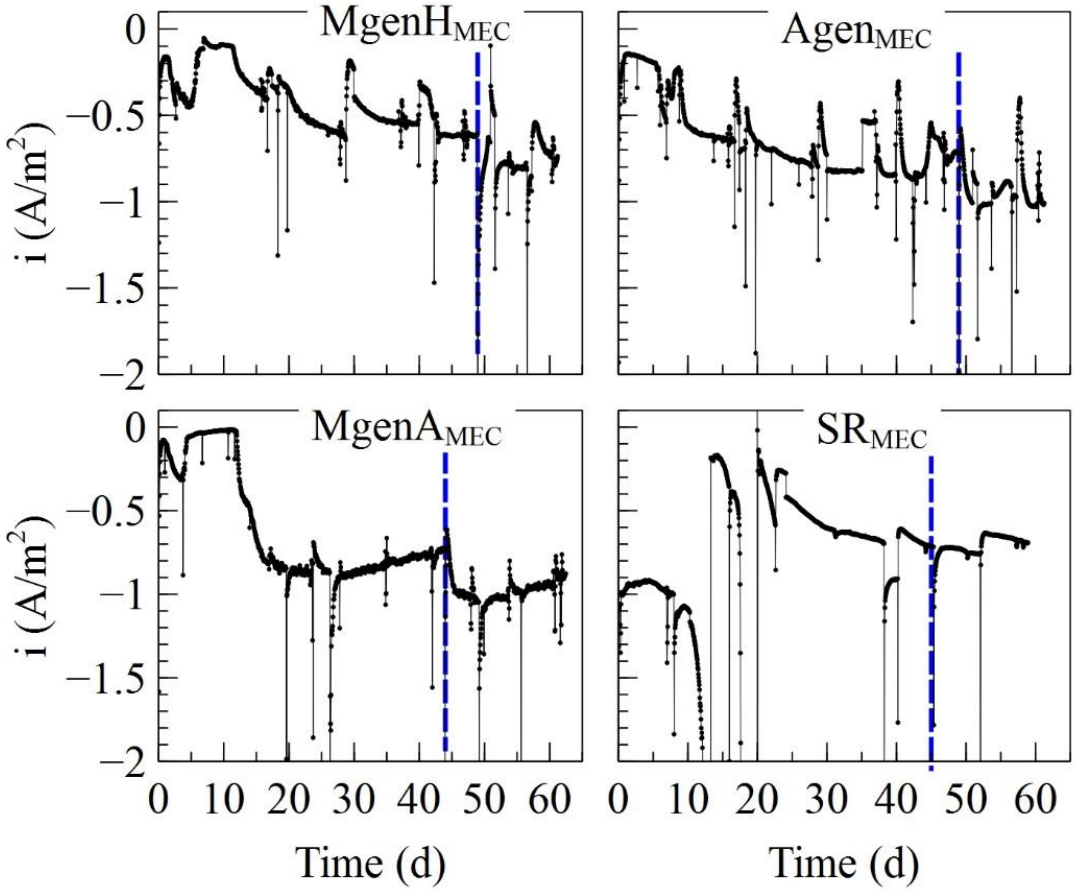

Figure 19. Current generation in MECs inoculated by MgenH1, Agen1, MgenA1, and SR1. The cathode potential was lowered from -0.65 V to -0.8 V on day 49 in MgenH and Agen, and on day 44 in MgenA and SR MECs as indicated by the dashed vertical lines.

In summary, acetate-fed bioanodes which were enriched in the single chamber MFC (paper II) showed cathodic response and produced a noticeable current density immediately after the potential was changed. However, the acetate-fed bioanodes enriched in double chamber MEC (paper III) generated very low cathodic current for about 170 days after changing the potential to -0.65 V vs SHE. We speculated that the microorganisms growing near the air cathode in the single chamber MFC (paper II) could seed the anode with a diverse microbial community before switching the potential. Moreover, oxygen diffusion through the air cathode and through

the feeding process, led to formation of a distinctly different biofilm on the anode in the single chamber MFC compared to the anodes enriched in the double chamber MEC. This could explain the difference in biocathode start-up times for paper II and paper III. The electrode from the glucose-fed MFC (paper II) initially catalysed cathodic reactions with higher current density than the electrode from the acetate-fed MFC (paper II) and acetate-fed MEC (paper III). It is possible that bacteria possessing hydrogenases were present in the biofilm, particularly in the glucose-fed MFC. Fermentation of glucose can lead to generation of hydrogen. Hydrogenases may fortuitously catalyse cathodic reactions (Deutzmann et al., 2015). In fact, we showed that the pre-enriched hydrogenotrophic microorganisms are capable of generating high cathodic current after addition to a double chamber MEC (paper IV). Moreover, microbial community analysis of the cathode electrodes in paper III showed that hydrogenotrophic communities, particularly hydrogenotrophic methanogens developed on the biocathodes in both MECs and generated very high current densities at the end of the experiment (0.6 to 3.6 A/m^2).

Electrochemical test. Different CV tests were carried out for pre-enriched acetate-fed biocathodes. In paper II, at the start, both electrodes showed anodic peaks associated with the oxidation of organic substrate remaining in the reactors (Figure 20). Reactor 7 also showed much higher cathodic currents than reactor 3, corresponding to the higher cathodic current observed in that reactor during operation with constant potential in the beginning of the experiment. However, from day 11, both anodic and cathodic peaks disappeared from the voltammograms. In the last voltammogram on day 202, a small reversible peak with a midpoint potential of about -0.45 V vs SHE appeared. It was most pronounced in reactor 3.

