Bioelectrochemical methane production from CO_2

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This research was conducted under the auspices of the Graduate School Socio-Economic and Natural Sciences of the Environment (SENSE).

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 19 September 2014
at 4 p.m. in the Aula.

Mieke C. A. A. van Eerten-Jansen
Bioelectrochemical methane production from CO_2,
200 pages.

PhD thesis, Wageningen University, Wageningen, NL (2014)
With references, with summaries in Dutch and English

ISBN: 978-94-6257-006-1

Abstract

In a renewable energy system, renewable electricity that is produced in excess needs to be stored not to get lost. Besides, carbon-neutral fuels need to be produced to become independent of biomass and to avoid a further increase of CO_2-levels in the atmosphere. In this thesis, a methane-producing Bioelectrochemical System (BES) was studied: a technology that could produce CO_2-neutral methane without depending on biomass, and store excess renewable electricity in the form of methane. Key aspect of a methane-producing BES are microorganisms that grow on an electrode and catalyse the conversion of CO_2 and renewable electricity into renewable methane, the so-called methane-producing biocathode.

The aim of this thesis was to investigate the principles and perspectives of bioelectrochemical methane production, focussing on the main bottlenecks that limit system's performance. We show that the technology could be operated long-term (188 days) at a methane production rate of 6 L CH_4/m^3 reactor per day and energy efficiency of 3.1% at -0.55 V vs. NHE cathode potential. Internal resistance analysis showed that most energy was lost at the cathode. At the conditions studied, methane was mainly produced indirectly using hydrogen as electron donor. Bacteria that were present in the mixed culture biocathode likely produced the hydrogen. Based on the results obtained in this thesis, we discuss a possible first application of a methane-producing BES: upgrading CO_2 in biogas of anaerobic digestion to additional methane.

We show that BESs can also be used to produce medium chain fatty acids from acetate, using electricity as the electron donor. Selectivity and purification of the products are bottlenecks that need to be overcome for this application of the BES.

Keywords: bioelectrochemical system, CO_2 reduction, Microbial Electrolysis Cell, methane-producing biocathode, methane, undefined mixed cultures, carboxylic acids, Microbial Fuel Cell, MFC, MEC, BES, biomass efficiency

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Chapter 1

Introduction

1.1 Background

Our energy is nowadays mainly derived from fossil fuels

A growing world population and an increase in wealth worldwide has led to a growth in world-wide energy use from 256 EJ/year in 1973 to 532 EJ/year in 2010 [70], and is expected to grow to 623-710 EJ/year in 2035 [70] and to 802-867 EJ/year in 2050 [52, 85, 105, 129]. In 2010, the world energy consumption was mainly derived from fossil fuels (81.1%), of which oil accounted for 32.4%, coal/peat accounted for 27.3%, and natural gas accounted for 21.4% [70]. In 2035 and 2050, it is expected that energy remains to be mainly derived from fossil fuels, with fossil fuels contributing to 62-75% of the energy supply in 2035 [70] and to 65-79% of the energy supply in 2050 [52, 129]. Using fossil fuels has several drawbacks: (i) fossil fuels are consumed at a higher rate than they are generated, resulting in depletion of fossil fuels, (ii) consequently, carbon dioxide (CO_2) is emitted at a higher rate than its sequestration in fossil fuels, resulting in increased CO_2 concentrations in the atmosphere that could possibly lead to climate change, (iii) upon using fossil fuels pollutants are emitted, such as fine dust particles, NOx and SOx, being detrimental for the environment and human health, and (iv) fossil fuels are unevenly distributed, resulting in countries being dependent on sometimes politically instable regions [105]. Besides, fossil fuel prices are expected to rise [19], causing economic problems in non-fossil fuel-producing countries, such as countries in the EU or Japan. For reasons of energy security and environmental sustainability, there is therefore a need to search for renewable alternatives for energy supply [36]. To promote the use of renewable resources in our energy supply, the European Union developed an EU Energy Security and Solidarity Action Plan that states that in 2020 20% of the produced energy in the EU should be derived from renewable resources [41].

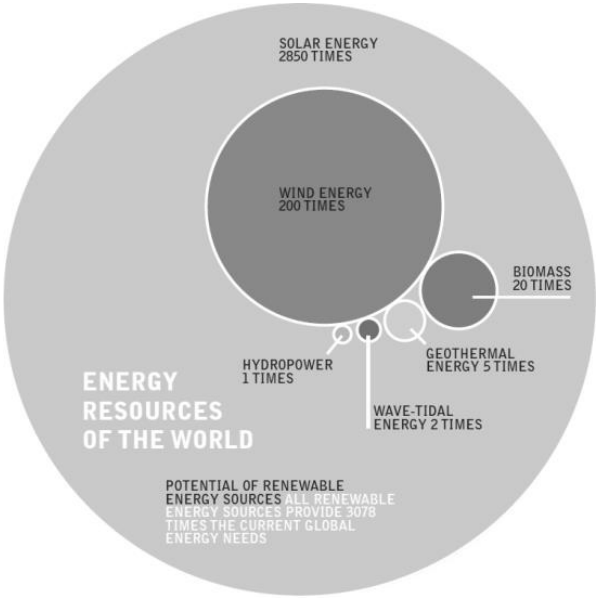

Figure 1.1. Potential of renewable resources to provide the 2005 global energy needs (from [51], reprinted with permission).

Renewable resources have the potential to fulfil our energy demand

Greenpeace has investigated the energy potential of a wide variety of renewable resources, such as solar, wind, biomass, geothermal, wave-tidal, and hydropower, and estimated that renewable resources have the potential to supply yearly 3078 times more energy than the 2005 global energy needs (Figure 1.1) [52]. Lewis and Nocera calculated that the sun can provide more energy in 1 hour to the earth than all energy consumed by humans in an entire year [85]. Despite the large potential of renewable resources to fulfil our energy demand, renewable resources accounted for only 13% of the 2008-2010 global energy supply [6, 52, 70]. Factors that limit implementation of renewable resources into our energy supply are economic factors, public acceptance,

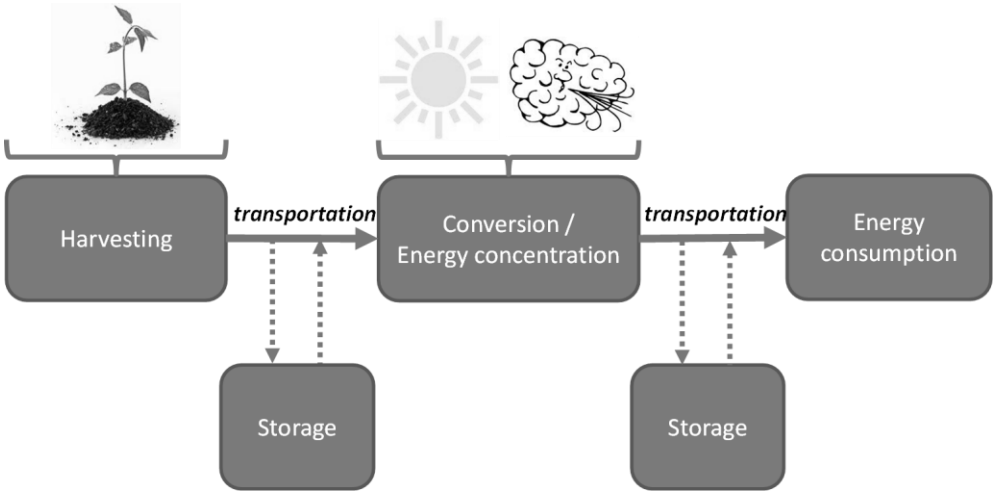

Figure 1.2. The renewable energy supply chain. Energy derived from renewable resources needs to be harvested, converted, transported and/or stored into an energy carrier that can be used at the right time and place for its end users. The energy carrier should preferably have a high energy density (energy concentration). Contrary to for instance solar and wind energy, biomass does not need to be converted into another energy carrier upon harvesting to be able to be stored.

sustainability concerns, system integration and infrastructure constraints, and the technical inaccessibility of the renewable resources due to for instance impermeable strata or limited availability of fertile land area [71]. Nevertheless, renewable resources that are technically accessible and economically feasible to harvest globally have been estimated to still count for 1.9-63 times more energy than the global energy needs [105]. Energy derived from renewable resources, however, is often not supplied in the right form, at the right time and/or at the right place to be consumed by its end users. For example, solar energy can only be harvested during daytime (time dependence) and solar electricity needs to be stored not to get lost (type of energy carrier dependence, see also the next paragraph), and for biomass production fertile land area is required (location dependence). For renewable resources to be integrated in our energy system, the energy contained in renewable resources should be converted and concentrated in an energy carrier that can be transported to the consumer, can be

stored when supply exceeds demand, and can be used for consumption by its end users (Figure 1.2) [98].

Moving from a chemical-based energy system towards an electrified energy system

A wide range of renewable energy technologies currently exists that can convert, transport, and store the energy contained in renewable resources into a useful energy carrier, that is hot bodies (e.g. hot air or water), electricity, and fuels (Table 1.1). Table 1.1 shows that all energy carriers currently consumed (hot bodies, electricity, and fuels) can be produced from renewable resources. Electricity is the only energy carrier that can be produced from all renewable resources, while fuels, at this point, can only be produced directly from biomass. Using renewable resources for energy production mismatches our current energy system, which is mainly based on the consumption of chemical energy carriers in the form of (fossil) fuels. To come to a renewable energy system, our current energy system is expected to move from a mainly chemical-based energy system towards a mainly electricity-based energy system [98]. One of the challenges in using renewable electricity as the main energy carrier in an energy system is that electricity from most renewable resources is produced intermittently. As a consequence, excess electricity, when supply does not meet demand, needs to be stored not to get lost [57, 98, 113]. There are currently several technologies via which electricity can be stored, for instance batteries, pumped hydro energy storage, compressed air energy storage, hydrogen production, and flywheels [57]. These technologies store electrical energy by converting it into another energy form, such as chemical energy (e.g. batteries, and hydrogen), potential energy (e.g. pumped hydro energy storage), mechanical and thermal energy (e.g. compressed air energy storage), and kinetic energy (e.g. flywheels), and convert the obtained energy forms back into

Table 1.1. Overview of renewable energy technologies that can directly convert the energy contained in renewable resources (left column) into a useful energy carrier, that is hot bodies, electricity, and fuels (top row). The conversion of energy contained in renewable resources via a multitude of renewable energy technologies (indirect conversion) have been excluded in this overview, but would increase the number of options for converting renewable resources into useful energy carriers.

Renewable resources	**Hot bodies (e.g. hot air or water)**	**Electricity**	**Fuels**
Sun	Direct/Passive heating Solar collector	Photovoltaics	-
Wind	-	Wind turbines	-
Biomass	Incineration	Bioelectrochemical Systems	Landfill gas production Anaerobic digestion Fermentation Pyrolysis Biodiesel production
Geothermal	Direct/Passive heating Aquifer Thermal Energy Storage Combined Heat and Power	Geothermal power plants	-
Ocean	-	Tidal power Wave power	-
Hydropower	-	Hydropower: potential-driven Hydropower: kinetic-driven	-
Salinity gradient	-	Pressure-retarded Osmosis Reversed Electrodialysis	-

electrical energy again when demand exceeds supply. The use of these electricity storage technologies is, however, criticized due to the use of precious metals, their costs, their impact on the environment, their efficiency, their lifetime and/or their limited capacity [57]. To assure energy security in an energy system with electricity as the main energy carrier, storage of renewable electricity is a key challenge in the future [113].

Renewable fuels are currently only produced directly from biomass

Renewable fuels are currently only produced directly from biomass (Table 1.1). Biomass can be converted into biogas consisting of ~40-60% methane via landfill, or consisting of ~60% methane and ~40% CO_2 via anaerobic digestion, into syngas consisting of H_2 and CO via pyrolysis, into a variety of alcohols and fatty acids via fermentation, and into biodiesel via transesterification (Table 1.1). Production of renewable fuels, such as hydrogen or hydrocarbons, via photocatalysis using solar radiation as energy source is still under development and not yet applied at full-scale (e.g. [12, 58]).

Biomass is a versatile source, and can be used for food, fuel, material and chemical production. However, there are discussions about whether sufficient biomass can be produced in a sustainable way to cover all the world's needs [25, 39, 43, 108, 160]. In other words, there are discussions whether there is enough land, water and nutrients to produce sufficient biomass in a sustainable way [108]. Biomass is currently the most used renewable energy resource, contributing to 52 EJ/year in 2009 (76-77% of the global renewable energy production in 2009-2010) [52, 70], and is expected to grow to 81-120 EJ/year in 2050, an increase of 55-131% [25, 52]. Although biomass is currently the most used renewable energy resource, renewable fuel production from biomass is still limited. Biomass is currently mainly landfilled or incinerated to produce

heat and electricity [25]. For a renewable energy system it is thus essential to make efficiently use of biomass and to develop renewable energy technologies that can convert non-biobased renewable resources into fuels.

Bioelectrochemical Systems to convert electricity into fuels

For a renewable energy system it is thus crucial that (excess) renewable electricity is stored not to get lost, and that new renewable energy technologies are developed that can produce fuels without depending on biomass. Bioelectrochemical systems (BESs) are a novel technology that meets both criteria. Key principle of a BES is the use of microorganisms as catalysts for a wide diversity of oxidation and reduction reactions.

The field of bioelectrochemistry started after the discovery that microorganisms can directly transfer electrons to electrodes [115]. A more recent finding is electron transfer in the opposite direction, from electrode to microorganism [53]. By powering microorganisms with electricity, a wide range of applications are possible, such as bioremediation of metal and organic contaminants, and microbial electrosynthesis, that is the production of fuels and chemicals [62, 93]. Using microorganisms as catalysts on electrodes instead of chemical catalysts is more sustainable, as they are self-regenerating, the BES can be operated at mild operating conditions (at neutral pH and mesophilic temperatures), and non-noble, cheap electrodes can be used [8, 31]. To date, microbial electrosynthesis in BESs has led to the production of a wide variety of fuels and chemicals, such as hydrogen [87, 130], methane [23], hydrogen peroxide [133], acetate and 2-oxobutyrate [107], alkalinity [117], ethanol [151], butyrate [24], and ammonium [80].

The conversion of CO_2 into methane is an attractive application of microbial electrosynthesis [16, 23] (Figure 1.3). Advantages of this application are that CO_2-neutral methane could be produced, independent of biomass. Additionally, methane

can be used as an energy carrier for renewable electricity from sun and wind, when supply exceeds demand [23]. Moreover, compared to landfill gas or biogas of anaerobic digestion a gas enriched in methane could be obtained that does not require further processing to be directly injected in the national gas grid [91]. Finally, the infrastructure for transport, storage and consumption of methane is already in place [23].

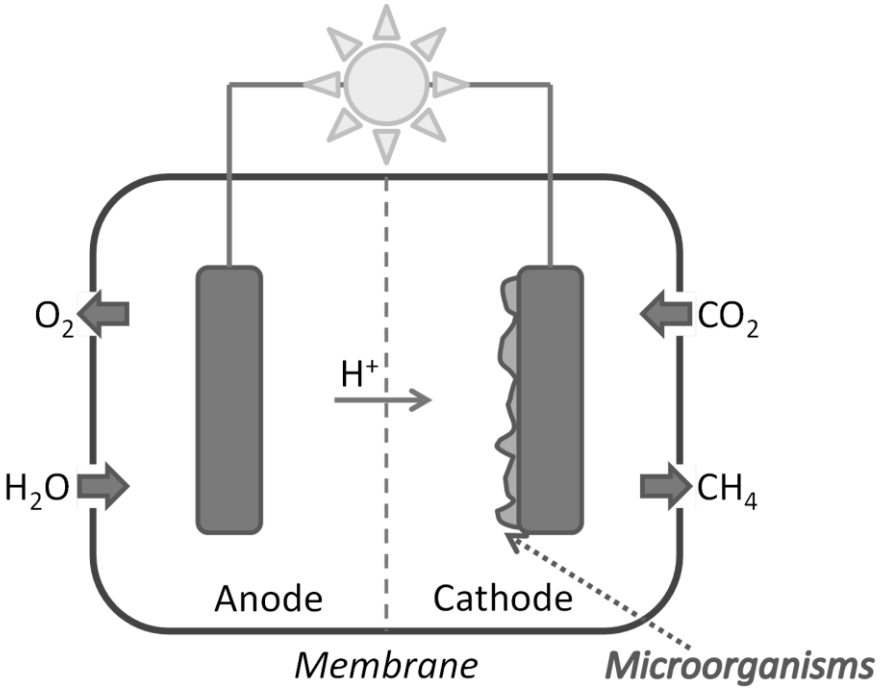

Figure 1.3. A methane-producing bioelectrochemical system consists of two electrodes, anode and cathode, separated by a membrane to produce pure products [59]. At the anode, water is oxidized to yield oxygen, protons and electrons. The electrons are transferred to the anode, and flow via an external electrical circuit to the cathode, while the protons migrate through the membrane towards the cathode to maintain charge neutrality. At the cathode, microorganisms that grow on the electrode catalyse the reduction of CO_2, together with the protons and electrons, to methane. Bioelectrochemical methane production requires an input of electrical energy, as depicted by the sun.

1.2 Aim of this thesis

A methane-producing BES holds promise as a renewable energy technology that could produce CO_2-neutral methane without depending on biomass, and store excess renewable electricity in the form of methane. A methane-producing BES is, however, a new technology that was still at its infancy at the start of this thesis. For a methane-producing BES to be applied into a renewable energy system, a more thorough understanding of the technology is needed. Therefore, the aim of this thesis was to investigate the principles and perspectives of bioelectrochemical methane production from CO_2. Focus was on the main bottlenecks limiting system's performance.

1.3 Methane production from CO_2 in Bioelectrochemical Systems

Principle of a methane-producing Bioelectrochemical System

A methane-producing BES consists of two electrodes, cathode and anode, separated by a membrane to produce pure products [59] (Figure 1.3). At the anode, an oxidation reaction takes place yielding, amongst others, protons and electrons. Renewable electron donors that can be oxidized at the anode are water (*e.g.* [23]), inorganic compounds such as sulphide [119], and a variety of organic compounds in for instance wastewaters, sediments and sludges, processed energy crops, plant rhizodeposits, and food and garbage slurries (*e.g.* [17, 88, 122, 123, 152]). The most commonly used renewable electron donors in methane-producing BESs are organics in (waste)water (typically represented by acetate, CH_3COO^-) that are oxidized by microorganisms growing at the anode, and water itself, which is oxidized via an electrolysis reaction. It should be noted that using organics in (waste)water as electron donor, bioelectrochemical methane production is dependent on biomass. Therefore, water was used as electron donor in this thesis. Water is oxidized at the anode

according to

$$4H_2O \rightarrow 2O_2 + 8H^+ + 8e^- \quad (1.1).$$

The electrons are transferred to the anode, and flow via an external electrical circuit to the cathode, while protons/cations migrate through the membrane towards the cathode to maintain charge neutrality. At the cathode, microorganisms that grow on the electrode catalyse the reduction of CO_2, together with the protons and electrons, to methane, according to

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (1.2).$$

CO_2 in this equation resembles all forms of carbonic acid: CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}.

The overall reaction of converting CO_2 to methane using water as electron donor is depicted in eq. 1.3.

$$CO_2 + 2H_2O \rightarrow CH_4 + 2O_2 \quad (1.3)$$

Thermodynamics of a methane-producing Bioelectrochemical System

The bioelectrochemical reactions described in the previous paragraph require energy to occur. The maximum amount of energy that needs to be supplied to let the bioelectrochemical reactions occur, can be described by the Gibb's free energy, according to

$$\Delta G_r = \Delta G_r^0 + RTln(\Pi) \quad (1.4)$$

where ΔG_r is the Gibb's free energy of the reaction (J) at specified conditions, ΔG_r^0 is the Gibb's free energy of the reaction (J) at standard conditions (T = 298.15 K, and 1 M or 1 bar for all species in the reaction), R is the universal gas constant (8.314 J/mole per K), T is the temperature (K), and Π is the reaction quotient (unitless).

For a reaction that is represented by v_A A + v_B B → v_C C + v_D D, the reaction quotient in dilute systems, as is the case for BES, is defined as

$$\Pi = \frac{[C]^{v_C} \cdot [D]^{v_D}}{[A]^{v_A} \cdot [B]^{v_B}} \quad (1.5)$$

where [i] is the concentration of species i, and v_i is the reaction coefficient of species i [9]. Note that in the case of half reactions, the reaction should be written as a reduction reaction, that is as an electron-consuming reaction.