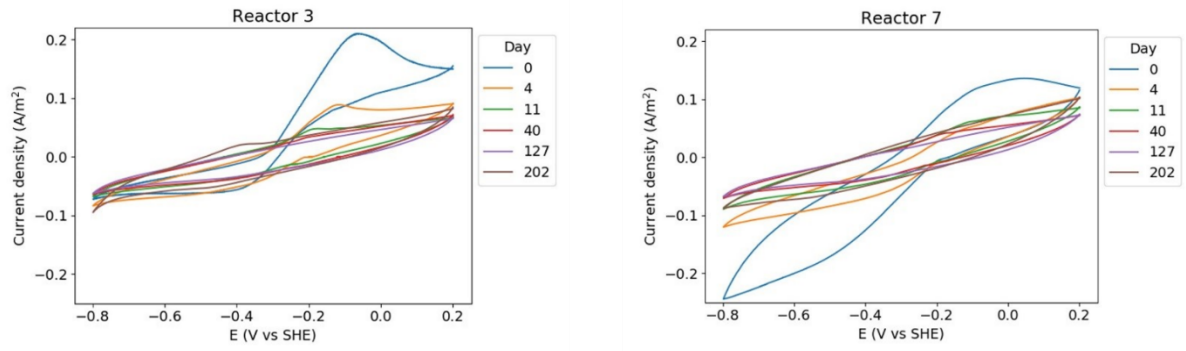

Figure 20. Cyclic voltammograms for microbial electrodes reactors 3 and 7 converted to biocathodes.

The results from the CV tests for MEC1 (paper III) are shown in Figure 21.The first two tests were carried out before lowering the potential. The CV test after 71 days showed an increase in current at -0.2 V. This type of anodic peak is usually seen in CVs with acetate-fed bioanodes (e.g. Modin and Fukushi, 2012), and indicates that acetate is being oxidized bioelectrochemically. Directly after switching the potential, after 81 days of incubation, the CV tests still showed some catalytic waves. An anodic peak at around -0.2 V indicated that the acetate oxidizing biofilm still responded to the CV. There are also some cathodic peaks and the onset of H_2 evolution, at the potential of -1 V, appears to be slightly shifted to a more positive potential in comparison to the CVs from day 1. From day 250 and onwards, the CVs have a different shape, showing several reduction peaks. The onset of H_2 evolution is markedly shifted from -1 V vs SHE to a more positive potential, especially on day 325 and 404. Reduction peaks were observed at potentials of -0.24 V, -0.4 V and -0.6 V.

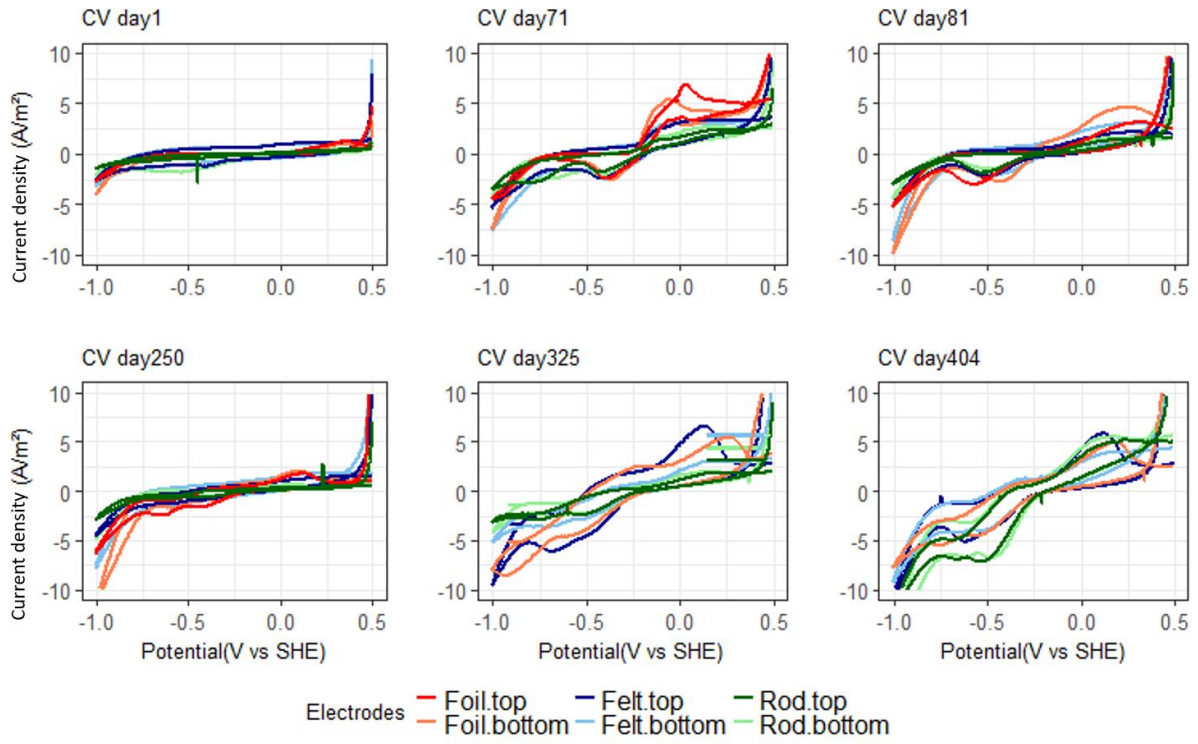

Figure 21. Six different CVs for MEC1. The first two tests were done before switching the potential from -0.2 to -0.65 V vs SHE, and the other four tests were done after switching the potential. One graphite foil electrode was removed from MEC1 on day 313 due to technical problems.