The Gibb's free energy of reactions at standard conditions is tabulated (e.g. [4, 159]), or can be calculated using tabulated Gibb's free energies of formation of all species involved in the reaction (e.g. [4, 159]). In the latter case, the Gibb's free energies of formation of the oxidized species is subtracted from the Gibb's free energies of formation of the reduced species, taking into account the stoichiometry. A negative Gibb's free energy of reaction means that the reaction occurs spontaneously, while a positive Gibb's free energy of reaction means that additional energy is required for the reaction to occur.

In the field of bioelectrochemistry, it is more common to convert the Gibb's free energy of reaction to an electrode potential, the driving force of the reaction. The electrode potential of a reaction can be calculated according to

$$E = \frac{-\Delta G_r}{n \cdot F} \quad (1.6)$$

where E is the electrode potential needed for the reaction to occur (V), n is the number of moles involved in the reaction (mole e^-), and F is Faraday's constant (96485 C/mole e^-).

Similarly, the electrode potential at standard conditions can be calculated

$$E^0 = \frac{-\Delta G_r^0}{n \cdot F} \quad (1.7)$$

where E^0 is the electrode potential at standard conditions (V).

Combining equations 1.4, 1.5, 1.6 and 1.7, yields the Nernst-equation

$$E = E^0 + RTln(\frac{[C]^{\nu_C} \cdot [D]^{\nu_D}}{[A]^{\nu_A} \cdot [B]^{\nu_B}}) \quad (1.8),$$

via which directly the electrode potential of a bioelectrochemical reaction can be calculated. The electrode potentials are reported versus a Normal Hydrogen Electrode (NHE), an electrode that is defined to have 0 V electrode potential at standard conditions (298.15 K, pH_2 = 1 bar, $[H^+]$ = 1 M). From the anode and cathode electrode potentials, the thermodynamic cell voltage (E_{cell}, V) can be calculated according to

$$E_{cell} = E_{cat} - E_{an} \quad (1.9).$$

Analogous to the Gibb's free energy of reaction, a positive cell voltage means that the reaction occurs spontaneously, while a negative cell voltage means that additional energy is required for the reaction to occur.

The Gibb's free energy of reaction and the electrode potentials of the oxidation and reduction reactions that occur in a methane-producing BES are reported in Table 1.2. The reduction of CO_2 to methane occurs at a lower electrode potential than the oxidation of water. The thermodynamic calculations also reveal that the transfer of electrons from water to CO_2 to form methane is a non-spontaneous process, as indicated by a positive Gibb's free energy of reaction and a negative thermodynamic cell voltage (Table 1.2), meaning that an additional electrical energy input is required. The added electrical energy is not lost, but the electrical energy is converted into energy carrier methane. When methane is combusted back to CO_2, this energy is

Table 1.2. The electrode potential of the oxidation and reduction reactions of a methane-producing BES calculated with the Nernst equation (eq. 1.8) at standard conditions (298.15K and 1M/1 bar for all species in the half reaction) and at pH 7.

Anode oxidation reaction	**ΔG_r (kJ/reaction)**	**E^0 (V vs. NHE)**	**E (V vs. NHE)**
$2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$	-629.8	1.23	0.82
Cathode reduction reaction		**E^0 (V vs. NHE)**	**E (V vs. NHE)**
$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$	188.2	0.17	0.24
Cell voltage		**E^0 (V)**	**E (V)**
$CO_2 + 2H_2O \rightarrow CH_4 + 2O_2$	818.0	-1.06	-1.06

released (eq. 1.10 depicts methane combustion, which is the opposite of bioelectrochemical methane production using water as electron donor (eq. 1.3)).

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (1.10)$$

In reality, however, for bioelectrochemical methane production (eq. 1.3) to occur in a bioelectrochemical system, more electrical energy than the Gibb's free energy of reaction or the thermodynamic cell voltage needs to be added, due to energy losses in the BES as is explained below.

Performance of a methane-producing Bioelectrochemical System

The performance of BESs is often indicated by two key characteristics: the production rate and the energy efficiency, the efficiency of capturing the added electrical energy into the formed fuel (chemical energy). As methane production is directly linked to electron transfer from the anode to the cathode (assuming all electrons end up in methane), the current density, the rate of electron transfer, is a commonly used parameter to approximate the methane production rate. Methane production rate, energy efficiency and current density are used throughout this thesis to indicate the performance of methane-producing BESs.

The methane production rate is influenced by, for instance, reactor design, the material properties and design of the electrodes, the microbial community at the cathode, and the operational conditions (e.g. [27, 90, 111]). How these factors affect the methane production rate is investigated and discussed throughout this thesis.

The energy efficiency of a methane-producing BES is the product of coulombic efficiency and voltage efficiency [59]. Coulombic efficiency is the efficiency of capturing the electrons from the electric current and from the reduced electron donor at the anode in methane. Other microbial processes that compete for electrons and

biomass growth lower the coulombic efficiency as electrons are lost for methane production [90]. Also cross-over of products (through the membrane) towards the other compartment lower the coulombic efficiency [27]. Voltage efficiency is the amount of external electrical energy that ends up in the methane. Energy losses that cause the voltage efficiency to decrease are: (i) ohmic losses, the resistance to the flow of electrons through the electrodes and the electrical circuit, and to the flow of ions through the electrolytes and membrane, (ii) activation losses, energy needed for an oxidation/reduction reaction to occur at the electrode, (iii) microbial metabolic losses, energy needed by the microbial community for maintaining their metabolism, and (iv) concentration losses, the development of concentration gradients of substrates towards or products away from the electrode that limit current production [90]. Most of these energy losses are current dependent: the higher the current density (and thus methane production rate) is, the higher these energy losses are [89]. Consequently, at increasing current densities, the energy input per m^3 methane increases and the energy efficiency decreases [89]. In a BES thus a trade-off exists between production rate and energy efficiency: a higher electrical energy input results in a higher production rate, but also in a lower energy efficiency, and vice versa. Minimizing the energy input per m^3 methane, while maintaining an acceptable methane production rate is thus essential for application of a methane-producing BES. Investigating the energy losses of a methane-producing BES in order to minimize them is of utmost importance for applying the technology.

1.4 Thesis outline

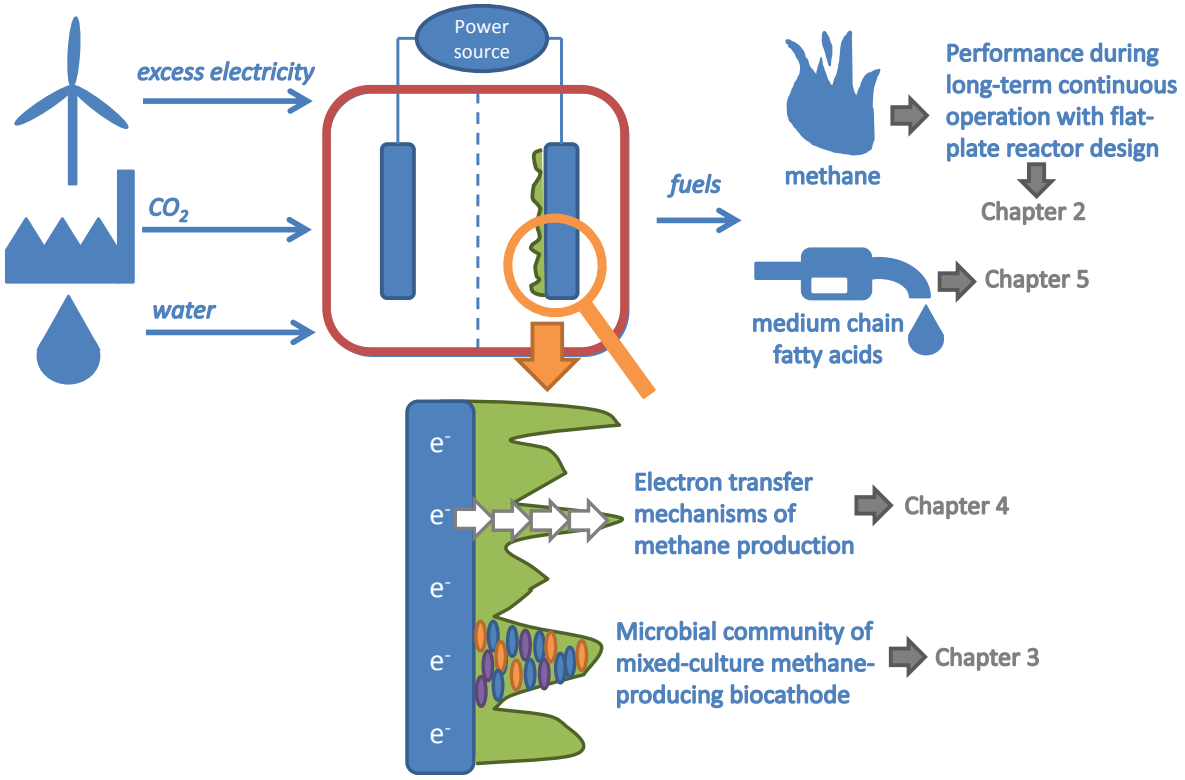

Figure 1.4. Schematic representation of the outline of this PhD thesis. In this thesis the principles and perspectives of bioelectrochemical methane production from CO_2 were investigated. Focus was on the main bottlenecks limiting system's performance, such as reactor design and operation conditions (**Chapter 2**), the microbial community of the methane-producing biocathode (**Chapter 3**), and the electron transfer mechanisms of bioelectrochemical methane production (**Chapter 4**). Besides, bioelectrochemical production of medium chain fatty acids was investigated as an alternative microbial electrosynthesis process (**Chapter 5**).

The aim of this thesis was to investigate the principles and perspectives of bioelectrochemical methane production from CO_2 with focus on the main bottlenecks that limit system's performance. In **Chapter 2** we demonstrate long-term and continuous bioelectrochemical methane production in a flat-plate BES design with two different electron donors at the anode and at different applied cathode potentials.

An internal resistance analysis was performed to identify factors that limit performance. Since microorganisms are the catalysts for bioelectrochemical methane production, in **Chapter 3** the microbial community of a mixed culture methane-producing biocathode was investigated, and the role of the detected community members in methane production was discussed. The electron transfer mechanisms for bioelectrochemical methane production, and thus the role of *Bacteria* in methane production, were investigated in **Chapter 4** by selectively inhibiting the methanogenic *Archaea*. In **Chapter 5**, it was investigated whether cathodes could also be used to supply electrons or hydrogen *in-situ* to mixed cultures to bioelectrochemically produce medium chain fatty acids from acetate. Finally, in **Chapter 6** the present performance of methane-producing BESs is evaluated. The main bottlenecks that limit system's performance as found in this thesis, are discussed. The feasibility of production of methane and higher-value organics, such as medium chain fatty acids, in BES will be discussed.

Chapter 2

Microbial Electrolysis Cells for production of methane from CO_2: long-term performance and perspectives

This chapter has been published as:

Mieke C. A. A. Van Eerten-Jansen, Annemiek Ter Heijne, Cees J. N. Buisman, Hubertus V. M. Hamelers (2012), *Microbial Electrolysis Cells for production of methane from CO_2: long-term performance and perspectives*, International Journal of Energy Research 36 (6): 809-819, doi 10.1002/er.1954.

Abstract

A methane producing Microbial Electrolysis Cell (MEC) is a technology to convert CO_2 into methane, using electricity as an energy source and microorganisms as the catalyst. A methane producing MEC provides the possibility to increase the fuel yield per hectare of land area, when the CO_2 produced in biofuel production processes is converted to additional fuel methane. Besides increasing fuel yield per hectare of land area, this also results in more efficient use of land area, water, and nutrients. In this research, the performance of a methane producing MEC was studied for 188 days in a flat plate MEC design. Methane production rate and energy efficiency of the methane producing MEC were investigated with time to elucidate the main bottlenecks limiting system performance. Using water as the electron donor at the anode during continuous operation, methane production rate was 0.006 m^3/m^3 per day at a cathode potential of -0.55 V vs. NHE with a coulombic efficiency of 23.1%. External electrical energy input was 73.5 kWh/m^3 methane, resulting in a voltage efficiency of 13.4%. Consequently, overall energy efficiency was 3.1%. The maximum achieved energy efficiency was obtained in a yield test and was 51.3%. Analysis of internal resistance showed that on the short-term, cathode and anode losses were dominant, but with time, also pH gradient and transport losses became more important. The results obtained in this study are used to discuss the possible contribution of methane producing MECs to increase in fuel yield per hectare of land area.

Keywords: Microbial Electrolysis Cell, CO_2 reduction, methane, biomass efficiency, land use

2.1 Introduction

There is a societal need for the production of fuels from alternative, renewable energy sources to substitute fossil fuels. Organic material (biomass) is an attractive feedstock for the production of biofuels, as it is often locally available, it could contribute to reducing greenhouse gas emissions when produced and utilized in a sustainable way, and biomass can be easily stored [66]. The current biofuel debate, however, shows that it is questionable whether sufficient biomass can be produced in a sustainable way to cover all the world's fuel needs [39, 43]. To achieve a higher and more sustainable biofuel production, it is therefore of importance that the fuel yield per hectare of the available land area should be increased, while water and nutrients should be used as efficiently as possible [108].

The amount of biomass used for energy production has increased considerably from 648 Mtoe in 1973 to 1179 Mtoe in 2007 [68]. In the conversion of biomass into biofuel, e.g. fermentation of sugars into ethanol or anaerobic digestion of acetate into methane, most of the oxygen atoms present in organic material need to be removed to produce a high energy density fuel. These oxygen atoms are removed in the form of CO_2 [110]. For example, in case of fermentation of sugars into ethanol, for each mole of ethanol produced, 1 mole of CO_2 is formed. Similarly, in case of anaerobic digestion of acetate into methane, for each mole of methane produced, 1 mole of CO_2 is formed. In this process of removing oxygen atoms in the form of CO_2 part of the carbon present in biomass is lost [110]. If the by-product CO_2 is converted into additional fuel, this would increase the fuel yield per hectare of land area. This increases not only the fuel yield land use efficiency, but also the efficiency of use of nutrients and water. This research therefore investigates whether a Microbial Electrolysis Cell (MEC) is a suitable technology to convert CO_2 to additional fuel.

An MEC is a novel technology that uses renewable electricity as the energy source

for the production of fuels and chemicals, such as hydrogen [132], ethanol [151], and hydrogen peroxide [133]. A recently developed application for MECs is to produce methane from CO_2 using microorganisms as the catalyst, with input of electrical energy [23]. A methane producing MEC consists of two electrodes, anode and cathode, separated by a membrane. The membrane is essential to produce pure products [59]. At the anode, an oxidation reaction takes place, e.g. the oxidation of acetate or water, yielding CO_2 or O_2 respectively, and protons and electrons. Electrons are released to the anode and flow through an external electrical circuit to the cathode, while protons and cations migrate through the membrane to the cathode to maintain electroneutrality. At the cathode, the protons and electrons are used to produce methane. The reaction at the cathode is catalysed by electrochemically active microorganisms, i.e. hydrogenotrophic methanogens [23], and the cathode is therefore called a biocathode. In a methane producing MEC, the overall reaction is thermodynamically not favourable and needs electrical energy to drive the reaction [90].

Hydrogenotrophic methanogens can catalyse methane production from CO_2 in an MEC via two mechanisms: (i) direct extracellular electron transfer (eq. 2.1), i.e. the electrons are directly taken up from the electrode and used to reduce the CO_2 to methane,

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$

(E_{cat} = -0.24 V vs. NHE [23]) (2.1)

All reported potentials are standard potentials under biological relevant conditions at pH 7 and 25°C.

and (ii) indirect extracellular electron transfer (eq. 2.2 and 2.3), i.e. with intermediate production of hydrogen [23, 168]. In this mechanism first hydrogen is produced at the cathode either electrochemically or bio-electrochemically (eq. 2.2).

$2H^+ + 2e^- \rightarrow H_2$ (E_{cat} = -0.41 V vs. NHE [89]) (2.2)

This hydrogen is used together with CO_2 to produce methane (eq. 2.3).

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (2.3)

Methane production via direct extracellular electron transfer is considered the most energy-efficient process, as the standard potential of hydrogen production via indirect extracellular electron transfer (E_{cat} = -0.41 V vs. NHE) is lower than of methane production via direct extracellular electron transfer (E_{cat} = -0.24 V vs. NHE). The protons and electrons needed for the reduction reaction at the cathode are produced by oxidizing water at the anode (eq. 2.4).

$4H_2O \rightarrow 2O_2 + 8H^+ + 8e^-$ (E_{an} = 0.81 V vs. NHE [90]) (2.4)

The overall process is the production of methane from CO_2 and water (eq. 2.5).

$CO_2 + 2H_2O \rightarrow CH_4 + 2O_2$ (E_{cell} = -1.05 to -1.22 V vs. NHE) (2.5)

The minimum thermodynamic energy input required is thus achieved when employing direct reduction of CO_2 to methane, and is 32.7 MJ electrical energy per m^3 of methane (9.1 kWh/m^3 methane) under biologically relevant conditions (pH 7 and 25°C).

This paper studies whether a methane producing MEC is a suitable technology for increasing the fuel yield per hectare of land area. Previous studies on methane producing MECs focused on the mechanism of methane production and showed that direct reduction of CO_2 to methane coupled to water oxidation is feasible with an additional energy input on top of the thermodynamic energy input [23, 168]. However, the different types of energy losses occurring in the MEC that lead to this additional energy input were not extensively studied. Insight in these energy losses is essential to improve the performance of methane producing MECs, and consequently to increase the fuel yield per hectare of land area. This study therefore investigated the nature and extent of energy losses in a methane producing MEC and their effects on MEC energy

efficiency and methane production rates.

The oxygen produced at the anode could negatively affect the performance of methane producing MECs. The oxygen might diffuse to the cathode [78] and could lead to parasitic reactions either via direct reduction to water at the cathode or via oxidizing the methane produced. Oxygen is also known to inhibit hydrogenotrophic methanogens [47] and might decrease the methane production rate. To understand a possible negative role of oxygen, the methane producing MEC was first operated for 83 days using hexacyanoferrate(II) oxidation at the anode and subsequently for 105 days using water oxidation at the anode. The performance of the biocathode in both periods was analysed using polarization curves and yield tests. The overall performance of the methane producing MEC was determined by analysing the resistances of the different elements of the methane producing MEC, i.e. anode, cathode, membrane, and electrolyte, and by analysing the overall energy efficiency. The results obtained in this study were finally used to discuss the possible contribution of methane producing MECs to increase in fuel yield per hectare of land area.

2.2 Materials and methods

Experimental set-up

The experiment was performed in the same electrochemical cell as described in Sleutels *et al.* [144] with a total volume of 0.56 L using a cation exchange membrane (0.7 mm, Ralex CM, Mega a.s., Czech Republic). The anode was made of platinum coated (50 g/m^2) titanium mesh (projected surface area 250 cm^2, thickness 1 mm, specific surface area 1.7 m^2/m^2 – Magneto Special Anodes BV, The Netherlands). The cathode was made of graphite felt (projected surface area 0.025 m^2, thickness 3 mm – FMI Composites Ltd., Scotland). Both the anode and cathode compartment were equipped with an Ag/AgCl 3 M KCl reference electrode (+0.205 V vs. NHE; ProSense QiS, The Netherlands). The electrochemical cell was connected to a PC via a Fieldpoint FP-AI-110 module (National Instruments, United States) and every 60 seconds cell voltage, current, and cathode and anode potential were recorded using LabVIEW 7.1 (National Instruments, United States). A luminescent dissolved oxygen probe (LDO10101, Hach, USA) was installed in the catholyte, and every 30 minutes dissolved oxygen in the catholyte was measured. The system was operated in a temperature controlled chamber at 30ºC.

Electrolytes and microorganisms

Oxygen, product of water oxidation at the anode, can possibly affect methanogens at the cathode [47]. To prevent oxygen to affect biofilm development at the cathode at the start of the experiment, hexacyanoferrate(II) was oxidized at the anode. Because hexacyanoferrate(II) is not sustainable as it is not self-regenerating [90], it was changed to water oxidation on day 83. At start, anolyte consisted of 100 mM potassium hexacyanoferrate(II) and was circulated at 1.5 mL/s. The anolyte was refreshed

regularly to avoid depletion of electron donor. The anolyte was changed to demineralized water containing 20 mM potassium phosphate buffer on day 83. The catholyte influent consisted of a 20 mM potassium phosphate buffer, macronutrients (280 mg/L NH_4Cl, 5.7 mg/L $CaCl_2$, 10 mg/L $MgSO_4 \cdot 7H_2O$, 90 mg/L $MgCl_2 \cdot 6H_2O$), and 1 mL/L of a micronutrients and vitamin solution same as [155]. The catholyte influent was supplemented with 5 g/L $NaHCO_3$ as carbon source, as at operating pH 7 CO_2 is mainly present as HCO_3^-, and circulated at 1.5 mL/s. The catholyte was continuously refreshed at a rate of 0.1 mL/min (Stepdos 03RC, KNF, Germany). 5 gram of anaerobic sludge, obtained from an UASB treating distillery wastewater (Nedalco, The Netherlands), was used as inoculum and the electrochemical cell was flushed with pure nitrogen (>99.9992%) for 30 minutes before applying a cell voltage. pH of the catholyte was controlled at pH 7±0.1 through a pH controller (Liquisis M CPM 253, Endress+Hauser, Switzerland) using 1 M HCl.

MEC operation

During long-term MEC operation there is a risk on malfunctioning of the reference electrodes in the electrochemical cell. To prevent damage to the methane producing biocathode as a result of malfunctioning of the reference electrode, cell voltage was controlled instead of cathode potential. The electrochemical cell was connected to a power supply (MCP94, Bank Elektronik, Germany). Cell voltage was adjusted to obtain the desired cathode potential, when cathode potential deviated >20 mV from the desired cathode potential.

The experiment was started using a biocathode from an already running MEC using hexacyanoferrate(II) oxidation at the anode. This biocathode had a current density of 0.3 A/m^2 and was operated at an E_{cat} of -0.8 V vs. NHE. At the start of the experiment (day 0) this biocathode was inoculated to make sure that sufficient biomass

was present. From day 0 to day 83, with hexacyanoferrate(II) as the anolyte, the cathode potential was controlled at -0.7 V vs. NHE. After switching the anolyte to water (day 83 to 177), the cathode potential was -0.55 V vs. NHE. This potential was higher than with hexacyanoferrate(II), and is the result of the maximum range in applied cell voltage of the potentiostat, which was limited to -2 V. This range limited the cathode potential as the anode potential required for water oxidation was higher than the anode potential required for hexacyanoferrate(II) oxidation. On day 188, the experiment was finished due to leakages in the MEC.

Polarization tests

Polarization tests were made using an IviumStat potentiostat with a Peripheral Port Expander (Ivium Technologies, Eindhoven, The Netherlands). The applied cathode potential was decreased from -0.4 V to -1.0 V with steps of 0.1 V, each step lasting 10 minutes, using hexacyanoferrate(II) oxidation at the anode. When using water oxidation at the anode, the cell voltage was controlled instead of cathode potential. In this case, the applied cell voltage was decreased from -1.0 V to -2.0 V with steps of 0.1 V, each step lasting 10 minutes, and cathode potential was continuously recorded versus a reference electrode. This resulted in a cathode potential at highest -0.21 V vs. NHE and at lowest -0.78 V vs. NHE using water oxidation at the anode. Current was recorded each second and the last ten data points at each cathode potential were averaged and plotted in the polarization curve.

Gas analysis

Gas composition of the cathode gas phase was measured with two different gas chromatographs, same as [149], to measure all gasses present. Gas production was measured with a gas flow meter (Milligascounter®, Ritter, Germany).

Methane production rate was calculated from the measured gas production and gas composition using the mass balance equation described by [131]:

$$V_{CH_4,t} = V_{CH_4,t-1} + \left(V_{T,t} - V_{T,t-1}\right) \cdot \frac{\left(c_{CH_4,t} + c_{CH_4,t-1}\right)}{2} + V_{cat} \cdot \left(c_{CH_4,t} + c_{CH_4,t-1}\right) \quad (2.6)$$

with $V_{CH4,t}$ and $V_{CH4,t-1}$ the cumulative methane gas production (L CH_4) on sample time t and previous sample time t-1, respectively, $V_{T,t}$ and $V_{T,t-1}$ the total gas production measured with a gas flow meter (L) on sample time t and previous sample time t-1, respectively, $c_{CH_4,t}$ and $c_{CH_4,t-1}$ the measured methane fractions in the cathode gas phase (-) on sample time t and previous sample time t-1, respectively, and V_{cat} the cathode headspace volume (0.7 L).

Yield tests

To compare performance of the methane producing biocathode between the two anolytes hexacyanoferrate(II) and water, a methane yield tests of 8h was performed on day 70 (13 days before switching anolytes), and day 101 (18 days after switching anolytes). Before the yield test was started, the cathode compartment was flushed with pure nitrogen (>99.9992%) for 30 minutes. During the yield test, the cathode potential was controlled at -0.7 V vs. NHE using an IviumStat potentiostat. Catholyte was continuously refreshed as described for continuous operation, and at the start and end of the yield test the cathode gas phase was analysed for methane as described.

Energy efficiency

The two most important parameters to describe performance of the MEC are methane production rate and energy efficiency. Methane production rate was determined as described in yield tests. Energy efficiency (eq. 2.7) of a methane

producing MEC is the product of coulombic efficiency (eq. 2.8) and voltage efficiency (eq. 2.9) [59].

$$\eta_{energy} = \eta_{CE} \cdot \eta_{voltage} = \frac{-\Delta G_{CH_4} \cdot V_{CH_4}}{V_m \cdot E_{cell} \cdot \int_{t=0}^{t} Idt} \quad (2.7)$$

Coulombic efficiency (η_{CE}, %), the efficiency of capturing the electrons from the electric current in methane, was calculated via

$$\eta_{CE} = \frac{V_{CH_4} \cdot F \cdot n}{V_m \cdot \int_{t=0}^{t} Idt} \quad (2.8),$$

with V_{CH_4} the cumulative methane gas production (m^3 CH_4), F the Faradays constant (96485 C/mole e^-), n the moles of electrons per mole of methane (8 mole e^-/mole CH_4), V_m the molar volume (0.0252 m^3/mole), I the current (A), and t the time (s).

Voltage efficiency, the amount of external electrical energy that ends up in methane, was calculated via the Gibb's free energy of oxidation of methane over the electrical energy input of the MEC

$$\eta_{voltage} = \frac{E_{emf}}{E_{cell}} = \frac{-\Delta G_{CH_4}}{E_{cell} \cdot n \cdot F} \quad (2.9),$$

with E_{emf} the so called reversible energy loss (V), i.e. the electrical energy converted into chemical energy in the form of methane, E_{cell} the energy input of the MEC, i.e. the applied cell voltage (V), and ΔG_{CH4} the Gibb's free energy of oxidation of methane (890.4 kJ/mole CH_4 [120]).

To improve the performance of the methane producing MEC, factors that affect the energy efficiency should be identified. Therefore, the effect of oxygen diffusion through the membrane on MEC performance was calculated, and an internal resistance analysis of the methane producing MEC was performed as described hereafter.

Oxygen diffusion through membrane

The coulombic efficiency reflects to which extent other electron consuming reactions, so called parasitic reactions, than the preferred CO_2 reduction reaction take place in the electrochemical cell. Parasitic oxidation occurs when oxygen produced at the anode diffuses to the cathode, where it oxidizes methane and/or hydrogen to CO_2 and/or water. This results in a decrease in methane production rate and a lower coulombic efficiency (smaller part of the electrons ending up in methane). Parasitic reduction occurs when oxygen is directly reduced to water at the cathode. The contribution of parasitic oxidation or reduction to coulombic efficiency can be estimated by calculating the oxygen diffusion flux (J_{O2} in mole O_2/m^2 per second) over the cation exchange membrane using Fick's law

$$J_{O_2} = \mathrm{D}_{O_2} \cdot \frac{(c_{O_2,an} - c_{O_2,cat})}{\delta} \quad (2.10)$$

with D_{O2} the diffusion coefficient of oxygen determined for a CMI-7000 cation exchange membrane (Membrane International Inc., USA) ($4.3 \cdot 10^{-10}$ m^2/s) [78], $c_{O_2,an}$ and $c_{O_2,cat}$ the dissolved oxygen concentration at the anode and cathode (mole O_2/m^3), respectively, and δ the thickness of the membrane as supplied by the manufacturer ($0.7 \cdot 10^{-3}$ m). The oxygen diffusion flux is inversely proportional to the thickness of the membrane. The estimated rate of methane oxidation ($r_{CH4,ox}$ in m^3 CH_4/m^3 per day) due to this flux of oxygen is

$$r_{CH_4,ox} = \frac{J_{O_2} \cdot A \cdot V_m \cdot t}{2 \cdot V_{reactor}} \quad (2.11)$$

with A the membrane surface area (0.025 m^2), t the time (d), $V_{reactor}$ the total reactor volume (0.56 L), and taking into account that 2 moles of oxygen are consumed per mole methane.

Internal resistance analysis

The applied cell voltage consists of a thermodynamically calculated cell voltage needed to produce methane under biologically relevant conditions, the reversible energy loss E_{emf}, and internal energy losses, the so called irreversible energy losses [144]. Irreversible energy losses, energy lost as a result of the resistances of different parts of the MEC, consist of the pH gradient over the membrane ($E_{\Delta pH}$), cathode overpotential (η_{cat}), anode overpotential (η_{an}), ionic losses (E_{ionic}), and transport losses (E_T), that were calculated according to [144]

$$E_{cell} = E_{emf} - E_{\Delta pH} - \eta_{cat} - \eta_{an} - E_{ionic} - E_T \quad (2.12)$$

with $E_{emf} = E_{cat} - E_{an}$ (V). Cathode (E_{cat}) and anode (E_{an}) potential were calculated using the Nernst equation under experimentally relevant conditions ($E^0_{Fe(II)}$= 0.361 V vs. NHE, T = 303 K, $[Fe(CN)_6^{4-}]$ is assumed on average to be equal to $[Fe(CN)_6^{3-}]$ [90], $E^0_{H_2O}$= 1.229 V vs. NHE, $pO_2 = 0.28 \cdot 10^5$ Pa (average measured liquid dissolved oxygen concentration cathode was 12.4 mg O_2/L (day 83-177)), E^0_{cat} = 0.169 V vs. NHE, $pCH_4 = 0.075 \cdot 10^5$ Pa (pressure cathode compartment was 1.005 bar with an average measured methane concentration of 8.78% (day 0-188)), $[HCO_3^-]$ = 0.06 M, $[H^+] = 10^{-7}$ M). As anolyte and catholyte pH changed with time, these were included in the irreversible energy losses calculations.

$$E_{an,Fe(II)} = E^0_{Fe(II)} - \frac{R \cdot T}{F} \cdot \ln\left(\frac{[Fe(CN)_6^{4-}]}{[Fe(CN)_6^{3-}]}\right) = 0.36V\ vs.\ NHE \quad (2.13)$$

$$E_{an,H_2O} = E^0_{H_2O} - \frac{R \cdot T}{4 \cdot F} \cdot \ln\left(\frac{1}{pO_2 \cdot [H^+]^4}\right) = 0.87\ V\ vs.\ NHE \quad (2.14)$$

$$E_{cat} = E^0_{cat} - \frac{R \cdot T}{8 \cdot F} \cdot \ln\left(\frac{pCH_4}{[HCO_3^-] \cdot [H^+]^9}\right) = -0.34\ V\ vs.\ NHE \quad (2.15)$$

Ionic and transport losses were calculated from all other potential losses using equation 2.12. Parameters measured in time to calculate potential losses were anode and cathode potential, catholyte and anolyte pH, and cell voltage. At a constant

applied cell voltage, the current density that is produced by MECs depends on the internal resistance of the MEC. Partial resistances (R_i in $\Omega \cdot m^2$) were calculated by dividing the calculated potential loss by the current density, and total internal resistance was the sum of the partial resistances [144].

For analysis of internal resistance during continuous operation, six representative periods were chosen. These six periods were:

1) at the start of the experiment using hexacyanoferrate(II) oxidation (days 2-6, indicated as 'start Fe'),
2) in the middle of stable operation using hexacyanoferrate(II) oxidation (days 50-57, indicated as 'middle Fe'),
3) before switching anolytes (day 83, indicated as 'end Fe'),
4) after switching anolytes (day 83, indicated as 'start water'),
5) in the middle of stable operation using water oxidation (days 125-132, indicated as 'middle water'), and
6) at the end of the experiment using water oxidation (days 167-176, indicated as 'end water').

2.3 Results and discussion

Biocathode performance

Polarization tests are a useful tool to give insight in the development of the methane producing biocathode with time. A higher current density at the same cathode potential indicates an increase in performance. When using hexacyanoferrate (day 0-83) at the anode, during continuous operation cathode potential was controlled at -0.7 V vs. NHE, and current density was on average 0.78 ± 0.20 A/m^2. The polarization curves as shown in Figure 2.1 show that the biocathode had similar

performance on days 6 and 83, indicating stable performance. The current density obtained in the polarization curves at -0.7 V vs. NHE is well within the range obtained during continuous operation. After changing the anode reaction to water oxidation and increasing the cathode potential to -0.55 V vs. NHE during continuous operation, current density was on average 0.25±0.04 A/m^2. Similar to using hexacyanoferrate(II) oxidation at the anode, the polarization curves show that the biocathode had similar performance on days 132 and 177, indicating stable performance, and the current density obtained in the polarization curves at -0.55 V vs. NHE is in line with the current density obtained during continuous operation.

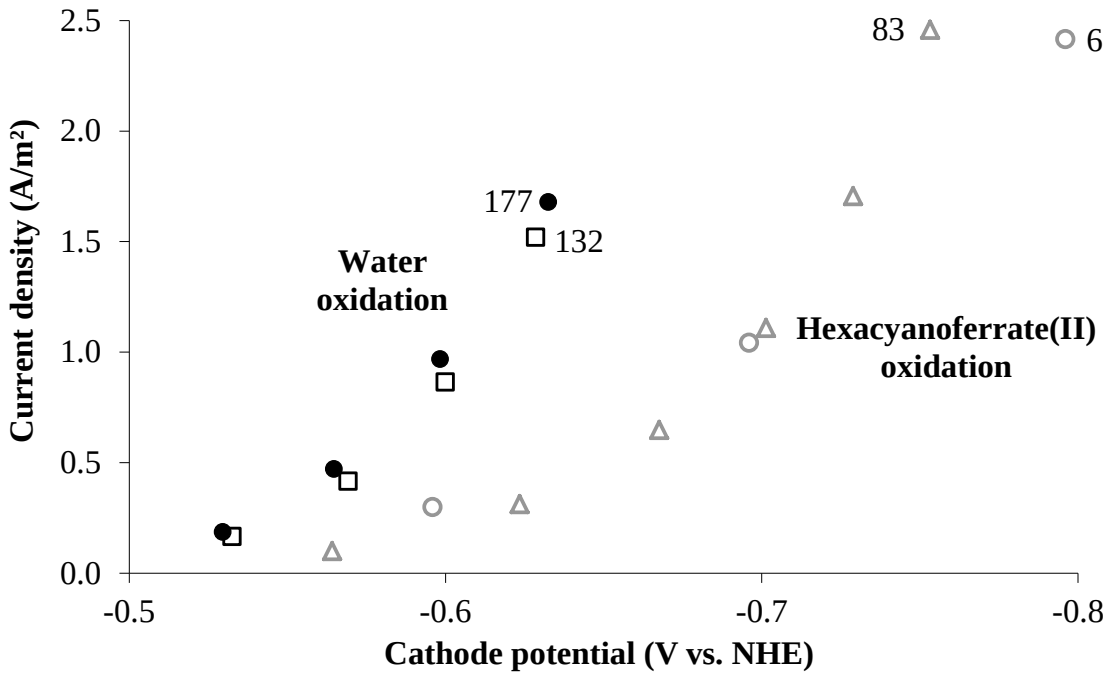

Figure 2.1. Polarization tests give insight in the performance of a methane producing biocathode in time (operation day indicated in figure) using hexacyanoferrate(II) (grey symbols) or water (black symbols) oxidation at the anode.

Figure 2.1 also shows that at the same cathode potential the biocathode performed better when using water oxidation compared to hexacyanoferrate(II) oxidation. The higher current density using water oxidation compared to hexacyanoferrate(II) oxidation could be the result of oxygen diffusion over the membrane resulting in parasitic reactions at the cathode, or the result of better performance of the biomass on the biocathode. Presence of oxygen at the cathode could lead to increased current production through direct oxygen reduction at the cathode. Whether parasitic reactions at the cathode are a plausible explanation for the better performance using water oxidation can be verified by calculating the oxygen flux over the cation exchange membrane (eq. 2.10 and 2.11). Dissolved oxygen concentration at the anode was on average 0.95 mg/L using hexacyanoferrate(II) oxidation, and 12.4 mg/L using water oxidation, while dissolved oxygen concentration at the cathode was always 0 mg/L. This leads to an oxygen flux over the membrane of $1.8 \cdot 10^{-8}$ mole O_2/m^2 per second using hexacyanoferrate(II) oxidation, and $2.4 \cdot 10^{-7}$ mole O_2/m^2 per second using water oxidation. This flux of oxygen can consume electrons at a rate of 0.007 A/m^2 using hexacyanoferrate(II) oxidation, and 0.09 A/m^2 using water oxidation. This is equal to 0.6% of the measured current density at cathode potential -0.7 V vs. NHE during the polarization test using hexacyanoferrate(II) oxidation and 37% of the measured current density at cathode potential -0.55 V vs. NHE during the polarization test using water oxidation (Figure 2.1). These calculations show that oxygen diffusion over the membrane can explain part of the higher current density using water oxidation.

After correction for the additional current density caused by possible oxygen diffusion over the membrane, current density using water oxidation was still higher than when using hexacyanoferrate(II) oxidation (Figure 2.1). This suggests that the higher controlled cathode potential during continuous operation also led to an increased current density. Using water oxidation, during continuous operation cathode

potential was -0.55 V vs. NHE, while during hexacyanoferrate(II) oxidation the cathode potential was -0.7 V vs. NHE. The cathode potential of -0.55 V vs. NHE was higher than used in previous studies, where potentials below -0.7 V vs. NHE were investigated [23, 28, 120, 168]. At this high cathode potential, direct CO_2 reduction to methane is energetically more favourable than CO_2 reduction via hydrogen: eq. 2.1 and 2.2 show a higher potential for direct CO_2 reduction compared to CO_2 reduction via hydrogen, meaning that at -0.55 V vs. NHE more energy is to be gained by the microorganisms via direct reduction of CO_2. Previous study indeed revealed that at cathode potentials higher than -0.75 V vs. NHE, methane was mainly produced via direct CO_2 reduction, while at lower cathode potentials methane can also be produced indirectly via hydrogen [168]. In this study, at a cathode potential of -0.55 V vs. NHE, direct reduction of CO_2 was the most likely process, although we did not further investigate the mechanisms.

To further study the performance of the biocathode when using water oxidation at the anode compared to hexacyanoferrate(II) oxidation, yield tests were performed, in which the cathode potential was controlled at the same value of -0.7 V vs. NHE. This was done on day 70 (13 days before switching anolytes) and day 101 (18 days after switching anolytes). Table 2.1 shows that during the yield test, the current density was 40% higher when using water oxidation compared to hexacyanoferrate(II) oxidation. This is in agreement to what was found during polarization tests (Figure 2.1). Methane production rate was 30% higher when using water oxidation compared to hexacyanoferrate(II) oxidation, and this was confirmed by the slightly lower coulombic efficiency for water oxidation compared to hexacyanoferrate(II) oxidation in the yield tests. The coulombic efficiency of hexacyanoferrate(II) oxidation in the yield test being higher than 100% could be a result of biomass degradation and oxidation or storage of electrons in the microorganisms [45]. The better performance

of the biocathode when using water oxidation could also be a result of the lower applied cathode potential in the yield test (-0.7 V vs. NHE) compared to the potential at which the biofilm was acclimatized during continuous operation (-0.55 V vs. NHE). This change in cathode potential could have affected the functioning of the biocathode.

The polarization curves and yield tests reveal that oxygen via parasitic reactions decreases the coulombic efficiency of the biocathode, however, it has no obvious negative effect on the methane production rate. This might be explained by the fact that the oxygen reacts away either via direct reduction to water at the cathode or via oxidizing the methane produced, and therefore cannot affect the methanogens present at the biocathode.