Figure 22 shows different CV tests that were carried out during the operation of the four MECs (paper IV). In all MECs, biological activity on the biocathode was observed directly after inoculation at day 1. In $MgenH_{MEC}$, the hydrogen evolution peak at -1 V versus SHE was improved over the operation time according the next two CV test carried at day 20 and day 48. After 60 days, the final CV test showed that the hydrogen evolution peak at -1 V versus SHE was shifted slightly towards more positive potential (-0.9 V vs SHE) and the current generation increased noticeably. One reason for this could be that the MEC was operated at -0.8 V versus SHE during the last 12 days. In acetogens enriched MEC ($Agen_{MEC}$), the current increased slightly at the potential of -1 V vs SHE after 20 days. However, at the end of the MEC operation, the current was generated much less at -1 V vs SHE compared to previous CV tests even though the MEC was operated at -0.8 V vs SHE the last 12 days of the experiment. In SR_{MEC}, high cathodic reduction peak was observed at -0.55 V versus SHE after inoculation. The next CV tests carried out at day 26, day 54, and day 66, showed a clear reduction peak at -0.42 V, -0.22 V, and -0.36 V versus SHE, respectively. The current that was generated at -1 V versus SHE at day 26 and day 54, increased noticeably and shifted more towards -0.9 V versus SHE compared to the beginning of the experiment. However, at day 66 the current that was generated at -1 V versus SHE was decreased markedly. The CV tests for MEC inoculated by acetate utilizing methanogens ($MgenA_{MEC}$) did not show any improvement over the operation period even though the current was generated up to 1 A/m^2 (Figure 19). This indicates that the microbial community was electrochemically active on the cathode, however, catalyzing cathodic reactions did not improve over the time.

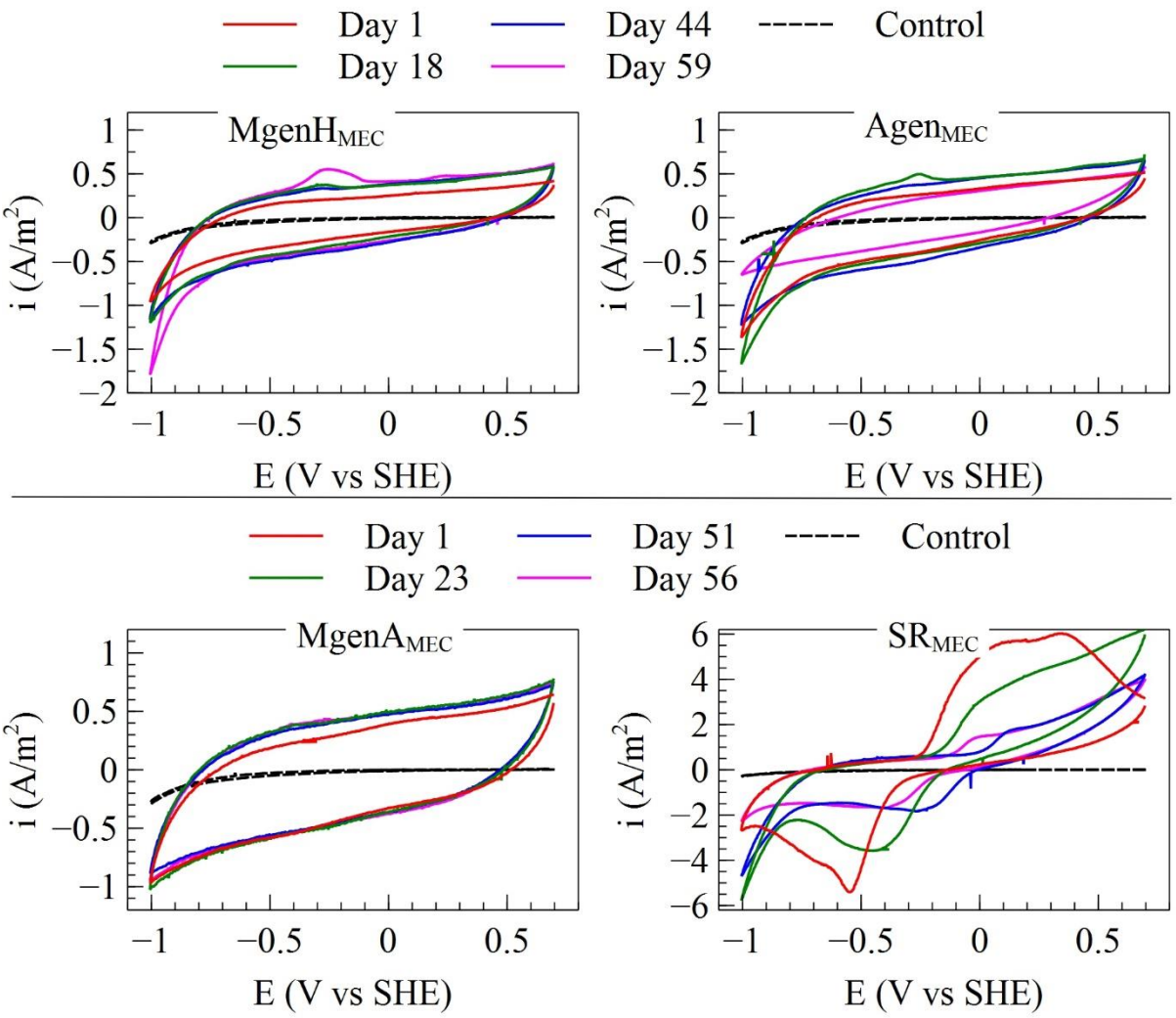

Figure 22. Five different CV tests carried out for MgenH-, Agen-, MgenA-, and SR1-MEC. The control CV was carried out without the presence of microorganisms.