Long-term current generation and methane production in flat-plate MEC

Current and methane were produced continuously in the flat plate MEC for 188 days (Figure 2.2). When using hexacyanoferrate (day 0-83), cathode potential was controlled at -0.7 V vs. NHE, and current density ranged between 0.4 A/m^2 and 2.5 A/m^2, being on average 0.78±0.20 A/m^2. Methane production ranged between 0.01-0.10 m^3 CH_4/m^3 per day, being on average 0.05±0.03 m^3 CH_4/m^3 per day. After changing the anode reaction to water oxidation and increasing the cathode potential to -0.55 V vs. NHE, current density decreased and ranged between 0.2 A/m^2 and 0.6 A/m^2 (day 83-188), being on average 0.25±0.04 A/m^2. Methane production ranged between $3 \cdot 10^{-4}$-0.025 m^3 CH_4/m^3 per day, being on average 0.006±0.008 m^3 CH_4/m^3 per day. This decrease in gas production rate when switching the anolyte from hexacyanoferrate(II) to water is in line with the lower current density (Figure 2.2).

Methane production rate was on average a factor 8 higher using

Table 2.1. Performance of a methane producing biocathode with hexacyanoferrate(II) or water oxidation at the anode during continuous operation and for yield tests.

	Hexacyanoferrate(II) oxidation			**Water oxidation**		
	Continuous		**Yield test**	**Continuous**		**Yield test**
Operation period	**Start Fe**	**Middle Fe**	**Day 70**	**Middle water**	**End water**	**Day 101**
E_{cat} (V)	-0.69	-0.71	-0.69	-0.55	-0.55	-0.70
j (A/m^2)	0.87	0.80	1.09	0.25	0.21	1.75
r_{CH4} (m^3/m^3 per day)	0.071	0.033	0.15	0.005	0.006	0.21
$\eta_{coulombic}$ (%)	64.9	33.2	104.6	17.5	23.1	95.2
Energy input (kWh/m^3)	15.4	31.6	16.6	97.2	73.5	18.2
$\eta_{voltage}$ (%)	63.7	31.1	59.3	10.1	13.4	53.9
η_{energy} (%)	42.6	10.3	62.0	1.8	3.1	51.3

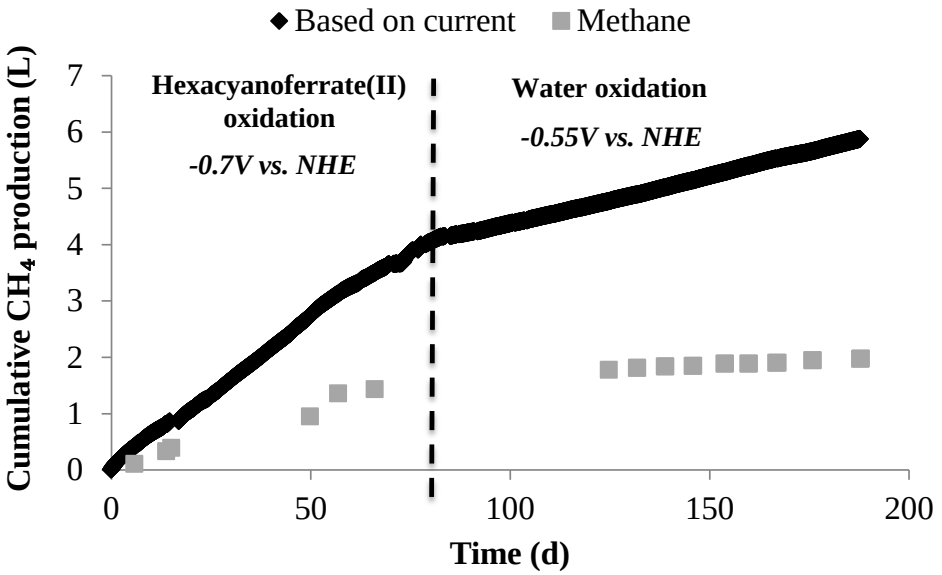

Figure 2.2. Cumulative methane production with time. The black diamonds indicate methane production calculated from the current, while the grey squares indicate measured methane production. Anolyte was changed from hexacyanoferrate(II) to water on day 83, as indicated by the dashed line.

hexacyanoferrate(II) as electron donor compared to water during continuous operation (Table 2.1). Part of the explanation for the lower methane production rate when using water oxidation is the higher cathode potential and consequently the lower current density, however, this cannot be the only explanation, as the current density was only a factor 3-4 lower. Parasitic reactions that consume electrons will also lead to a lower methane production rate. These parasitic reactions are reflected in the coulombic efficiency, which was about a factor 1.5-4 lower when using water oxidation compared to hexacyanoferrate(II) oxidation during continuous operation (Table 2.1). Whether parasitic reactions are a plausible explanation for the lower coulombic efficiency can be verified by calculating the oxygen diffusion flux over the cation exchange membrane. Average dissolved oxygen concentration at the anode was 12.3 mg/L using water oxidation, while dissolved oxygen concentration was always

0 mg/L at the cathode. This leads to an oxygen flux over the membrane of $2.4 \cdot 10^{-7}$ mole O_2/m^2 per second. This flux of oxygen can oxidize methane at a rate of 0.012 m^3 CH_4/m^3 per day. If we calculate the expected methane production rate using the measured methane production rate in Table 2.1, and taking into account the methane lost due to oxidation by oxygen, then the methane production rate using water oxidation was 0.005 + 0.012 = 0.017 m^3 CH_4/m^3 per day (middle water) and 0.006 + 0.012 = 0.018 m^3 CH_4/m^3 per day (end water). This would result in a maximum coulombic efficiency of 57.8% (middle water) and 67.5% (end water), which is comparable to the coulombic efficiency for hexacyanoferrate(II) oxidation. These calculations show that parasitic reactions can explain part of the differences in coulombic efficiency between both anolytes.

Parasitic reactions alone, however, cannot explain why coulombic efficiency is considerably lower than 100% for both anolytes during continuous operation. It is believed that part of the methane is lost due to diffusion from the cathode to the anode, similar to hydrogen losses in MECs [89, 130], and therefore less methane is measured in the cathode gas phase than expected from the measured current. The coulombic efficiency can likely be improved by using a membrane that is less permeable for gases. This results in less methane diffusion from the cathode to the anode, and less oxygen diffusion from the anode to cathode, and consequently in less parasitic reactions.

Methane production rates reported for methane producing bio-electrochemical systems using water oxidation are 0.012-0.015 m^3 CH_4/m^3 per day for two-compartment MECs at cathode potentials between -0.8 V and -0.9 V vs. NHE [23, 168]. The methane production rates during continuous operation using hexacyanoferrate(II) oxidation, 0.033-0.071 m^3 CH_4/m^3 per day, were higher than previously reported methane production rates. Methane production rates during

continuous operation using water oxidation were somewhat lower, 0.005-0.006 m^3 CH_4/m^3 per day, however, this is expected as the results are obtained at a cathode potential of >0.25 V higher than used in other studies.

Voltage efficiency and energy efficiency

During continuous operation using hexacyanoferrate(II) oxidation at the anode, the energy input was 15.4 kWh/m^3 methane (start Fe; based on E_{cell} = -1.21 V) and 31.6 kWh/m^3 methane (middle Fe; based on E_{cell} = -1.23 V) (Table 2.1). Using water oxidation at the anode, the energy input was 97.2 kWh/m^3 methane (middle water; based on E_{cell} = -1.99 V) and 73.5 kWh/m^3 methane (end water; based on E_{cell} = -1.99 V) (Table 2.1). Gibb's free energy of methane oxidation is 9.8 kWh/m^3 methane [120], so voltage efficiency was 63.7% (start Fe) and 31.1% (middle Fe) using hexacyanoferrate(II) oxidation, and 10.1% (middle water) and 13.4% (end water) using water oxidation.

Energy efficiency (eq. 2.7) is the product of coulombic efficiency (eq. 2.8) and voltage efficiency (eq. 2.9) [59]. The energy efficiency was 42.6% (start Fe) and 10.3% (middle Fe) using hexacyanoferrate(II) oxidation, and 1.8% (middle water) and 3.1% (end water) using water oxidation (Table 2.1).

It should be noted that the energy efficiency using hexacyanoferrate(II) oxidation at the anode does not include the energy required for regeneration of hexacyanoferrate(II), which is essential for hexacyanoferrate(II) to be a sustainable electron donor for use in practical applications [90]. The energy efficiency using hexacyanoferrate(II) oxidation at the anode, however, reveals the potential performance of a methane producing biocathode using an efficient anode, and is useful to study the effect of oxygen diffusion over the membrane on the performance of a methane producing MEC.

The yield tests show the maximum achieved voltage efficiency and energy efficiency. The energy input in a yield test using hexacyanoferrate(II) oxidation was 16.6 kWh/m^3 methane (day 70), leading to a voltage efficiency of 59.3% and an energy efficiency of 62.0% (Table 2.1). The energy input in a yield test using water oxidation was 18.2 kWh/m^3 methane (day 101), leading to a voltage efficiency of 53.9% and an energy efficiency of 51.3% (Table 2.1).

Identifying sources of irreversible energy losses

To study how energy efficiency can be improved, analysis of irreversible energy losses during continuous operation of the methane producing MEC is essential. At a constant applied cell voltage, the current density that is produced by MECs depends on the internal resistance of the MEC [144]. Therefore, partial resistances were calculated to identify which processes contributed most to the total internal resistance of the MEC. These partial resistances represent cathode losses, anode losses, losses due to the pH gradient over the membrane, and transport & ionic losses.

Figure 2.3 shows an increase in total internal resistance with time for both anolytes. This causes a decrease in current density with time. Current density decreased from 1.15 A/m^2 to 0.30 A/m^2 (from start Fe to end Fe) using hexacyanoferrate(II) oxidation, and from 0.30 A/m^2 to 0.25 A/m^2 (from start water to end water) using water oxidation. From Figure 2.3, also two main effects can be seen: (i) in case of hexacyanoferrate(II) oxidation, the total internal resistance was considerably lower than with water oxidation, and was mainly caused by the cathode, and (ii) in case of water oxidation, in the beginning anode and cathode contributed most to the total internal resistance, while pH and transport & ionic losses increased with time.

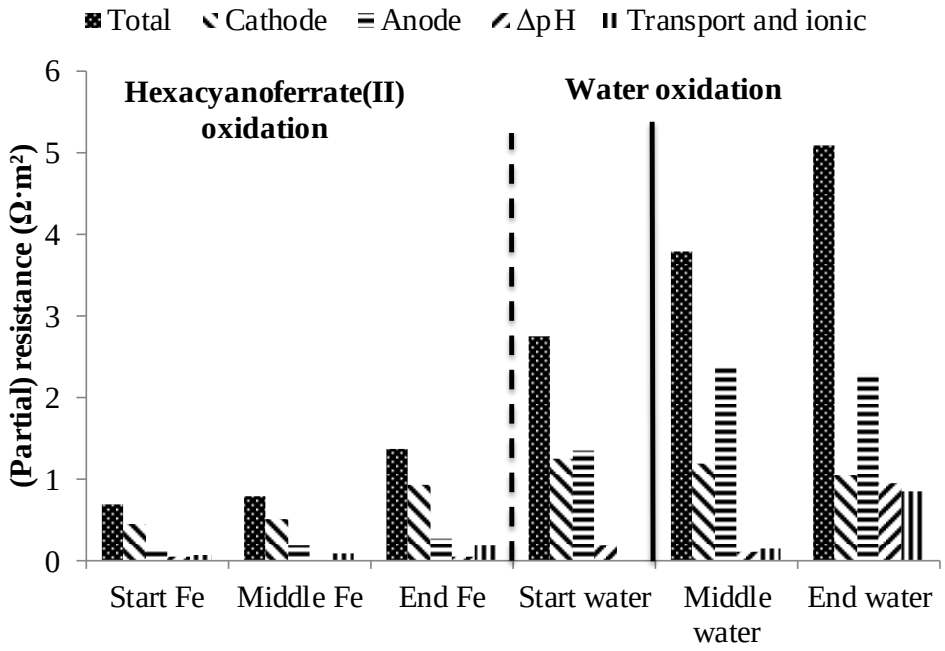

Figure 2.3. Total internal resistance, and partial resistances were measured in time for hexacyanoferrate(II) and water oxidation at the anode for continuous operation. Anolyte was changed from hexacyanoferrate(II) to water on day 83, as indicated by the dashed line. Anolyte was refreshed on day 115, as indicated by the solid line.

In case of hexacyanoferrate(II) oxidation, the cathode resistance was at least 0.43 $\Omega \cdot m^2$ (start Fe). Immediately after switching anolyte hexacyanoferrate(II) for water, both the cathode and anode contributed most to total internal resistance (Figure 2.3). Cathode resistance was at least 1.04 $\Omega \cdot m^2$ (end water), while anode resistance was at least 1.33 $\Omega \cdot m^2$ (start water). The sudden increase in anode resistance after switching anolytes could be attributed to the poor catalytic properties of graphite for water oxidation [13]. The increase in anode and cathode resistances with time may be caused by increasing product concentrations (O_2, methane, and protons) near or inside the electrode. This could negatively affect reaction kinetics, and gas accumulation inside the electrode could result in less available effective cathode surface area. Transport & ionic resistances increased with time from 0.015 $\Omega \cdot m^2$ (start water) to 0.83 $\Omega \cdot m^2$ (end

water), despite refreshing the anolyte (day 115). In this same time period, anolyte conductivity increased from 0.3 S/m (start water) to 0.7 S/m (end water), while catholyte conductivity decreased from 2.1 S/m (start water) to 1.0 S/m (end water). This is equal to a decrease in ionic resistance from 0.01 $\Omega \cdot m^2$ (start water) to 0.006 $\Omega \cdot m^2$ (end water), using the equation described by Sleutels *et al.* [144] and assuming the distance between the electrodes and the membrane is 5 mm. These calculations show that the transport & ionic resistance mainly consists of transport losses, and not so much of ionic losses due to limited conductivity of the electrolyte, which is in line with Sleutels *et al.* [144]. The resistance due to the development of a pH-gradient over the membrane increased in the same time period from 0.17 $\Omega \cdot m^2$ (start water) to 0.94 $\Omega \cdot m^2$ (end water), with the most prominent increase at the end using water oxidation, as the anolyte acidified due to proton production from pH 6.25 (start water; cathode pH 7.09) to 3.37 (end water; cathode pH 7.10). At the end using water oxidation, all resistances contributed to a similar extent to the total internal resistance.

Increased methane yield per hectare of land area

At this point, the methane producing MEC is still in its early stage of development. For an estimation of the potential of a methane producing MEC, two crucial inputs need to be considered: renewable electrical energy and CO_2. There are several possible sources and technologies for renewable electricity production: PV cells using solar energy, wind turbines using wind energy, or reverse electrodialysis using the energy from mixing salt and fresh water [114]. A suitable source of CO_2 should (i) be of renewable origin to be independent of fossil fuels, and (ii) contain high concentrations of CO_2, preferably without oxygen present. Gas streams of fermentation processes (renewable fuel production technologies) are therefore an attractive CO_2 source for methane producing MECs. As an example, we will discuss biogas produced via

anaerobic digestion of biomass. Biogas consists of both CH_4 and CO_2, which are produced in a 1:1 ratio. To add a higher energetic and economic value to the biogas, it needs to be upgraded, which means that the CO_2 content needs to be lowered, and the methane content needs to be increased. Conventionally, CO_2 is removed by scrubbing the CO_2-rich gas with an aqueous solution containing chemicals (hydroxide, amines, etc.) [158]. By contrast, a methane producing MEC does not only lower the CO_2 content of biogas, but furthermore, converts CO_2 into additional methane. As roughly half of the biogas consists of CO_2, the methane yield from anaerobic digestion could be doubled using a methane producing MEC.

Figure 2.4 shows the relative methane yield per hectare of land area per year for combined anaerobic digestion and methane producing MECs using electricity from PV cells. A relative methane yield >1 indicates that more methane is produced via the combined processes compared to anaerobic digestion alone. The relative methane yield is shown as a function of the surface area used for PV cells in combination with a methane producing MEC, and the energy efficiency of a methane producing MEC. We assume that PV cells convert the incoming solar radiation of 150 W/m^2 [152] into electricity at an efficiency of 10% [77], and that biogas consists of equal parts of methane and CO_2. In the hypothetical situation of 100% energy efficiency, which means that no energy losses occur in the MEC system and the energy input consists only of the reversible thermodynamic energy input, to double the methane yield, 5% of the land area needs to be covered with PV cells in combination with methane-producing MECs (meaning that 95% of the surface area is used for biomass growth, resulting in a lower CO_2 yield per hectare, and a relative methane yield <2). The methane producing MEC should have an energy efficiency higher than 5.5% to increase methane yield per hectare of land area compared to anaerobic digestion alone (Figure 2.4).

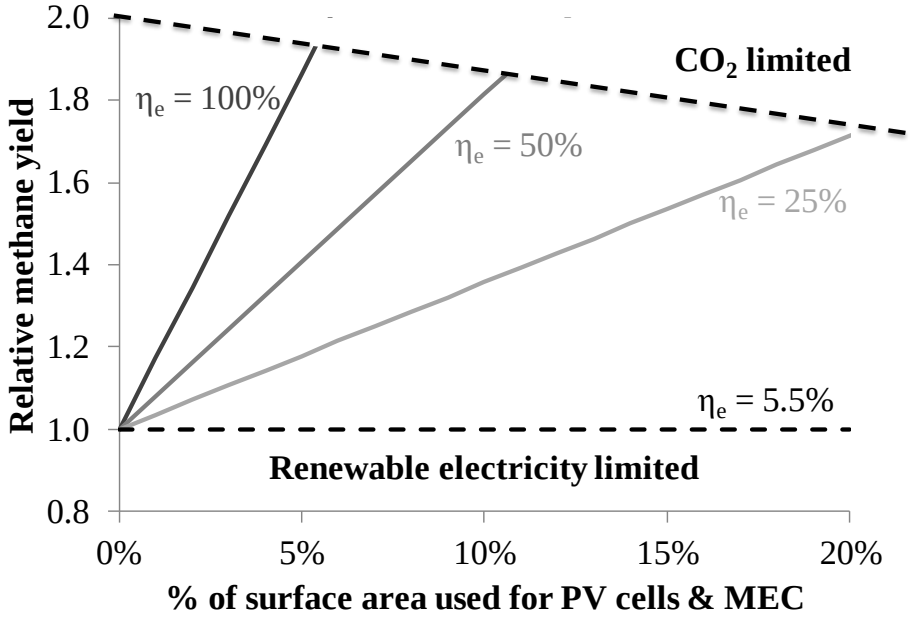

Figure 2.4. Combining anaerobic digestion and methane producing MECs leads to an improved methane yield per hectare of land area. The energy efficiency (η_e) of the methane producing MEC is calculated as the ratio between the thermodynamic energy input based on the Gibb's free energy of the reaction (9.8 kWh/m^3 methane) divided by the actual electrical energy input. A relative methane yield >1 indicates that more methane is produced than via anaerobic digestion alone. Thus, at an energy efficiency above 5.5%, the combination of anaerobic digestion and methane producing MECs leads to an increased methane yield per hectare of land per year compared to anaerobic digestion.

It is important to note that the use of water oxidation at the anode is essential for reaching an additional methane yield compared to existing biomass conversion technologies. Methane producing MECs that use biomass in the form of acetate as the electron donor at the anode, and that recycle the produced CO_2 from the anode to the cathode, produce the same mix of CO_2 and methane as anaerobic digestion. Oxidation of one mole of acetate at the anode results in 8 moles of electrons and 2 moles of CO_2, the latter being present in the form of bicarbonate at biologically relevant pH 7 (eq. 2.16). The formed 8 moles of electrons can be used to reduce only one mole of CO_2 (or bicarbonate) to methane (eq. 2.17). The overall reaction in a methane

producing MEC using acetate oxidation at the anode is thus limited by the 8 moles of electrons present in one mole of acetate. Overall, 1 mole of acetate results in 1 mole of methane produced at the cathode, and 1 mole of CO_2 produced at the anode (eq. 2.18).

Organic matter oxidation at anode [90]:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (2.16)$$

Carbon dioxide reduction at cathode:

$$HCO_3^- + 9H^+ + 8e^- \rightarrow CH_4 + 3H_2O \quad (2.17)$$

Overall reaction methane producing MEC using organic matter oxidation at the anode:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (2.18)$$

In contrast, when using water oxidation at the anode, the amount of electrons is in principle unlimited, meaning that sufficient electrons can be produced to reduce all the CO_2 present into methane, leading to a theoretically double methane yield. Methane producing MECs that use an electron source other than biomass are therefore the only way to achieve higher methane yields per hectare of land area compared to anaerobic digestion.

Perspectives

This study showed that an MEC is suitable to convert CO_2 to methane, with a biocathode that continuously converted CO_2 to methane for 188 days. The maximum achieved energy efficiency in this study was 51.3%, obtained during the yield test using water oxidation. An energy efficiency of 51.3% would increase the methane yield per hectare of land area by a factor 1.8 when covering 10% of the land area with PV cells

(Figure 2.4). For these calculations, it is assumed that the methane production rate of methane producing MECs is the same as the CO_2 production rate of anaerobic digestion (5 m^3/m^3 per day), whereas the maximum achieved methane production rate in this study was still a factor 25 lower. A considerable reduction in internal resistance is still needed to reach sufficiently high conversion rates. The internal resistance analysis shows that several improvements can be made to reduce the internal resistance and to increase energy efficiency. First, high-surface electrode materials with good catalytic properties for water oxidation and with good properties for biofilm development [155] for catalysis of CO_2 reduction should be used to decrease cathode and anode losses [90]. Second, by directing the flow through the porous electrode, the surface area can be effectively used and mass transfer losses are decreased [82, 90, 145]. Third, decreasing the distances between membrane and electrodes minimizes mass transfer losses even further [20, 90]. Finally, to increase the coulombic efficiency and the methane content of the gas, a membrane that is less permeable for gases should be used. Implementing these improvements will bring a methane producing MEC closer to its potential for increasing the methane yield land use efficiency, and consequently to increase water, and nutrient efficiency.

2.4 Acknowledgement

This research was funded by the SenterNovem "Energie Onderzoek Subsidie: Nieuw Energie Onderzoek" program (NEOT05005).

Chapter 3

Microbial community analysis of a methane-producing biocathode in a bioelectrochemical system

This chapter has been published as:

Mieke C. A. A. Van Eerten-Jansen, Anna B. Veldhoen, Caroline M. Plugge, Alfons J. M. Stams, Cees J. N. Buisman, Annemiek Ter Heijne (2013), *Microbial community analysis of a methane-producing biocathode in a bioelectrochemical system*, Archaea, Article ID 481784, 12 pages, doi 10.1155/2013/481784.

Abstract

A methane-producing biocathode that converts CO_2 into methane was studied electrochemically and microbiologically. The biocathode produced methane at a maximum rate of 5.1 L CH_4/m^2 projected cathode per day (1.6 A/m^2) at -0.7 V vs. NHE cathode potential and 3.0 L CH_4/m^2 projected cathode per day (0.9 A/m^2) at -0.6 V vs. NHE cathode potential. The microbial community at the biocathode was dominated by three phylotypes of archaea and six phylotypes of bacteria. The archaeal phylotypes were most closely related to *Methanobacterium palustre* and *Methanobacterium aarhusense*. Besides methanogenic archaea, bacteria seemed to be associated with methane production, producing hydrogen as an intermediate. Biomass density varied greatly, with part of the carbon electrode covered with a dense biofilm, while only clusters of cells were found on other parts. Based on our results, we discuss how inoculum enrichment and changing operational conditions may help to increase biomass density and to select for microorganisms that produce methane.

Keywords: Microbial Electrolysis Cell, CO_2 reduction, methanogenic archaea, *Methanobacterium palustre*, electromethanogenesis, biocathode

3.1 Introduction

In bioelectrochemical systems (BES), microorganisms catalyse oxidation and reduction reactions to produce or use electricity. Recently, it has been discovered that microorganisms can accept electrons from an electrode [38] to bioremediate metal and organic contaminants, or for microbial electrosynthesis to produce fuels and chemicals. Using microorganisms as catalysts on an electrode instead of chemical catalysts is innovative and sustainable: the microorganisms are self-regenerating, the BES can be operated at ambient conditions (at neutral pH and low temperature), and low-cost carbon electrodes can be used [8, 31]. Microbial electrosynthesis in BES has been described for the production of, for example, hydrogen [130], hydrogen peroxide [133], caustic [117], acetate and 2-oxobutyrate [100, 107], ethanol [151], ammonium [80], butyrate [24], or caproate and caprylate [164].

Another attractive application of microbial electrosynthesis is the conversion of CO_2 into methane [23]. Besides producing carbon-neutral methane, BES can convert excess renewable electricity from sun and wind into methane as an energy carrier [23]. Moreover, the infrastructure for transport, storage and consumption of methane is already in place [23].

To improve the performance of a methane-producing BES, focus so far was mainly on BES design [21, 28, 120, 163]. However, another key challenge is understanding the methane-producing microbial communities in order to improve the methane production rate and energy efficiency [27]. The microbial consortium (types of microorganisms, community composition, and interaction between microorganisms) and the biomass density (the number of microorganisms at a specified electrode surface or reactor volume that take part in these processes) will influence the performance of methane-producing biocathodes [27]. Selecting for electrochemically active microorganisms that produce methane, and operating the BES under the most

favourable conditions for the selected microorganisms may help to further improve the performance of a methane-producing BES [27].

The microbial composition of a methane-producing biocathode using enriched cultures as inoculum is scarcely documented [23, 100]. Cheng and co-workers obtained an enriched mixed-culture methane-producing biocathode after inoculating the cathode with the effluent of an existing bio-anode [23]. Therefore, it was known beforehand that electrochemically active microorganisms would be present in the biofilm. The methane-producing biocathode consisted of an enriched consortium dominated by *Methanobacterium palustre*, which accounted for 87% of the total number of cells. A biocathode inoculated with a pure culture of *M. palustre*, however, produced less methane than the mixed-culture biocathode [23]. The role of the other detected microbial community members in methane production was not investigated. Marshall and co-workers obtained an enriched mixed-culture methane-producing biocathode after inoculating the cathode with brewery wastewater sludge that was pretreated at -0.59 V vs. NHE cathode potential and that produced methane at this potential [100]. The microbial community at the methane-producing biocathode consisted of methanogens related to *Methanobacterium* sp. (>93%) and *Methanobrevibacter* (~5%), and bacteria related to the *Sphingobacteriales* WCHB1-69 family (37.7%), the *Spirochaetaceae* family (17.4%), and the *Synergistaceae* family (11.1%) [100]. It is possible that the bacteria related to the *Sphingobacteriales* family catalysed bioelectrochemical hydrogen production [100], but the role of the other bacteria was not investigated.

Although the microbial community of methane-producing biocathodes has been described before, the possible roles of the detected community members in methane production remain unclear. In this study, the electrochemical performance and microbial community of a mixed-culture methane-producing biocathode was investigated to illuminate the possible role of the detected community members in

methane production.

3.2 Materials and methods

Electrochemical cell

A flat plate electrochemical cell (1.24 L total volume) was used with a cathode and anode volume of 0.62 L each (described in more detail in [156]), and using a cation exchange membrane (fumasep FKB, FuMA-Tech GmbH, Germany). Both the anode and cathode were made of graphite felt (19x19 cm, thickness 3 mm – FMI Composites Ltd., Scotland) having an effective geometric channel surface area of 290 cm^2 each. The electrolytes flowed parallel to the electrodes through serpentine flow channels in both the anode and cathode compartment (Figure 3.1). The anode and cathode compartments were equipped with an Ag/AgCl, 3 M KCl reference electrode (+0.205 V vs. NHE; ProSense QiS, The Netherlands). The reference electrode was connected to a glass capillary with a membrane tip that was inserted at the top of the cathode near the outlet (the glass capillary was positioned 5 mm from the graphite felt; Figure 1). The cathode headspace was connected to a gas flow meter (Milligascounter®, Ritter, Germany) via an injection port containing a septum. The cathode headspace volume was on average 750±250 mL, and varied due to the batch-wise feeding of the catholyte. The experiment was performed in a temperature controlled chamber at 30ºC.

Electrolytes and microorganisms

The anolyte consisted of 50 mM potassium hexacyanoferrate(II) in de-ionized water. The anolyte was recirculated over a 10 L vessel at 1.5 mL/s and replenished on days 16, 28 and 49 to avoid depletion of electron donor. The catholyte consisted of 20

mM potassium phosphate buffer, macronutrients (280 mg/L NH_4Cl, 5.7 mg/L $CaCl_2$, 10 mg/L $MgSO_4 \cdot 7H_2O$, 90 mg/L $MgCl_2 \cdot 6H_2O$), and 1 mL/L of a micronutrients solution and 1 mL/L of a vitamin solution as described in [155]. To the catholyte 5 g/L $NaHCO_3$ was added as a carbon source, since at the operating conditions of pH 7, CO_2 is predominantly present as bicarbonate. The catholyte was recirculated over a 0.5 L vessel at 1.5 mL/s. To avoid depletion of substrate, every two to three days 250-350 mL catholyte in the electrochemical cell was replaced by 250 mL fresh catholyte under continuous flushing with nitrogen gas. The cathode was inoculated with 5 gram of anaerobic sludge obtained from an upflow anaerobic sludge blanket reactor treating distillery wastewater (Royal Nedalco, The Netherlands). After inoculation, the system was flushed with pure nitrogen (>99.9992%) for 30 minutes before applying a cell voltage and starting the experiment. The pH of the catholyte was controlled at pH 7±0.1 by a pH controller (Liquisis M CPM 253, Endress+Hauser, Switzerland) using 1 M HCl.

Electrochemical cell operation

The electrochemical cell was connected to a potentiostat (MCP94, Bank Elektronik Intelligent Controls GmbH, Germany) using a two-electrode configuration where the cathode was connected to the work electrode, and the anode was connected to both the counter electrode and the reference electrode. From the beginning, the electrochemical cell was operated at cathode potential -0.7 V vs. NHE, as at this cathode potential methane can be both produced directly or via hydrogen as an intermediate [168]. From the moment that only methane and no hydrogen were detected in the cathode gas phase (day 24), the cathode potential was changed to -0.6 V vs. NHE to decrease the energy input of the electrochemical cell. The cathode potential was controlled via the cell voltage as described in [163]. The cell voltage was

adjusted when the cathode potential deviated > 20 mV from the desired cathode potential. The experiment was terminated on day 57, due to leakages at the anode.

Analysis and calculations

The electrochemical cell was connected to a PC via a Fieldpoint FP-AI-110 module (National Instruments, United States), and every 60 seconds cell voltage, current, and cathode and anode potential were measured using LabVIEW 7.1 (National Instruments, United States). Daily averages were calculated and reported throughout this manuscript.

Gas composition of the cathode gas phase was measured on days 0, 12, 24, 33, 51, and 57 with two different gas chromatographs to measure methane, hydrogen and oxygen. Gas samples were taken with a 100 μL gastight syringe (Hamilton, USA) from the injection port near the gas flow meter. Hydrogen was measured with an HP 5890A gas chromatograph by injecting 100 μL of gas-sample on a molsieve column (30 m x 0.53 mm x 0.25 mm) with thermal conductivity detection (TCD). The oven temperature was 40°C, the injection gate 110°C and the TCD 150°C. The carrier gas was argon and had a flow rate of 20 mL/min. Methane and oxygen were measured with a Finsons Instruments GC 8340 gas chromatograph. Gas (100 μL) was split (1:1) over a molsieve column (30 m x 0.53 mm x 25 mm) and a porabond Q column (25 m x 0.53 mm x 10 mm). The oven temperature was 40°C, injection gate 110°C and the TCD 90°C. The carrier gas was helium and had a flow rate of 45 mL/min. Gas composition was measured immediately after replenishing the catholyte and just before the next catholyte replenishment. The time between catholyte replenishments was two to three days. Gas production was continuously measured with a gas flow meter (Milligascounter®, Ritter, Germany). Methane production was calculated using the total gas production and the measured methane fractions, as in [131].

To compare methane production rates with those reported in the literature, all rates were calculated at standard temperature and pressure (STP, 273.15 K and 1 atm) and with respect to the projected cathode surface area (eq. 3.1) or total reactor volume (eq. 3.2), according to

$$r_{CH_4}^{STP} = \frac{V_{CH_4,t} \cdot T_{STP} \cdot p}{A_{cat} \cdot \Delta t \cdot T \cdot p_{STP}} \quad (3.1)$$

$$r_{CH_4}^{STP} = \frac{V_{CH_4,t} \cdot T_{STP} \cdot p}{V_{reactor} \cdot \Delta t \cdot T \cdot p_{STP}} \quad (3.2)$$

where $r_{CH_4}^{STP}$ is the methane production rate at STP (L CH_4/m^2 projected cathode per day or L CH_4/L reactor per day), $V_{CH,t}$ is the cumulative methane gas production (L) on sample time t, A_{cat} is the projected cathode surface area (0.029 m^2), $V_{reactor}$ is the total reactor volume (1.24 L), t is the time (s), T is the temperature used in this study (303 K), p is the pressure used in this study (1.005 atm), and T_{STP} and p_{STP} are the temperature and pressure at STP, 273.15 K and 1 atm respectively. Cathodic electron efficiency, the efficiency of capturing the electrons from the electric current in methane, was calculated as in [163]:

$$\eta_{CE} = \frac{V_{CH_4,t} \cdot F \cdot n}{V_m \cdot \int_{t=0}^{t} I dt} \cdot 100\% \quad (3.3)$$

where F is Faraday constant (96485 C/mole e^-), n is the moles of electrons per mole of methane (8 mole e^-/mole CH_4), V_m is the molar volume (22.7 L/mole at 273.15 K and 1 atm), I is the current (A), and t is the time (s).

Microbiological characterization of the methane-producing biocathode

At the end of the experiment (day 57), the cathode was cut into samples of about 1x1 cm^2. These samples were used to characterize the microorganisms that had developed at the methane-producing biocathode. The samples were taken at three different locations at the cathode: where the catholyte entered the electrochemical cell

(referred to as "entrance"), the center of the cathode (referred to as "center") and where the catholyte left the electrochemical cell (referred to as "outlet"). The locations of the samples are indicated in Figure 3.1.

The microorganisms present at the biocathode were identified using the molecular techniques described below. The amount of volatile suspended solids (VSS) was quantified using the modified Hartree-Lowry protein analysis. The morphology and distribution of microorganisms at the biocathode were visualized by Fluorescence Microscopy and Scanning Electron Microscopy.

Microbial community analysis

Community analysis was performed at Nadicom GmbH Microbiology Services (Germany). Total genomic DNA was extracted from the 1x1 cm^2 cathode sample taken in the high flow zone (Figure 3.1) of the center of the biocathode using the DNA extraction kit from Applichem (Germany) according to manufacturer's instructions. PCR amplification of the bacterial 16S rRNA gene was performed corresponding to Standard Operating Procedure (SOP) AD-01, using primers 27f and 1492r [153]. PCR protocols for amplification were: initial denaturation for 5 minutes at 95°C, followed by 28 cycles of denaturation (20 seconds at 94°C), annealing (30 seconds at 55°C) and extension (60 seconds at 72°C), followed by a final extension (10 minutes at 72°C). The amplicons were stored at 8°C until further analysis. For the identification of methanogenic *Archaea* (indicated as "methanogens" in the rest of the manuscript), PCR was performed corresponding to SOP AD-01-1 [94], using primers Ar109f and Ar907r to amplify archaeal 16S rRNA. PCR protocols for amplification were: initial denaturation for 5 minutes at 94°C, followed by 28 cycles of denaturation (60 seconds at 94°C), annealing (60 seconds at 52°C) and extension (90 seconds at 72°C), followed by a final extension (6 minutes at 72°C) [94]. The samples were stored

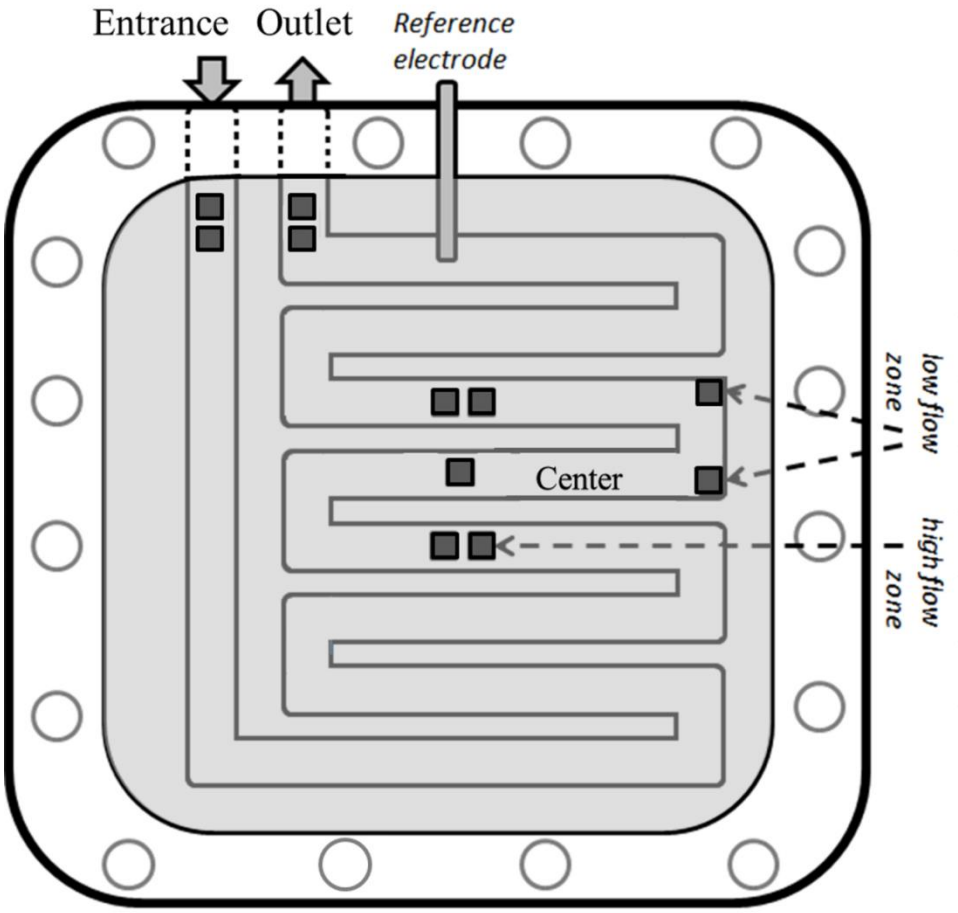

Figure 3.1. Schematic representation of the cathode chamber with the locations of the samples (dark grey squares) used for microbiological analysis. Samples were about 1x1 cm^2. The high flow zone is where the cathode was in contact with the straight part of the flow channels, while the low flow zone is where the cathode was located in a dead zone. The graphite felt electrode (light grey) was placed between two supportive flow channel plates. The total projected surface area of cathode was 361 cm^2, while the effective geometric channel surface area was 290 cm^2.

at 4°C until further analysis. Archaeal and bacterial PCR amplicons were purified with the ChargeSwitch PCR Clean-Up kit (Invitrogen, USA) according to manufacturer's instructions and cloned into *E. coli* JM109 by using the TOPO TA Cloning Kit (Invitrogen, USA). After blue/white screening, positive colonies were transferred to LB medium containing 100 μg/mL ampicilin and grown overnight at 37°C. Plasmid DNA was isolated using the PureLink Quick Plasmid Miniprep Kit (Invitrogen, USA) according to manufacturer's instructions. The PCR product quality was checked by agarose-gel-electrophoresis (1%) stained with ethidium bromide. PCR products with a size of 1.7 Kb were screened with a specific digestion using enzyme *MSP*1. Clones showing a unique band pattern were sequenced via cycle sequencing. The obtained sequences were compared to reference sequences in the NCBI BLAST database (blast.ncbi.nlm.nih.gov). A phylogenetic classification was obtained, together with the degree of similarity to the reference sequences. Sequences retrieved in this study are accessible in the GenBank database under the accession numbers: KC821541-KC821549.