Microbial community analysis. Figure 23 shows the microbial communities developed on bioanodes and biocathodes in paper III. The community on the bioanodes in MEC1 (paper III), after 71 days of operation, had shifted considerably to a community dominated by *Geobacter* sp (>40%) despite that the inoculum contained a diverse community of bacteria distributed among the phyla *Spirochaetes, Bacteroidetes,* and *Proteobacteria*. Both *Geobacter* spp. as well as *Desulfovibrio* spp. which were also abundant on the bioanodes, have previously been shown to catalyze hydrogen production on biocathodes (Croese et al., 2011, Geelhoed and Stams, 2011). On the biocathodes, after 363 days of operation, the most abundant sequences were affiliated to the genus *Methanobacterium* (phylum *Euryarchaeota*) in both MECs (paper III), which are known as hydrogenotrophic methanogens (Shlimon et al., 2004), with a relative abundance over 50%. In summary, the microbial communities developed on the biocathodes after reversing the potential was completely distinct compared to the bioanodes before reversing the potential (paper III). This confirmed that the biofilm developed on the bioanodes did not facilitate cathodic reactions for 170 days after reversing the potential, instead, a biofilm with different microbial community grew on the biocathodes.

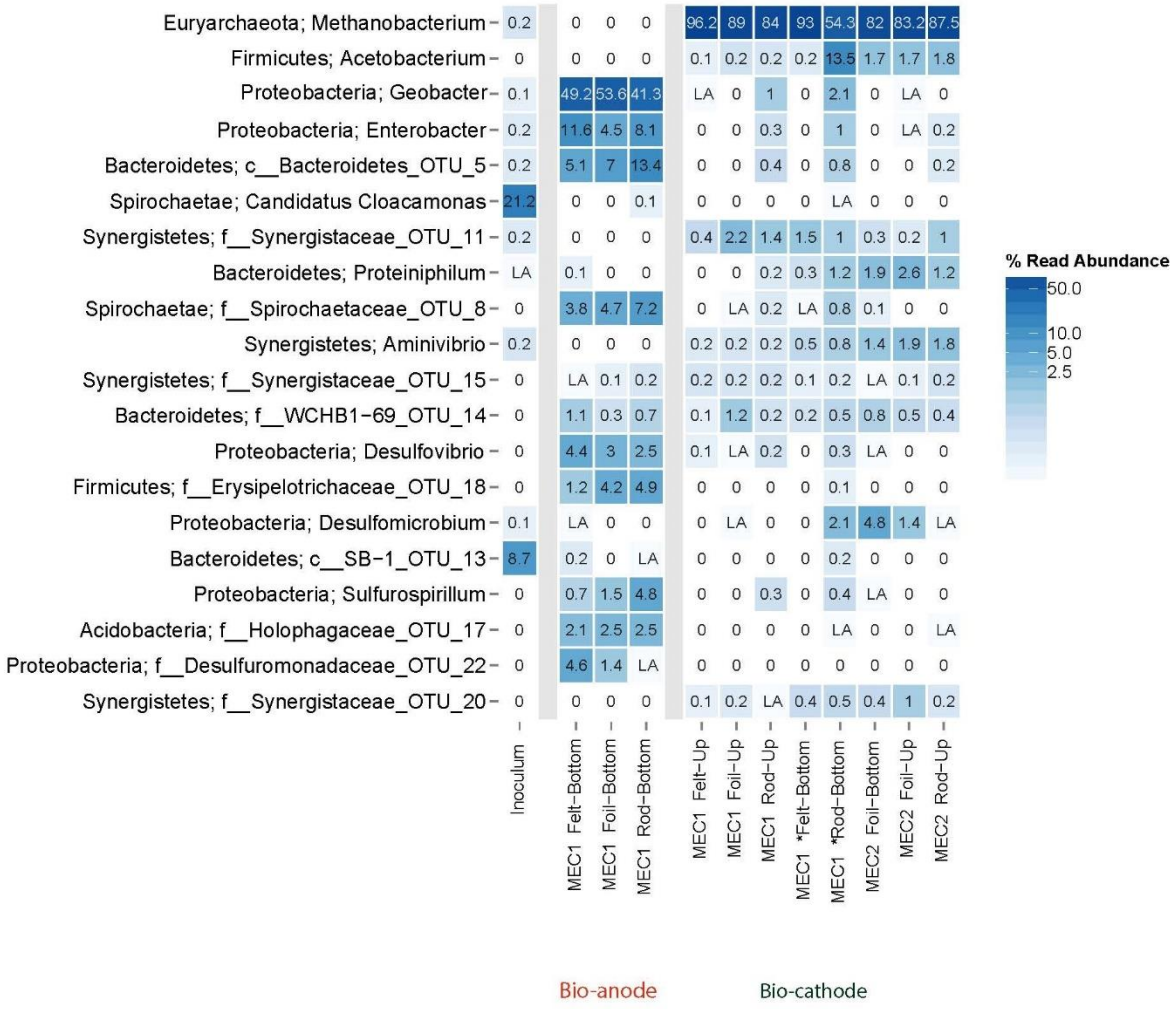

Figure 23. Relative abundance of the 20 most abundant 16S rRNA gene sequences in the bioanodes, biocathodes, and inoculum. LA refers to abundances < 0.1 %. *Electrodes that were placed in MEC1 after removing bioanodes.