Modified Hartree-Lowry analysis

The modified Hartree–Lowry method was used to determine the protein concentration per m^2 of biocathode in order to quantify the biomass density (expressed as volatile suspended solids (VSS) per m^2 projected cathode surface area). The modified Hartree-Lowry method was applied to two entrance samples, two center samples, and two outlet samples to investigate the effect of location on microbial cell concentration (Figure 3.1). For all samples the exact surface area of the sample was measured. Each sample was transferred in a 2 ml vial, suspended in 1 mL 1 M NaOH, and mixed vigorously for 30 seconds to make sure biomass was suspended and not attached to the graphite felt. The vial was left at 46°C for 35 min to hydrolyse the cells,

and the sample was subsequently neutralized with 1 mL 1 M HCl. The samples were once again mixed vigorously for 30 seconds. The suspension was filtered over a 2 μm filter paper (Whatman 589/3, GE Healthcare, UK), to separate hydrolysed cells from graphite fibres. To 0.5 mL filtrate, 2.5 mL filtered Lowry medium (19.6 g/L Na_2CO_3, 0.20 g/L $Na_3C_6H_5O_7$, and 0.1 g/L $CuSO_4.5H_2O$) was added and the solution was mixed vigorously. After 15 minutes, 0.25 mL Folin-Ciocalteu's phenol reagent was added to the solution, and again the solution was mixed vigorously. After 25 minutes the solution was spectrophotometrically analysed at 650 nm (XION 500 spectrophotometer, Hach Lange Germany). The biomass density (g VSS/m^2 projected cathode surface area) was calculated according to

$$\rho_{VSS} = \frac{C_{protein} \cdot V_{spec} \cdot 4}{0.25 \cdot A_{felt}} \quad (3.4)$$

where $C_{protein}$ is the spectrophotometrically analysed protein concentration derived from calibration measurements in which Bovine Serum Albumin was used as reference protein (g/mL), V_{spec} is the total sample volume that was spectrophotometrically analysed (3.25 mL), 4 is the dilution factor of the original sample, 0.25 is the conversion of g protein to g VSS, and A_{felt} is the surface area of the biocathode sample (m^2).

Fluorescence microscopy

Methanogenic archaea have a low-potential electron carrier coenzyme F_{420} that emits a blue-green autofluorescence when exposed to ultraviolet light at a wavelength of 420 nm. Therefore, immediately after dismantling the methane-producing BES (day 57), two samples of the high flow zone of the center of the biocathode (Figure 3.1) were observed under a UV fluorescence microscope (Leica DMR FC4 microscope with Leica DFC340 FX camera, Germany) with filter cube I3 to identify the presence of active methanogens. The 3D-structure and the 3 mm thickness of the graphite felt

electrode made it impossible to observe the intact biocathode under the UV fluorescence microscope. Therefore, graphite fibres were taken from the graphite felt electrode and observed under the UV fluorescence microscope.

Scanning electron microscopy

Two samples from a low flow zone (where the cathode was located in a dead zone) and two samples of a high flow zone (where the cathode was in contact with the straight part of the flow channels) at the center of the biocathode (Figure 3.1) were analysed using Scanning Electron Microscopy. The biocathode samples were fixed for 2 hours in 2.5% glutaraldehyde in PBS (130 mM NaCl in 10 mM phosphate buffer pH 7.2). After fixation, the samples were washed with PBS 3 times for 15 minutes per washing step. The samples were dehydrated through a series of ethanol baths of increasing concentrations: 10, 25, 50, 75, and 90% (v/v), 20 minutes each, and finally in 100% (v/v) ethanol for 30 minutes. The samples were dried in a desiccator and finally sputter coated with a 5 nm thin gold layer before visualization under High Vacuum with a JSM-6480 LV Scanning Electron Microscope (Jeol, Japan) at 10 kV accelerating voltage.

3.3 Results and Discussion

Performance of the methane-producing biocathode

Current consumption started directly after applying a cathode potential at the start of the experiment. Current density increased to 1.6 A/m^2 projected cathode (day 24, Figure 3.2A). On day 24, solely methane and no hydrogen was detected in the cathode gas phase, indicating an active methane-producing biocathode. On day 24, the cathode potential was changed from -0.7 V to -0.6 V vs. NHE. After changing the cathode

potential, current density was rather constant, with a daily average of 0.60 ± 0.16 A/m^2 projected cathode.

Along with electric current consumption, hydrogen and methane were produced at the cathode. On day 12, only hydrogen (35.7% H_2 (v/v)) was detected in the cathode headspace. On day 24, only methane was detected in the cathode headspace (29.5% CH_4 (v/v)). Maximum methane production rate was 5.1 L CH_4/m^2 projected cathode per day (119 mL CH_4/L reactor per day, at standard temperature and pressure, STP) at -0.7 V vs. NHE cathode potential (1.6 A/m^2, day 24, Figure 3.2B). Maximum methane production rate was 3.0 L CH_4/ m^2 projected cathode per day (69 mL CH_4/L reactor per day, at STP) at -0.6 V vs. NHE cathode potential (0.9 A/m^2, day 51, Figure 3.2B).

Cathodic electron efficiency, the efficiency of capturing the electrons from the electric current in methane, increased from the start of the experiment from 0% (day 0) to 6% (day 12) to 99% (day 24) at -0.7 V vs. NHE cathode potential. If the hydrogen produced at day 12 were included, the cathodic electron efficiency would increase to 49%, assuming 4 moles of hydrogen are required per mole of methane. After changing the cathode potential to -0.6 V vs. NHE, cathodic electron efficiency was 92 ± 29% (average of days 33, 51 and 57) (Figure 3.2B). Cathodic efficiencies of >100% have been reported previously [21, 163] and have been explained by biomass degradation and oxidation [163] or oxidation of carbon stored inside the biomass [45].

Table 3.1. Overview of the operational parameters, inoculum, and performance of methane-producing biocathodes that use CO_2 as electron acceptor.

Microbial catalysts	Operational mode	Applied voltage (V (vs. NHE))	Current density[b] (A/m^2)	Methane production rate[c] (L/m^2 per day)	Ref.
Defined enriched cultures – dominant microbe *Methanobacterium palustre*	two-chamber, batch-fed	-0.8 V E_{cat}	1.8	4.5	[23]
Methanobacterium palustre ATCC BAA-1077	two-chamber, batch-fed	-0.8 V E_{cat}	0.07	0.26	[23]
Undefined enriched cultures	single-chamber, batch-fed	-0.807 V E_{cell}	n.r.	n.r.	[28]
Undefined enriched cultures	single-chamber, continuously-fed	-0.813 V E_{cell}	n.r.	n.r.	[28]
Undefined enriched cultures	two-chamber, batch-fed	-0.9 V E_{cat}	2.9	9.2	[168]
Undefined enriched cultures	single-chamber, continuously-fed	-0.9 V E_{cell}	5.8	1.8	[120]
Undefined enriched cultures	two-chamber, continuously-fed	-1.4 V E_{cat}	4.1	8.7	[21]
Undefined mixed cultures	two-chamber, continuously-fed	-0.7 V E_{cat}	0.87	1.4	[163]
Undefined mixed cultures	two-chamber, continuously-fed	-0.55 V E_{cat}	0.21	0.12	[163]
Defined enriched cultures – dominant microbe *Methanobacterium* sp.	two-chamber, batch-fed	-0.59 V E_{cat}	n.r.	n.r.	[100]
Undefined mixed cultures	single-chamber, batch-fed	-1.25 E_{cell}	n.r.	10	[79]

Undefined mixed cultures	two-chamber, continuously-fed	-0.93 V E_{cat}	0.10	0.24	[170]
Undefined enriched cultures	two-chamber, batch-fed	-1.15 V E_{cat}	15	24	[75]
Methanobacterium thermoautotrophicus	single-chamber, batch-fed	-1.5 V E_{cell}	n.r.	1.0	[136]
Defined mixed cultures – dominant microbe *Methanobacterium* sp.	two-chamber, batch-fed	-0.7 V E_{cat}	1.6	4.1	This study
Defined mixed cultures – dominant microbe *Methanobacterium* sp.	two-chamber, batch-fed	-0.6 V E_{cat}	0.9	2.3	This study

[a] Applied cathode potential (vs. NHE) or applied cell voltage when the cathode potential was not reported.

[b] Calculated using the projected cathode surface area.

[c] Calculated at standard temperature and pressure (273.15 K and 1 atm) using the projected cathode surface area.

n.r. = not reported

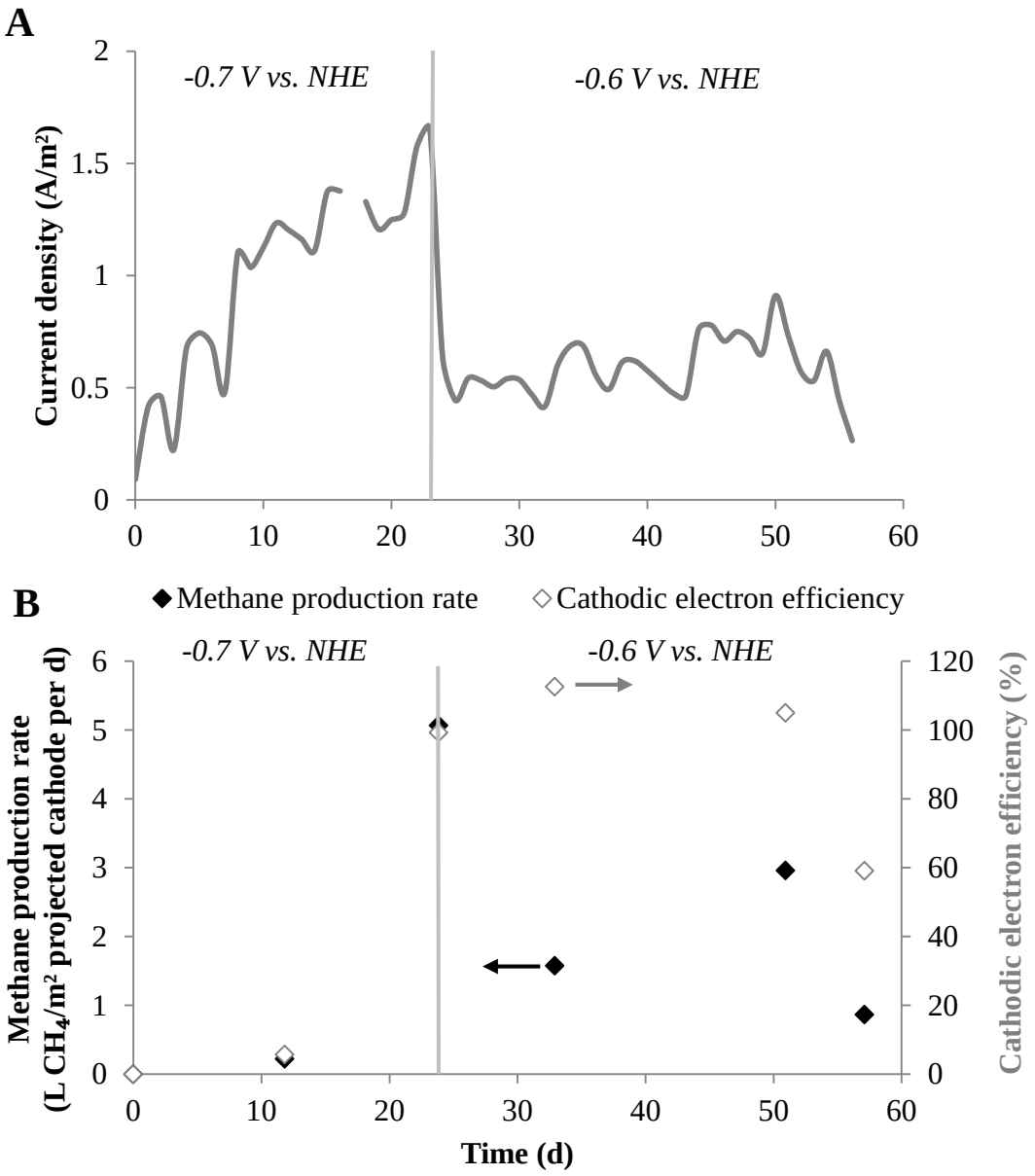

Figure 3.2. (A) Current density (daily averages), and (B) methane production rate and cathodic electron efficiency with time. Cathode potential was changed from -0.7 V vs. NHE to -0.6 V vs. NHE (day 24), as indicated by the grey vertical line.

Reported methane production rates for methane-producing biocathodes in BES are between 0.12 and 24 L CH_4/m^2 projected cathode per day (0.07 to 15 A/m^2) at ≤ -0.55 V vs. NHE cathode potential (Table 3.1). The methane production rates and current densities of the biocathode in this study were in the range of reported methane production rates and current densities. Nearly all of the reported studies used undefined enriched or mixed cultures as inoculum for the methane-producing biocathode. In these studies, the microbial populations were not analysed. Therefore, it is not clear how the microbial community composition affected the performance of the methane-producing biocathode, and via which mechanisms methane was produced. To optimize methane production in BES, key challenges are to select for microorganisms that produce methane at high rate and to operate the BES under their most favourable conditions [27]. This study therefore investigated the microbial community at a methane-producing biocathode and their possible role in bioelectrochemical methane production.

Characterization of the methane-producing microbial community at the biocathode

Samples from the biocathode were used to characterize the composition of the microbial community. The microorganisms that were present at the center of the methane-producing biocathode are reported in Table 3.2. Three phylotypes of archaea and six phylotypes of bacteria were identified in the methane-producing biocathode.

Archaeal 16S rRNA gene sequences were similar to the hydrogen-consuming *Methanobacterium palustre* strain DSM 3108 (98%, KC821542 and KC821543) and the hydrogen-consuming *Methanobacterium aarhusense* strain H2-LR (96%, KC821541).

Bacterial 16S rRNA gene sequences were similar to *Methylocystis* sp. SC2 strain SC2 (98%, KC821549), *Acidovorax caeni* strain R-24608 or *Hydrogenophaga caeni* strain EMB71 (98%, KC821548), *Desulfovibrio putealis* strain B7-43 (97%, KC821546),

Table 3.2. Archaeal and bacterial 16S rRNA gene sequences of the methane-producing biocathode and their similarity with their closest cultured relative. The NCBI accession number is given in parentheses.

	Clone number	Closest relative	Similarity (%)	Closest cultured relative	Similarity (%)	GenBank number
Archaea	1379-1A8r	*Methanobacterium palustre* strain 21 (DQ649333.1) or strain Z2 (DQ649332.1)	99	*Methanobacterium palustre* strain DSM 3108 (NR_041713.1)	98	KC821542
	1379-1A19r	*Methanobacterium palustre* strain 21 (DQ649333.1) or strain Z2 (DQ649332.1)	99	*Methanobacterium palustre* strain DSM 3108 (NR_041713.1)	98	KC821543
	1379-1A1	Uncultured *Methanobacteriaceae* archaeon clone LrhA43 (AJ879024.1)	99	*Methanobacterium aarhusense* strain H2-LR (NR_042895.1)	96	KC821541
Bacteria	1379-1-24r	*Methylocystis* sp. WRS (AY007196.1)	99	*Methylocystis* sp. SC2 strain SC2 (NR_074220.1)	98	KC821549
	1379-1-23r	*Ottowia* sp. RB1-10B (EU882843.1) or *O. pentelensis* strain RB3-7 (EU518930.1)	99	*Acidovorax caeni* strain R-24608 (NR_042427.1) or *Hydrogenophaga caeni* strain EMB71 (NR_043769.1)	98	KC821548
	1379-1-6r	Uncultured delta proteobacterium clone MBNTA-bac1 (DQ205193.1)	98	*Desulfovibrio putealis* strain B7-43 (NR_029118.1)	97	KC821546
	1379-1-2	Uncultured bacterium clone YC50 (GU062460.1)	99	*Petrimonas sulfuriphila* strain BN3 (NR_042987.1)	96	KC821544
	1379-1-5r	Uncultured *Bacteroides* sp. clone 30-S33 (JX462549.1)	99	*Petrimonas sulfuriphila* strain BN3 (NR_042987.1)	95	KC821545
	1379-1-17	*Ottowia* sp. RB1-10B (EU882843.1) or *O. pentelensis* strain RB3-7 (EU518930.1)	99	*Ottowia thiooxydans* strain K11 (NR_029001.1)	95	KC821547

Petrimonas sulfuriphila strain BN3 (96%, KC821544; and 95%, KC821545), and *Ottowia thiooxydans* (95%, KC821547).

Possible role of microorganisms in bioelectrochemical methane production

The methane-producing biocathode analysed in this study contained three phylotypes of archaea: two phylotypes were closely related to *Methanobacterium palustre* and the other phylotype to *Methanobacterium aarhusense*. *Methanobacterium palustre* can use hydrogen as an electron donor [185], although direct use of the electrode as electron donor has also been suggested [23]. *Methanobacterium palustre* has previously been identified as the dominant microorganism, accounting for 87% of the total cells, in a mixed-culture methane-producing biocathode inoculated with effluent of a bio-anode that was fed acetate [23]. *Methanobacterium aarhusense* can only use hydrogen as electron donor [142]. (Bio-)electrochemical production of hydrogen has been reported previously at the cathode potential used in this study [132]. It is likely that the phylotypes that were closely related to *M. palustre* and *M. aarhusense* used hydrogen as electron donor for bioelectrochemical production of methane. While at the start of the experiment hydrogen was detected in the cathode gas phase, no hydrogen was detected in the cathode gas phase once the biocathode had developed. In the experimental set-up, it could not be distinguished whether *M. palustre* also used the electrode as electron donor.

The methane-producing biocathode contained six phylotypes of bacteria. Bacteria identified in our biocathode that may be associated with bioelectrochemical production of methane were closely related to *Desulfovibrio putealis*, *Hydrogenophaga caeni* and *Methylocystis* sp. *Desulfovibrio putealis* is a strict anaerobic microorganism that is able to use hydrogen, organic acids or alcohol as an electron donor and sulfate as an electron acceptor [10]. However, it can only grow with hydrogen as electron donor

when acetate is provided as carbon source [10]. Several *Desulfovibrio* sp. are able to catalyse bioelectrochemical hydrogen production at cathode potentials ≤ -0.44 V vs. NHE (*e.g.* [8, 31]). In this study, the applied cathode potential was ≤ -0.6 V vs. NHE, being in the range of applied potentials at which *Desulfovibrio* sp. are reported to be electrochemically active. Therefore, we hypothesize that the phylotype that is closely related to *D. putealis* may have produced hydrogen, which in turn could be consumed by the methanogens to produce methane. Future research could focus on bioelectrochemical production of methane by *M. palustre* in the presence and absence of *D. putealis* in order to identify the role of the later.

Hydrogenophaga caeni is an aerobic microorganism that is able to use hydrogen as an electron donor, however only when an organic carbon source is provided [26, 173]. *Hydrogenophaga* sp. have also been found at hydrogen-producing biocathodes [30], but their role in hydrogen production remains unclear. The phylotype that is closely related to *Hydrogenophaga caeni* may have catalysed hydrogen production, or may have been an oxygen scavenger, creating strict anoxic conditions that are essential for the methanogenic archaea.

Methylocystis sp. is a facultative aerobic microorganism that is able to use methane as the sole source of carbon and energy [33]. The phylotype that shows similarity to *Methylocystis* sp. may have consumed part of the methane, thereby lowering the methane production rate. However, as oxygen scavenger, it will also create the anoxic conditions that are essential for proliferation of methanogenic archaea. Another phylotype that may have been an oxygen scavenger is closely related to *Acidovorax caeni*. *Acidovorax caeni* is a facultative aerobic microorganism that is able to use carboxylic acids as carbon source [64]. Oxygen concentrations in the cathode gas phase were 0.6-3.5%. At these oxygen concentrations aerobic bacteria that may act as oxygen scavengers have a physiological advantage compared to strict anaerobes. This

physiological advantage is due to the higher reduction potential of oxygen reduction to water versus for instance carbon dioxide reduction to methane, respectively 1.229 V vs. NHE [90] and 0.169 V vs. NHE [23] (at STP and 1 M or 1 atm for all components involved in the reaction).

For some of the bacterial phylotypes, their role in bioelectrochemical methane production remains unclear. For example, *Ottowia thiooxydans* is a facultative anaerobic microorganism that is able to use nitrate or nitrite for growth, and able to oxidize thiosulfate and hydrogen to sulphate [148]. Also, *Petrimonas sulfuriphila* is a strictly anaerobic microorganism that is able to use sugars as carbon and energy source, and able to reduce sulphur with hydrogen to sulphide [50].

In this study, insight is given into the role of the detected community members in methane production. While the identified 16S rRNA sequences in our study most likely have similar substrate preferences as their closest relatives, this is not necessarily the case.

After 24 days, the biocathode produced only methane and no hydrogen. This start-up time is similar to reported start-up times for methane-producing biocathodes, i.e. 28 days at -0.59 V cathode potential [100] and one month at -0.8 V cathode potential [23]. 33 days after initiating the methane-producing biocathode, the microbial community of the methane-producing biocathode was investigated (day 57 of the experiment). Although the current density was rather stable after start-up (0.6 ± 0.15 A/m^2 projected cathode, Figure 3.2A) and only methane was detected, it remains unclear whether a stable microbial community was obtained.

Morphology and distribution of microorganisms at the biocathode

Microscopy techniques were used to give insight into the distribution of the microbial populations at the biocathode. With fluorescence microscopy, the presence

of active methanogens in the biocathode can be revealed (Figures 3.3A and 3.3B). The observed microorganisms were rod-shaped 1-3 μm long cells, with a few longer than 5 μm. The cells were attached to the graphite felt as single cells or as microcolonies (Figures 3.3A and 3.3B). Whereas fluorescence microscopy is generally used to reveal the presence of active methanogens, it should be noted that it may also reveal the presence of reduced cytochromes of bacteria [40].

Scanning electron microscopy (SEM) revealed a variety of rod-shaped microorganisms at the biocathode, varying in their rod form (both straight rods and spiral-shaped rods were observed), the ability to form filaments, and their size (Figures 3.3C, 3.3D and 3.3E). The length of the observed rods varied between <1 μm and 10 μm. The observed rod length was similar to that of the rod-shaped methanogenic archaea pictured by fluorescence microscopy (Figures 3.3A and 3.3B). No clear relationship was observed between the morphology and the location within the biocathode. Rod-shaped microorganisms varying in size from 1 to 5 μm have also been observed in a previous mixed-culture biocathode that simultaneously produced acetate and methane [100].

SEM also revealed that part of the graphite felt was covered with a dense biofilm (Figure 3.3C), while other parts of the graphite felt were only covered with clusters of cells (Figures 3.3D and 3.3E). Both were observed at the low flow zone (where the cathode was located in a dead zone) and the high flow zone (where the cathode was in contact with the straight part of the flow channels). Accumulation of gasses (methane and hydrogen) inside and near the graphite felt electrode may have been an obstacle to attachment, thereby hindering biofilm formation. Another explanation might be that larger cell aggregates, consisting of both anaerobic methanogens and aerobic microorganisms acting as oxygen scavengers, are required to create the strict anoxic conditions needed by the methanogens. The absence of biomass on parts of the

electrode could also be explained by a local excess of electron donor (e.g. hydrogen) near the electrode. As long as methanogens have access to electron donors further away from the electrode, there is no need to attach to the electrode and use the electrode as an electron donor.

Biomass density at the methane-producing biocathode

Based on the modified Hartree-Lowry analysis, it was found that the methane-producing biocathode contained on average 55.6±11.9 g VSS/m^2 projected cathode (n = 6 samples). This VSS density is in the range of reported VSS densities for bio-anodes, being 8-66 g VSS/m^2 projected anode surface area [86]. This is the first study to report the VSS density for a methane-producing biocathode. The VSS density, and thus the biomass density, were similar at different locations of the biocathode: 57 and 68 g VSS/m^2 projected cathode (n = 2 samples) where the catholyte entered the BES, 49 and 65 g VSS/m^2 projected cathode (n = 2 samples) at the center of the biocathode, and 39 and 60 g VSS/m^2 projected cathode (n = 2 samples) where the catholyte left the BES. No clear relationship was observed between biomass density and location at the biocathode. With the current experimental set-up, it could not be determined whether all biomass was active. The density of active biomass is an important parameter to improve conversion rates.

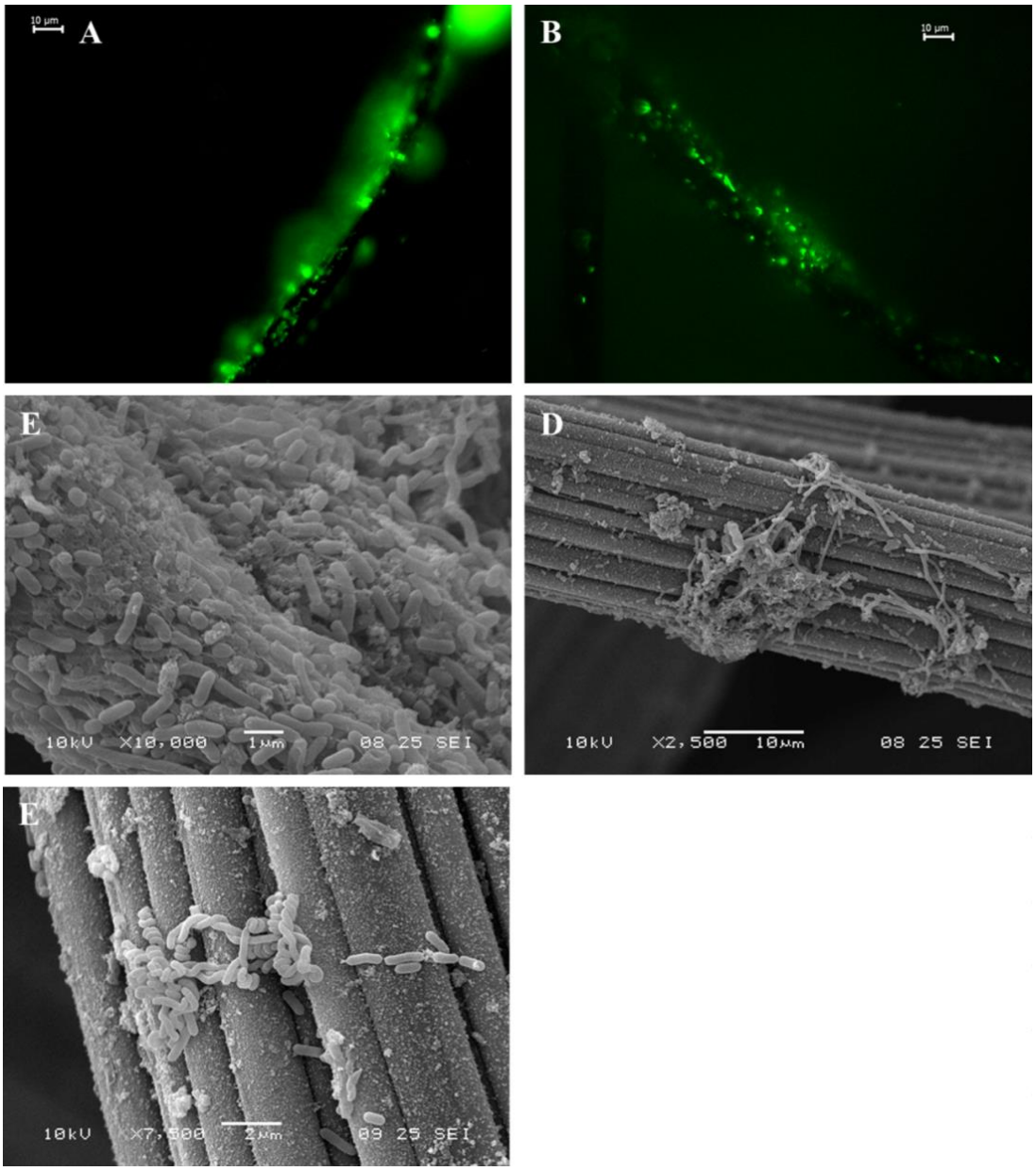

Figure 3.3. Fluorescence microscopy of the center of the biocathode revealed rod-shaped methanogens that were attached to the graphite felt fibres as single cells (A and B) or as microcolonies (A). Scanning electron microscopy (SEM) pictures of rod-shaped microorganisms at the center of the biocathode (C, D and E). Part of the graphite felt was covered with a dense biofilm (C), while parts were only covered with clusters of microbial cells (D and E).

Microbial and electrochemical methods to improve the performance of a methane-producing biocathode

Methane production rates can be improved by selecting for microorganisms that are involved in methane production or that create the optimal conditions for methane production. Selection strategies that could be used are: (i) enrich the inoculum prior to inoculation, (ii) add pure cultures of *Methanobacterium palustre* and *Methanobacterium aarhusense* to the mixed-culture inoculum to give them a competitive advantage during start-up of the biocathode, (iii) adjust operational conditions to the optimum growth conditions for the preferred microorganisms, and (iv) stimulate the growth of synergistic bacteria that might be involved in bioelectrochemical methane production. Methods to enrich the inoculum prior to inoculation include growing the microbial community with an electrode or hydrogen as electron donor, either in batch experiments with multiple feeding cycles or by using the effluent of well-performing BES [23, 75, 100, 120, 168, 170]. This study shows that the phylotypes that are closely related to *Methanobacterium* species produce the preferred end product methane. Isolating the *Methanobacterium* species and testing them as a pure culture would be a first step towards verifying if these methanogens do in fact play a role in methane production at the biocathode, as anticipated. Based on these results, enhancement of the number of cells of *Methanobacterium* species could be a strategy for increasing the methane production rate. Optimizing the operational conditions, such as cathode potential, pH, temperature, and mineral composition of the catholyte are known to positively affect BES performance [27]. Both *M. palustre* and *M. aarhusense* are mesophiles; *M. palustre* has its growth optimum at 37˚C (pH 7) [185] and *M. aarhusense* at 45˚C (pH 7.5-8) [142]. The temperature and pH used in this study were thus lower than the optimum conditions for *M. palustre* and *M. aarhusense*. Through adjusting the temperature and pH to the optimal growth conditions for methanogens, the

electrochemically active bacteria as well as bacteria that act as oxygen scavengers, the methane production rates might increase further. Finally, this study revealed that bacteria, such as *Desulfovibrio putealis*, might be involved in bioelectrochemical methane production through production of hydrogen as intermediate. Synergistic relationships between bacteria and methanogenic archaea were also demonstrated by Cheng and co-workers, who reported that a mixed culture methane-producing biocathode dominated by *Methanobacterium palustre* performed much better than a pure culture biocathode with *Methanobacterium palustre* [23]. Stimulating the growth of synergistic bacteria, through either increasing their cell numbers during inoculation or by adjusting the operational conditions, might be viable strategies to further improve bioelectrochemical methane production.

Besides selecting for microorganisms that are involved in methane production, increasing the biomass density could further improve methane production rate. This study shows that the cathode was only partly covered with microorganisms. Likely, increasing the coverage of the cathode with biomass and increasing the biomass density will improve the performance of a methane-producing biocathode [27]. An excess of electron donor (e.g. hydrogen) near the electrode could have made it unnecessary for the methanogens to attach to the electrode. Biomass density can be increased by growing suspended methanogenic biomass on an inert carrier material that is kept in the catholyte. Additionally, biofilm formation could have been hindered due to accumulation of gasses (methane and hydrogen) inside and near the graphite felt electrode. In this study, the catholyte flowed alongside the cathode. Using a flow-through electrode results in improved mass transfer of gasses away from the electrode and substrates towards the electrode [145], and may consequently yield improved biofilm formation. Biomass coverage can also be increased by changing the cathode surface or catholyte composition for improved attachment of the microorganisms.

Bacteria in natural systems usually have a net negative charge on the cell surface, resulting in electrostatic repulsion with negative charged surfaces, such as the cathode [15, 166]. Bacteria are, however, capable of adjusting their cell surface characteristics (charge and hydrophobicity) depending on the environment [15, 101]. Therefore, prolonged operation might improve bacterial adhesion. Bacterial adhesion could also be improved by changing the properties of the cathode surface, such as the hydrophobicity, and changing the catholyte composition, for example pH and conductivity (*e.g.* [127, 166]). Another method to improve biomass density on the electrodes would be applying high shear [116]. In addition to improved mass transfer and more effective use of the cathode surface area [145], using a flow through electrode results in more compact biocathodes.

3.4 Conclusions

A methane-producing biocathode was obtained that produced methane at a maximum rate of 5.1 L CH_4/m^2 projected cathode per day (1.6 A/m^2) at -0.7 V vs. NHE cathode potential and 3.0 L CH_4/m^2 projected cathode per day (0.9 A/m^2) at -0.6 V vs. NHE cathode potential. The microbial community at the methane-producing biocathode was dominated by three phylotypes of archaea and six phylotypes of bacteria. The archaeal phylotypes were most closely related to *Methanobacterium palustre* and *Methanobacterium aarhusense*. This study shows that, besides methanogenic archaea, bacteria may support methane production through production of hydrogen as intermediate or oxygen scavenging.

3.5 Acknowledgements

We thank Bert Hamelers for the fruitful discussions, Astrid Paulitsch-Fuchs and

Arie Zwijnenburg for their help with Scanning Electron Microscopy, Martin Liebensteiner for his help with autofluorescence microscopy, Christel Kampman for her help with the Hartree-Lowry protein analysis, and Nora Sutton for her help with submitting the rRNA sequences to Genbank and correcting the manuscript.

Chapter 4

Analysis of the mechanisms of bioelectrochemical methane production by mixed cultures

This chapter has been published as:

Mieke C. A. A. Van Eerten-Jansen, Nina C. Jansen, Caroline M. Plugge, Vinnie de Wilde, Cees J. N. Buisman, Annemiek Ter Heijne (2014), *Analysis of the mechanisms of bioelectrochemical methane production by mixed cultures*, Journal of Chemical Technology and Biotechnology, doi 10.1002/jctb.4413.

Abstract

BACKGROUND: In a methane-producing Bioelectrochemical System (BES) microorganisms grow on an electrode and catalyse the conversion of CO_2 and electricity into methane. Theoretically, methane can be produced bioelectrochemically from CO_2 via direct electron transfer or indirectly via hydrogen, acetate or formate. Understanding the electron transfer mechanisms could give insight in methods to steer the process towards higher rate.

RESULTS: In this study, the electron transfer mechanisms of bioelectrochemical methane production by mixed cultures were investigated. At a cathode potential of -0.7 V vs. Normal Hydrogen Electrode (NHE), average current density was 2.9 A/m^2 cathode and average methane production rate was 1.8 mole e^- eq/m^2 cathode per day (5.2 L CH_4/m^2 cathode per day). Methane was primarily produced indirectly via hydrogen and acetate. Methods to steer towards bioelectrochemical hydrogen and acetate production to further improve the performance of a methane-producing BES are discussed.

CONCLUSION: At cathode potentials equal or lower than -0.7 V vs. NHE and using mixed cultures, methane was primarily produced indirectly via hydrogen and acetate. (Bio)electrochemical hydrogen and acetate production rate could be increased by optimizing the cathode design and by enriching the microbial community. Consequently, the production rate of CO_2-neutral methane in a BES could be increased.

Keywords: biocathode, Microbial Electrolysis Cell, CO_2 reduction, direct electron transfer, hydrogen, acetate

4.1 Introduction

There is a societal need to replace fossil fuels with renewable fuels. Fossil fuel resources are finite because they are currently consumed at a higher rate than they are generated. Moreover, fossil fuel consumption leads to emission of CO_2 and pollutants, such as fine dust particles, NO_x and SO_x, that affect the environment and human health [105]. A bioelectrochemical system (BES) is a novel technology with which low-grade organic matter can be converted into electricity, or (renewable) electricity can be converted into fuels and chemicals [91]. The principle of BESs is excellently reviewed in [89-92, 118]. Key component of BESs are microorganisms that grow on an electrode and catalyse a wide range of oxidation and reduction reactions to produce or consume electricity [92, 93]. Besides the production of renewable electricity [90], fuels and chemicals produced in BESs are, for example, hydrogen [130], methane [23], hydrogen peroxide [133], caustic soda [117], acetate and 2-oxobutyrate [100, 107], ethanol [151], ammonium [80], butyrate [24], and caproate and caprylate [164].

Methane production from CO_2 is an attractive application of BESs for production of renewable fuels, since (i) CO_2-neutral methane could be obtained, independent from biomass, (ii) the infrastructure for transport, storage and consumption of methane is already in place [23], (iii) contrary to landfill gas or biogas of anaerobic digestion, a gas enriched in methane could be obtained that does not require further processing to be injected in the national gas grid [91], and (iv) methane can act as an energy carrier for renewable electricity from for instance the sun or wind, when supply exceeds demand [23]. For bioelectrochemical methane production, it is important to have more understanding of the electron transfer mechanisms that lead to methane production. Understanding these mechanisms could give insight in methods to steer the process, for instance, changing the operation parameters (e.g. cathode potential) or the microbial community.

Cheng and co-workers were the first to demonstrate methane production via direct electron transfer using enriched mixed cultures, obtained from an acetate-fed anode of an existing two-chamber bioelectrochemical system producing methane [22], or a pure culture of *Methanobacterium palustre* at cathode potentials ≤-0.6 V vs. a Normal Hydrogen Electrode (NHE) [23]. However, at the cathode potentials used, (bio)electrochemical production of hydrogen could not be excluded [93, 132], and there was no solid proof that methane production did not occur indirectly via hydrogen [99]. Villano and co-workers demonstrated with enriched mixed cultures that at cathode potentials equal or higher than -0.75 V vs. NHE methane was mainly produced via direct electron transfer and indirectly via bioelectrochemically produced hydrogen, while at cathode potentials lower than -0.75 V vs. NHE methane was mainly produced indirectly via electrochemically produced hydrogen [168]. However, in both studies methane production via acetate or formate was not investigated. Both acetate and formate can be produced (bio)electrochemically [107, 121] and in turn be converted into methane by methanogens [99].

The role of acetate and formate in methane production at a biocathode has been sparsely investigated. Nevin and co-workers were the first to report carbon dioxide reduction to acetate at -0.4 V vs. NHE cathode potential using *Sporomusa ovata* [107], and the same process has also been reported for mixed cultures at cathode potentials ≤-0.59 V vs. NHE [75, 100], but a possible role in methane formation was not investigated. Reda and co-workers reported carbon dioxide reduction to formate at cathode potentials between -0.4 and -0.8 V vs. NHE using FDH1, a tungsten-containing formate dehydrogenase, from *Syntrophobacter fumaroxidans* [121], but formate conversion into methane was not investigated. Marshall and co-workers were the first to report CO_2 reduction to a mixture of methane, hydrogen, acetate and formate by mixed cultures at -0.59 V vs. NHE cathode potential [100]. As described by Villano

and co-workers [168], the mixture of reduced end products was produced both via direct electron transfer and indirectly via hydrogen [100]. Contrary to Marshall and co-workers, this study aims to produce methane only. Marshall and co-workers did not investigate whether the produced acetate and formate played a role in methane formation, and whether the mechanisms could be steered to produce methane only.

The aim of this study was to investigate the mechanisms of electron transfer for bioelectrochemical methane production using mixed cultures at two different cathode potentials. The formation of intermediates hydrogen, acetate or formate was elucidated by selectively inhibiting the methanogens at the biocathode with 2-bromoethanesulfonate, a specific inhibiter of methanogenesis. It was hypothesized that by inhibiting solely methanogens and not bacteria, intermediates could be detected that play a role in indirect bioelectrochemical methane production. Besides, it was hypothesized that direct electron transfer is directly related to current density. Consequently, inhibiting methanogens would result in a direct decrease in current density in case methane would be produced via direct electron transfer.

4.2 Materials and methods

Electrochemical cell

Two flat-plate electrochemical reactors (0.56 L total reactor volume) as described in [144] were used. The anodes were made of platinum-coated (50 g/m^2) titanium-mesh (projected surface area 0.025 m^2, thickness 1 mm, specific surface area 1.7 m^2/m^2), and the cathodes were made of graphite felt (projected surface area 0.025 m^2, thickness 3 mm). The cathode was connected to three 14 carat golden wires that were the current collectors, same as [132]. Both the anode and cathode compartments (0.28 L each) were equipped with an Ag/AgCl 3 M KCl reference electrode (+0.205 V vs. NHE;

ProSense QiS, The Netherlands), and the compartments were separated from each other by a cation exchange membrane (Fumasep FKB, Fumatech GmbH, Germany). The cathode was operated as a flow-through electrode with spacer material (PETEX 07-4000/64, thickness 4 mm, 64% open, Sefar B.V., The Netherlands) placed between the membrane and the cathode, and the catholyte was directed away from the membrane through the spacer material and the cathode, same as [145]. The anolyte was directed through serpentine flow channels parallel to the electrode. The cathode gas phase was connected to a gas flow meter (Milligascounter, Ritter, Germany) via an injection port, while the anode gas phase was connected to a water lock. The total catholyte volume (electrochemical cell, tubing and recirculation vessel) was about 0.75 L, and the total cathode gas phase was about 0.36 L. The volumes changed slightly with time, and therefore the exact volumes were determined regularly and used for the calculations.

Inoculum and electrolytes

The electrochemical reactors were inoculated with anaerobic sludge obtained from an anaerobic digester treating municipal wastewater (Ede, The Netherlands). The anaerobic sludge was centrifuged (10 minutes, 3660 RPM, 20°C – Firlabo SW12R, Beun·de Ronde, The Netherlands) to remove large particles and the supernatant was inoculated in the catholyte. Inoculation occurred on day 0 (240 mL supernatant per cell) and on day 10 (100 mL supernatant per cell).