The microbial community developed on the biocathodes in the MECs inoculated by pre-enriched cultures (paper IV) varied depending on the source of inoculation (Figure 24). The cathode electrodes inoculated by hydrogenotrophic methanogens ($MgenH_{MEC}$) were dominated by *Methanobacterium* sp. (32%), *Stappia* sp. (27%), and *Thauera* (17%). The most abundant bacteria grew on the cathode in $MgenA_{MEC}$ were affiliated to *Rehaibacterium* (41%) and *Paracocccus* (38%). The biocathode enriched in $Agen_{MEC}$ contained a very diverse group of bacteria distributed among *Methanobacterium* sp. (31%), *Azoarcus* (17%), an SV belonging to the *Rhizobiales* family (SV14, 13%), *Thauera* (6.9%), and *Desulfovibrio* sp. (6.1%). The biocathode inoculated by sulphate-reducing enrichment cultures was dominated by two SV belonging to the *Coriobacteriaceae* family (55%) and *Methanobacterium* sp. (17%).

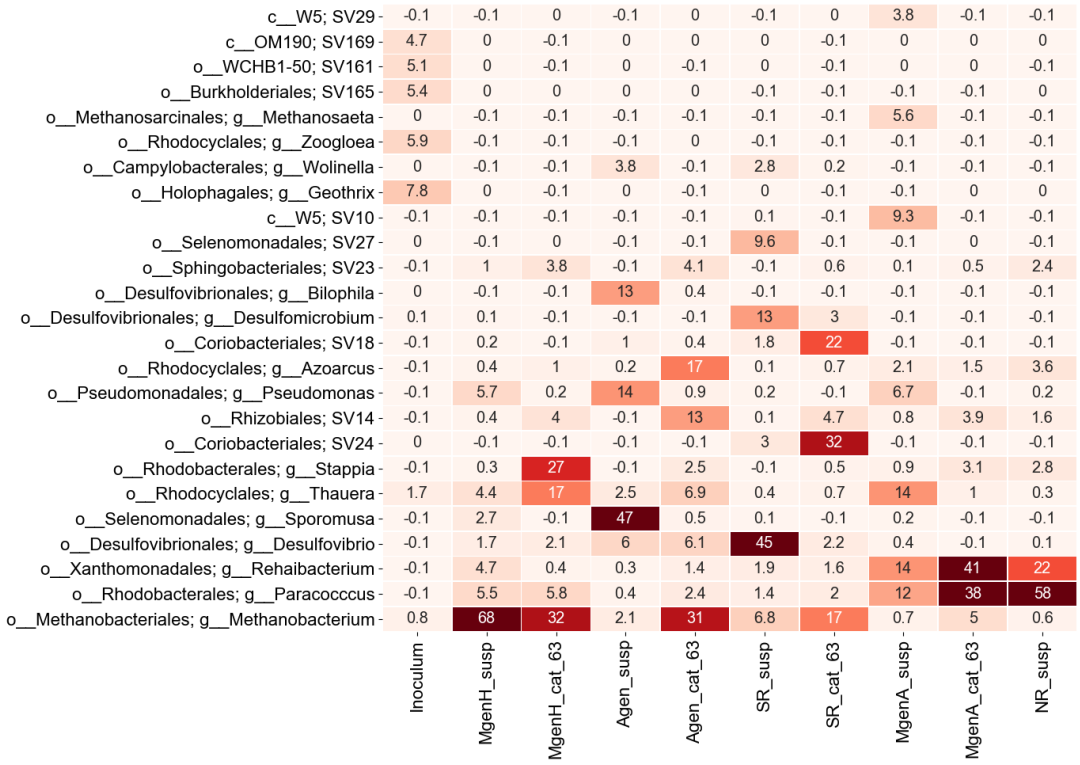

Figure 24. Relative abundance of the 25 most abundant taxa in the inoculum, enrichment, and biocathodes. SV followed by a number means that the sequence could not be classified to a known genus. -0.1 means that the relative abundance was less than 0.1% but higher than 0.

In many biocathodes, including both experiments (paper III & IV) presented in this thesis, *Methanobacterium* and *Methanobrevibacter* dominate the microbial communities (Siegert et al., 2015, Dykstra and Pavlostathis, 2017, Cheng et al., 2009, Van Eerten-Jansen et al., 2013b, Mateos et al., 2018). Previously, Cheng et al. (2009) showed that both a pure culture of *Methanobacterium palustre* and a mixed culture dominated by that archaeon could produce methane by reducing carbon dioxide, using a cathode as electron donor. Other studies have also found that *Methanobacterium* spp. and mixed cultures of hydrogenotrophic methanogens can produce methane either through direct electron transfer from the cathode or indirectly via hydrogen which are known as hydrogenotrophs (Van Eerten-Jansen et al., 2013b, Villano et al., 2010, Siegert et al., 2015). Furthermore, Aulenta et al. (2012) showed that *Desulfovibrio* sp., a known hydrogenotrophic microorganisms, can catalyze hydrogen production on biocathodes. We also showed that the biocathodes dominated by hydrogenotrophic microorganisms capable of catalyzing the cathodic reactions and can help to facilitate the start-up of the biocathodes. Indeed, the MECs operated in paper IV started to generate high current densities around 0.2 A/m^2 after addition of hydrogenotrophic microorganisms.

In summary, the biocathodes inoculated with hydrogenotrophic enrichment cultures showed better and faster electrochemical response compared to converted bioanodes capable of oxidizing acetate and glucose. This implies that the technique of cultivating microorganisms containing hydrogenases can help to facilitate start-up of the biocathodes. Moreover, it can help to reduce the transition time that is needed for the microbial communities to adopt to the cathodic conditions after changing the potential.

Chapter 5

5 Conclusions

Is it possible to store bioanodes?

- Yes, it is possible to store acetate-oxidizing bioanodes. Among the tested preservation methods, refrigeration was the best and acetone dehydration was the worst. However, we suggest that it is faster to enrich new bioanodes from a wastewater inoculum than to revive a bioanode that has been stored for five weeks. Furthermore, we observed that the microbial community was more diverse after storage compared to before storage and this seemed to negatively affect current generation.

Does starvation affect the performance of acetate- and glucose-fed bioanodes?

- Acetate- and glucose-fed bioanodes developed different microbial communities. The acetate-fed anodes were dominated by a *Desulfuromonas* sp., likely responsible for current generation. The glucose-fed anodes had a *Trichococcus* sp. and a species within *Deltaproteobacteria* in high abundance. The former likely fermented glucose into organic acids, which were used by the latter for current generation. Both communities could survive 10 days of starvation and immediate generate current once feeding was resumed. However, the acetate-fed bioanodes were more stable and only displayed a low reduction in peak current after each starvation period. The glucose-fed MFC has a more rapidly deteriorating performance throughout the experiment.

Is the transition from bioanode to biocathode possible?

- Conversion of bioanodes to biocathodes worked when bioanodes were enriched in single chamber MFCs (paper II). However, the cathodic current generation was highest the first two weeks after conversion into biocathodes, then it deteriorated until it stabilized at around 0.1 A/m^2. The bioanode enriched in a double chamber MEC (paper III) could not be converted to a biocathode. The reason could be that the dominating microorganisms on the bioanode in the MFC (*Desulfuromonas*) was different from the MEC (*Geobacter*). The microorganisms present in low abundance could also have contributed. In the single-chamber MFC, a diverse biofilm grew near that gas-diffusion cathode. This biofilm could have served as a diverse inoculum for the anode.

Do hydrogenotrophic enrichments help to facilitate start-up of biocathodes?

- Enrichment of specific cultures, for example hydrogenotrophic microorganisms, and using them as inoculum for MECs can help to facilitate start-up of biocathodes. In this study, MECs inoculated with hydrogenotrophic enrichment cultures started to generate current directly. In comparison, cathodic current generation was observed after 170 days lag time with pre-enriched acetate-oxidizing bioanodes and 80 days lag time with bare electrodes and wastewater/sludge inoculum (paper III). It seems that presence of hydrogenases, capable of catalysing the reversible reaction of $2H^+ + 2e^- \leftrightarrow H_2$ is a very important factor for start-up of biocathodes.

What about the other findings?

- Table 3 shows a summary of current generation and the dominating microorganisms on the bioanodes and the biocathodes in the MFCs/MECs presented in this thesis.

Table 3. Highlights of paper I, II, III, and IV.

	Current density	Abundant microorganisms on bioanode	Abundant microorganisms on biocathode
Acetate-fed MEC (paper I)	Anodic: 3.7 A/m^2	*Geobacter* sp.	*Acetobacterium* sp.
Acetate-fed MFC (paper II)	Anodic: 1.1-1.3 A/m^2	*Desulfuromonas* sp.	----
Glucose-fed MFC (paper II)	Anodic: 0.9-1.0 A/m^2	*Trichococcus* sp. *Desulfuromonadales*	----
Acetate-fed MFC (paper II): converted to biocathode	Cathodic: 0.1 A/m^2	-----	-----
Glucose-fed MFC (paper II): converted to biocathode	Cathodic: 0.1 A/m^2	-----	-----
MEC1 (paper III): converted to biocathode	Anodic: 2.5 A/m^2 Cathodic: 0.6-3.6 A/m^2	*Geobacter* sp.	*Methanobacterium* sp. *Acetobacterium* sp.
MEC2 (paper III): direct start-up of biocathode	Cathodic: 0.6-3.3 A/m^2	-----	*Methanobacterium* sp. *Acetobacterium* sp.
$MgenH_{MEC}$ (paper IV)	Cathodic: 0.6-0.8 A/m^2	-----	*Methanobacterium* sp.
$MgenA_{MEC}$ (paper IV)	Cathodic: 0.8-1 A/m^2	-----	*Rehaibacterium* *Paracocccus*
$Agen_{MEC}$ (paper IV)	Cathodic: 0.8-1 A/m^2	-----	*Methanobacterium* sp.
SR_{MEC} (paper IV)	Cathodic: 0.6-0.8 A/m^2	-----	*Coriobacteriaceae* *Methanobacterium* sp.

- For the acetate-oxidizing bioanodes in the MECs, the relative abundance of *Geobacter* sp., was related to current generation.
- Acetate-fed bioanodes in the MFCs were dominated by *Desulfuromonas* (62-77%) (paper II). However, the acetate-fed anodes in the MECs in paper I and III were dominated by *Geobacter*. The MFCs were operated with air-cathode and the anode in those reactors may at times have been exposed to microaerobic conditions. In contrast, the MECs were completely anaerobic. It can be speculated that this could have caused the difference in microbial community composition. Both *Desulfuromonas* and *Geobacter* belong to the *Desulfuromonadales* order, which have been found in the MFCs/MECs in other studies (Gao et al., 2017, Bond, 2010).
- Biocathodes in this thesis were dominated by *Methanobacterium* spp. (paper III and IV). *Acetobacterim* spp. (paper I and III) were also dominant, especially when methanogens were inhibited. Both genera are known as hydrogenotrophs. This shows the importance of hydrogenotrophic microorganisms for start-up of biocathodes.
- Based on the microbial community analysis, bioanodes and biocathodes are highly reproducible despite differences in the inoculum. *Desulfuromonadales* tend to be

enriched on bioanodes; *Methanobacterium* spp. and *Acetobacterium* spp. tend to be enriched on biocathodes.

- During long-term operation of biocathodes in paper III, all electrode materials had similar performance. Although carbon felt had a higher actual surface area than the graphite rod and graphite foil, it did not produce higher current.

The journey from bioanodes to biocathodes

During my PhD studies, I have learned that producing a bioanode was much easier and faster than producing a biocathode. Microbial communities developed on a bioanode were completely different from a biocathode even when both electrodes were placed in a same chamber. Functional ability and overall performance of bioanodes were completely different from biocathodes. After converting a bioanode to a biocathode, high performance could not be retained. During the conversion process, a significant transition in microbial communities took place. Consequently, new behaviours could be observed on newly converted biocathodes (i.e. current generation and chemical products). While my PhD studies was like a short magnificent journey into the world of bioanodes and biocathodes, it was a long journey for microorganisms inside an inoculum to survive either on an anode or on a cathode. This even became like an impossible survival journey for the microorganisms on an anode when the conversion to a cathode took place.

Chapter 6

6 Future research

The observations in the experiments described in this thesis have led to a number of questions that warrant further research:

- We found several groups of hydrogenotrophic bacteria on the biocathodes (paper III), however, we are not certain if they accepted electrons directly from the cathode or if H_2 served as an intermediate for production of methane and VFA.
- Biocatalyzed hydrogen production was observed on the biocathodes (paper III), but we are uncertain which microorganisms were responsible. Is it possible that the dominating hydrogenotrophic methanogens and acetogens (i.e. *Methanobacterium* spp. and *Acetobacterium* spp.) both catalysed H_2 and produced methane and acetate? Could cell debris and free enzymes from the microorganisms have catalysed H_2 generation? Further studies using pure culture could perhaps answer these questions.
- In paper II, we investigated 10 days starvation. Further studies with longer starvation intervals are necessary to determine how long the electroactive biofilms can maintain their capacities.
- In paper IV, hydrogenotrophic nitrate-reducing bacteria were enriched, however, we did not test the ability of the enrichment for start-up of biocathodes. Furthermore, there are also other hydrogenotrophic microorganisms that can use other electron acceptors (e.g. Fe^{3+}) which we did not investigate in this experiment.
- Storage and starvation experiments would also be interesting to carry out with pure cultures. Can the bioelectrochemical activity be recovered faster when there is only one species on the electrode surface?
- In the all experiments there was a possibility of oxygen leakage to the MECs/MFCs and we could not quantify the effect of this leakage on the productions and microbial community composition. For future experiments, reactors must design in a better way in order to provide complete anaerobic environment.
- Proper measurement of VFA and methane produced by biocathodes was done only in paper III. In the other experiments low amounts of O_2 may have leaked and caused oxidation of the products. Moreover, the reactors may not have been sealed properly and methane may have leaked out. Therefore, for further experiments, we suggest that the reactors must have a better designed in order to make it possible to quantify all products accurately.

Chapter 7

7 Potential applications

The research in this study is quite fundamental in nature. However, eventually it is desirable that research on BES leads to practical applications of the technology. In this section, I demonstrate four potential applications of MECs/MFCs. These applications could be solutions for energy storage and resource recovery from wastewater.

- MECs, particularly biocathodes, can be used for storing the electrical current as a biofuel (e.g. methane, acetate, and ethanol) or bioproduct. One possible bioproduct extracted from wastewater that has been studied extensively over the last decade is polyhydroxyalkanoates (PHA), which are pre-cursors for bioplastic. In conventional PHA production, microorganisms are exposed to feast and famine (feed and starvation) cycles in order to store PHA in their cells. Using a solid electrode as electron donor or electron acceptor during the feast and famine cycles could be a way of converting renewable electricity and CO_2 into PHA. This could e.g. be done via a biocathode producing H_2, CH_4, or acetate. I studied this application during my PhD, however, the production efficiency was very low and further improvement in reactor design is necessary (Saheb-Alam et al., 2017).
- The combination of MECs and anaerobic digestion is particularly interesting because with some external energy input, an MEC could improve the biogas (CH_4) yield from an anaerobic digester. Furthermore, the technique is important from an environmental perspective because it could decrease CO_2 emissions and improve degradation of organics in anaerobic digesters (Figure 25).

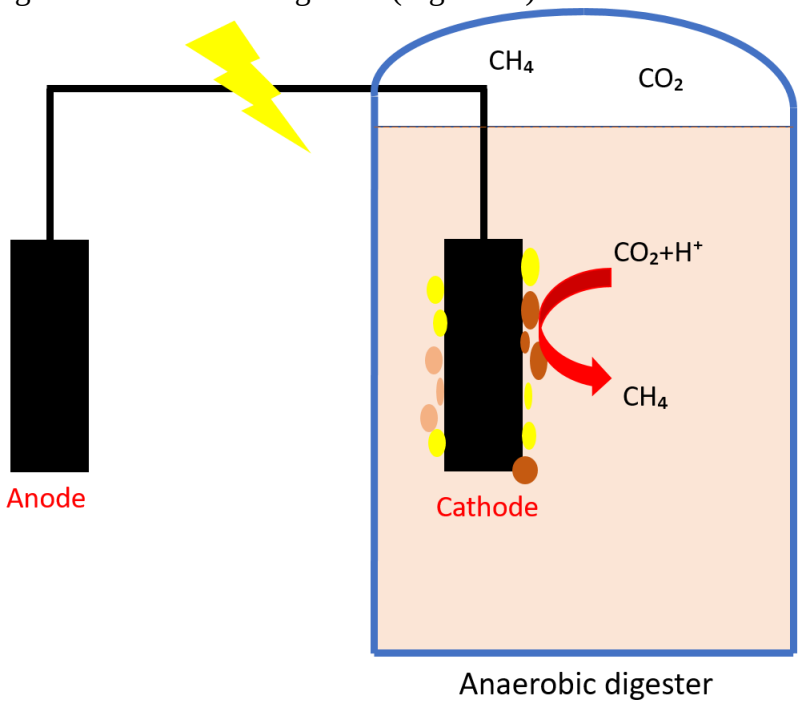

Figure 25. Schematic of a MEC using for increasing the biogas yield in anaerobic digester. CO_2 and H^+ can be converted to methane on the biocathode.

- Accumulation of VFA and concomitant decreasing pH is another common problem for operation of anaerobic digesters which often leads to reactor failure. One possible solution is to install MECs capable of oxidizing VFA on bioanodes inside the reactor. The MEC regulate the concentration of VFA whenever it reaches over the limit and also contributes to biogas generation at the cathode (Figure 26).

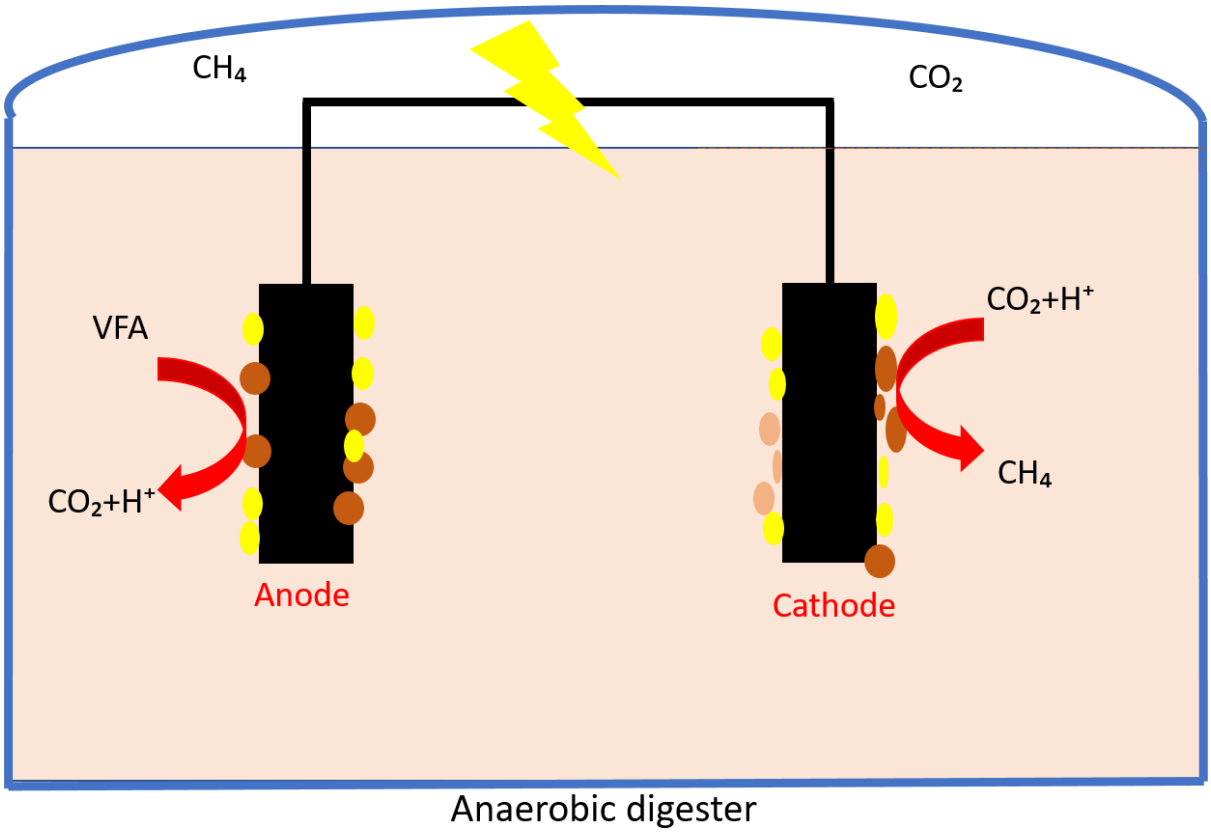

Figure 26. Schematic of a MFC using for oxidizing of VFA and consequently regulating pH in anaerobic digester.

- The standard method for measuring BOD usually takes 5 days and measurements are very uncertain. MFCs or MECs could be used as BOD sensors (Modin and Aulenta, 2017, Modin and Wilen, 2012). Bioanodes enriched by mixed microbial community has an ability of degrading a variety of organic substances. BOD concentration correlates with current generation. Using a MFC/MEC as BOD sensor is beneficial since it allows us to monitor BOD in real-time. Furthermore, MECs can be built in small scale which make it easy and convenient for practical use.

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