The catholyte consisted of 3.00 g/L K_2HPO_4 and 0.38 g/L KH_2PO_4 (20 mM potassium phosphate buffer pH 8), macronutrients (280 mg/L NH_4Cl, 5.7 mg/L $CaCl_2$, 10 mg/L $MgSO_4.7H_2O$, and 90 mg/L $MgCl_2.6H_2O$), and 1 mL/L of a micronutrients and vitamin solution same as [155], and was supplemented with 5 g/L $NaHCO_3$ as carbon source. The catholyte was recirculated at 40 mL/min (days 0 to

37) or 20 mL/min (from day 37 until the end of the experiment), and continuously refreshed at a rate of 0.2 mL/min. The pH of the catholyte was controlled at pH 7.0±0.1 through a pH controller (Liquisis M CPM 253, Endress + Hauser, Switzerland) using 0.5 M HCl. The anolyte consisted of 20 mM potassium phosphate buffer, adjusted to pH 2 with 37% (v/v) HCl. The anolyte was recirculated at 40 mL/min (days 0 to 37) or 20 mL/min (from day 37 until the end of the experiment), and replenished regularly. The system was flushed with pure nitrogen (>99.9992%) for at least three times its gas phase volume before applying a cell voltage.

Electrochemical cell operation

To investigate the effect of cathode potential on the mechanisms for electron transfer for bioelectrochemical methane production, one electrochemical reactor was operated at -0.7 V vs. NHE cathode potential (indicated as "BES -0.7 V" throughout the manuscript) and one electrochemical reactor was operated at -0.9 V vs. NHE cathode potential (indicated as "BES -0.9 V" throughout the manuscript). These cathode potentials were chosen, since at -0.7 V vs. NHE electron transfer can be both direct or indirectly via hydrogen, while at -0.9 V vs. NHE electron transfer is mainly indirectly via hydrogen [168]. The experimental conditions that were changed during the experiment are indicated in Table 4.1. Before the start of the experiment, the electrochemical reactors were rinsed with 70% ethanol (v/v) to disinfect the reactors. After inoculation, both electrochemical reactors were operated at -0.7 V vs. NHE cathode potential for the methane-producing biocathodes to develop under identical operational conditions. During the first days of the experiment oxygen could enter the system through a leakage, and therefore both BESs were re-inoculated with mixed cultures after 10 days. The re-inoculation is considered the start of the experiment (day 0). The cathode potential of BES -0.9 V was changed to -0.9 V vs. NHE on day 27,

Table 4.1. Description of the experimental conditions that were changed during the experiment.

Experimental condition	BES -0.7 V Day	BES -0.9 V Day
Inoculation with mixed cultures	-10	-10
Re-inoculation with mixed cultures	0	0
Pump speed was halved to 20 mL/min	27	27
Inhibition methanogens with 20 mM 2-BES	31	n.a.
Inhibition methanogens with 40 mM 2-BES	38	38
Experiment aborted	49	56

n.a. = not applicable

and the biocathode could adjust to this new cathode potential until day 38. On day 27, it was observed that a higher pressure was required for the catholyte to enter both BESs. To avoid this pressure build up to cause leakages, the speed of both catholyte and anolyte recirculation was reduced to 20 mL/min for both electrochemical cells (day 27). Finally, the methanogens in both electrochemical cells were inhibited with sodium 2-bromoethanesulfonate (2-BES), starting on day 31 for BES -0.7 V and day 38 for BES -0.9 V. 20 mM 2-BES proved not to be sufficient to fully inhibit methanogenesis, and therefore the 2-BES concentration was increased to 40 mM on day 38. After almost three weeks of 2-BES inhibition, the experiment was terminated for both electrochemical reactors. This manuscript focusses predominantly on the results obtained for BES -0.7 V, as the performance of BES -0.9 V was lower than expected. The ohmic resistance, measured via AC voltammetry at a frequency of 1000 Hz (10 mV amplitude; the measurement was performed in duplicate), of BES -0.9 V increased with time from 108 $m\Omega \cdot m^2$ cathode to 250 $m\Omega \cdot m^2$, while the ohmic resistance of BES -0.7 V was stable and on average 64 $m\Omega \cdot m^2$. Corroded and broken current collectors might be the cause for this higher ohmic resistance of BES -0.9 V, and consequently for the lower performance.

The electrochemical reactors were connected to a potentiostat (IviumStat, Ivium Technologies, The Netherlands) to control the cathode potential. The electrochemical reactors were controlled using a three-electrode set-up with the work electrode being the cathode, the counter electrode being the anode and the reference electrode being the cathode reference electrode.

The electrochemical reactors were connected to a PC via a Fieldpoint FP-AI-110 module (National Instruments, USA), and every 60 seconds cell voltage, current, cathode, anode and membrane potential were recorded using Labview 7.1 (National Instruments, USA). The electrochemical reactors were operated in a temperature controlled chamber at 31±1°C.

Analyses

The gas composition of the cathode gas phase was analysed two to three times per week. Gas samples were taken from the injection port, located in the cathode gas phase before the gas flow meter. The methane (2 mL gas sample) was analysed using gas chromatography (Shimadzu GC-2010) with a thermal conductivity detector (TCD) as described in [76]. The hydrogen (100 µL gas sample) was analysed using gas chromatography (Hewlett-Packard 5890A) with a TCD. 100 µL of gas was directed over a Molsieve 5A column (30 m x 0.53 mm x 15 µm, Varian, cat. nr. 7544) for 2 minutes using argon as carrier gas (20 mL/min, 220 kPa). The injection port temperature was 110°C, the oven temperature was 40°C, and the TCD temperature was 150°C. Total gas production was continuously measured with the gas flow meter.

Acetate and formate concentrations in the catholyte were analysed during 2-BES inhibition (for BES -0.7 V on day 40, and for BES -0.9 V on days 40 and 52). Acetate was analysed using gas chromatography (HP 5890 series II) with AT™ aquawax-DA glass column (30 m x 0.32 mm x 25 mm) and a flame ionization detector (FID).

Liquid samples were centrifuged for 5 minutes at 10.000 RPM and diluted twofold with 3% formic acid (w/w). Prepared sample (1 µL) was injected into the injection port at 200°C. The oven temperature was 130°C, the FID had a temperature of 280°C, and the sample run lasted 8.5 minutes. Formate was analysed spectrophotometrically according to [83].

Once per week the electrochemical reactor was characterized electrochemically by means of a polarisation test. The cathode potential was decreased stepwise from -0.4 V vs. NHE to -1.0 V vs. NHE with steps of 0.1 V, each step lasting 15 minutes. Current was measured each second, and the last 10 data points at each cathode potential were averaged and plotted in the polarisation curve. The standard deviation of the last 10 data points at each cathode potential was less than 5% of the average value.

Calculations

Methane and hydrogen production were calculated using the total gas production and the measured concentrations in the gas phase, according to

$$V_{product,t} = V_{product,t-1} + \left(V_{T,t} - V_{T,t-1}\right) \cdot \frac{\left(C_{product,t} + C_{product,t-1}\right)}{2} + V_{cat} \cdot \left(C_{CH_4,t} - C_{CH_4,t-1}\right) \quad (4.1)$$

with subscript "product" indicating the products methane or hydrogen, $V_{product,t}$ and $V_{product,t-1}$ the cumulative production (L) on sample time t and previous sample time t-1, $V_{T,t}$ and $V_{T,t-1}$ the total measured gas production (L) on sample time t and previous sample time t-1, $C_{product,t}$ and $C_{product,t-1}$ the measured concentrations on sample time t and previous sample time t-1 (L/L), and V_{cat} the total cathode gas phase volume for methane and hydrogen (L). The production rate (in L gas/m^2 cathode per day) was calculated from the cumulative production

$$r_{product} = \frac{V_{product,t}}{A_{cat} \cdot \Delta t} \quad (4.2)$$

with A_{cat} the projected cathode surface area (0.025 m^2). To compare the methane and hydrogen production rates with production rates reported in literature, all reported methane and hydrogen production rates were calculated at standard temperature and pressure (STP, 273.15 K and 1 atm) and with respect to the projected cathode surface area, according to

$$r_{product}^{STP} = r_{product} \cdot \frac{p \cdot T_{STP}}{p_{STP} \cdot T} \quad (4.3)$$

with $r_{product}^{STP}$ the production rate at STP (L/m^2 cathode per day), p the pressure used in this study (1.005 atm), T the temperature used in this study (304 K), and T_{STP} and p_{STP} the temperature and pressure at STP, 273.15 K and 1 atm respectively.

Cathodic electron efficiency, the efficiency of capturing the electrons from the electric current in gaseous products methane and hydrogen, was calculated according to

$$\eta_{CE} = \frac{V_{product,t} \cdot F \cdot n}{V_m \cdot \int_{t=0}^{t} I dt} \cdot 100\% \quad (4.4)$$

with F Faraday constant (96485 C/mole e^-), n the moles of electrons per mole of product (in mole e^-/mole product; n = 8 for methane and 2 for hydrogen), V_m the molar volume (25.1 L/mole at 304 K and 1 atm), I the current (A), and t the time (s). The average cathodic electron efficiency and its standard deviation were calculated for the period day 0 to 31, based on nine data points (the data of days 0, 3, 10, 18, 19, 21, 24, 26 and 31).

The minimum required electrical energy input for bioelectrochemical methane production (Q_{CH_4} in kWh/m^3 CH_4) was calculated from the standard cathode and anode potentials ($E^0_{cat/an}$ in V; at pH 7 for the catholyte and pH 3 for the anolyte, 298.15 K, 1 bar, and 1 M for all species, using the tabulated values in [159]), according

to

$$Q_{CH_4} = \frac{(E^0_{cat} - E^0_{an}) \cdot n \cdot F}{V_m \cdot 3.6 \cdot 10^6} \quad (4.5).$$

4.3 Results and discussion

Electrochemical performance of the methane-producing biocathode

Current density was 5.9 A/m^2 projected cathode surface area at the start of the experiment for the BES controlled at -0.7 V vs. NHE cathode potential (BES -0.7 V) (Figure 4.1). Current density decreased with time to a minimum value of 1.4 A/m^2 cathode (day 14), and increased again to an average current density of 2.6 A/m^2 cathode for the remainder of the experiment (day 14 to 49). Average current density of the methane-producing biocathode, before inhibition of the methanogens, was 2.9 A/m^2 cathode (day 0 to 31).

Biocathode development was also investigated with polarisation tests. The highest current densities were achieved for a non-inoculated electrode, hours before inoculation (control, Figure 4.2). After re-inoculation (day 0), biocathode performance decreased with time until day 24 after which it became stable, which is in line with the trend observed in the continuous current density measurements (Figure 4.1).

With polarisation tests also the total internal resistance of the BES was determined, the slope representing the total internal resistance [90]. The total internal resistance of the BES was on average 74 mΩ·m^2. The ohmic resistance contributed most to this total internal resistance, being on average 64 mΩ·m^2.

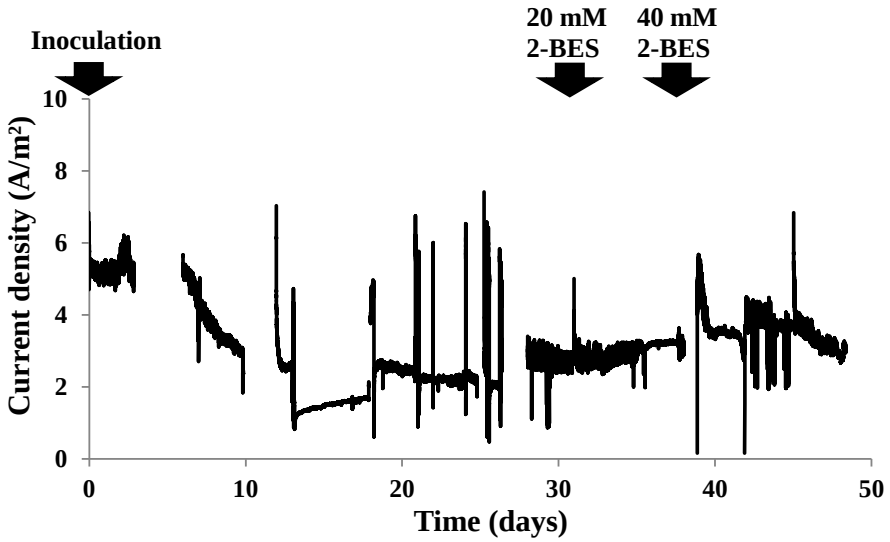

Figure 4.1. Current density with time for the BES controlled at -0.7 V vs. NHE cathode potential (BES -0.7 V). The peaks in current density occurred immediately after turning the potentiostat on, after being turned off for a short time. The experimental conditions that were changed during the experiment are indicated by the arrows (for a description of the experimental conditions that were changed with time, see Table 4.1).

Methane was produced concomitant with current consumption

Concomitant with current consumption, methane and hydrogen were produced. Immediately after first inoculation (day -10), hydrogen was detected in the cathode gas phase, reaching a fraction of 0.82 (v/v) on day -2. Methane fractions, on the other hand, were maximum 0.05 (v/v). After re-inoculation (day 0), however, only methane was produced, with fractions reaching up to 0.73 (v/v) (day 28) (Figure 4.3). The average methane production rate after second inoculation was 1.8±0.95 mole e^- eq/m^2 cathode per day (day 0 until 31; n = 16; Figure 4.3), and the maximum methane production rate was 3.8 mole e^- eq/m^2 cathode per day (day 4). At standard temperature and pressure (273.15 K and 1 atm), this is equal to an average methane production rate of 5.2±2.7 L CH_4/m^2 cathode per day, and a maximum methane production rate of 10.7 L /m^2 cathode per day. Maximum methane production rates

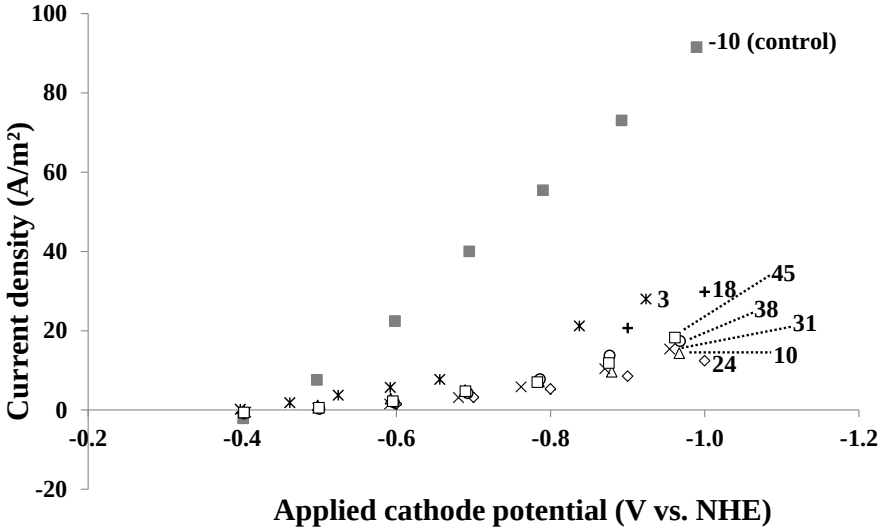

Figure 4.2. Polarisation tests revealed biocathode development with time for BES -0.7 V. The days at which polarisation tests were performed are displayed behind the measurements . A control measurement was performed with a non-inoculated electrode (grey squares) hours before first inoculation (day -10).

for mixed culture methane-producing biocathodes reported in literature are 0.1-3.7 mole e^- eq/m^2 cathode day or 0.12-23.8 L CH_4/m^2 cathode per day [21, 23, 28, 75, 79, 100, 120, 143, 163, 165, 168, 170]. The methane-producing biocathode obtained in this study had thus similar performance as mixed culture biocathodes reported in literature. Cathodic electron efficiency calculations revealed that up to 73% of the consumed current was recovered in methane (day 10). The total cathodic electron recovery fluctuated between 52% (day 18) and 74% (day 19) (Figure 4.4), similar to reported cathodic electron efficiencies in literature that range between 23% and 99% [21, 23, 75, 100, 143, 163, 165, 168, 170]. Electron sinks could be the diffusion of methane and hydrogen through the membrane towards the anode [130], biomass formation [90], temporary storage of electrons in microbial biomass [45], and the production of other reduced compounds.

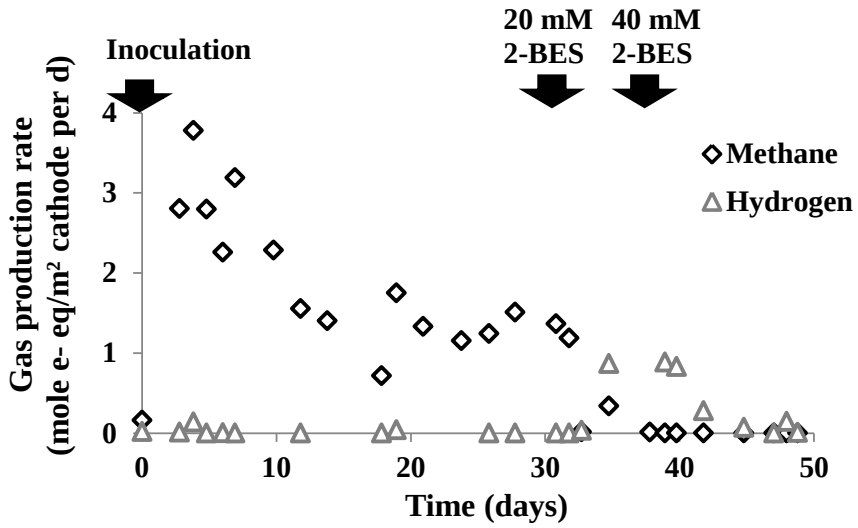

Figure 4.3. Methane and hydrogen production rate with time for BES -0.7 V. The experimental conditions that were changed during the experiment are indicated by the arrows (for a description of the experimental conditions that were changed with time, see Table 4.1).

Current density profile indicates formation of intermediates for indirect bioelectrochemical methane production

To investigate the mechanisms of electron transfer for bioelectrochemical methane production, the methanogens in the biocathode were inhibited with 2-BES, a specific inhibiter for methanogenesis. It was hypothesized that by inhibiting solely methanogens and not bacteria, intermediates could be detected that play a role in indirect bioelectrochemical methane production. Besides, it was hypothesized that direct electron transfer is directly related to current density. Consequently, inhibiting methanogens would result in a direct decrease in current density in case methane would be produced via direct electron transfer. In case methane is produced indirectly via intermediates, after inhibiting methanogens with 2-BES current density might decrease with time due to accumulation of the products. However, this decrease is

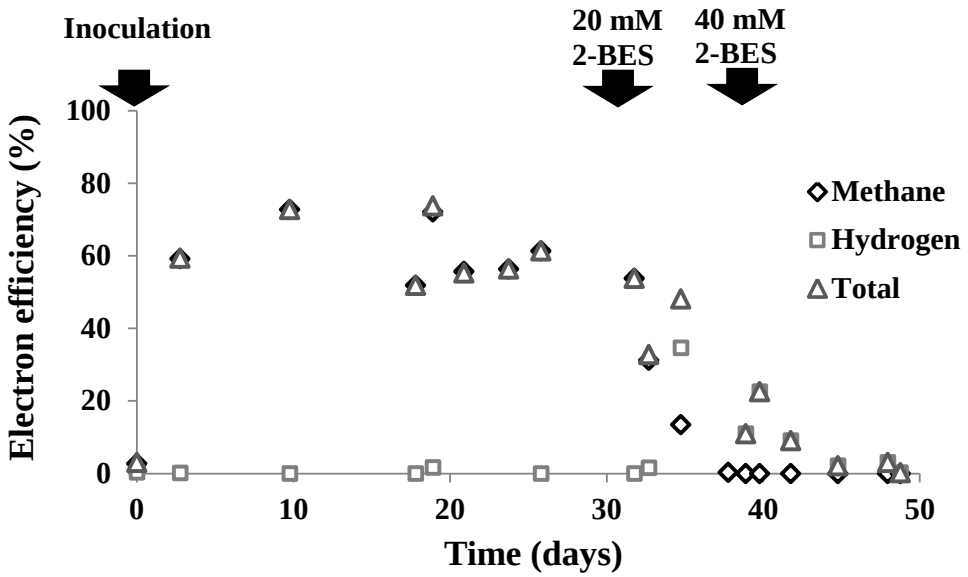

Figure 4.4. Cathodic electron efficiency for methane, hydrogen and both methane and hydrogen (total) with time for BES -0.7 V. The experimental conditions that were changed during the experiment are indicated by the arrows (for a description of the experimental conditions that were changed with time, see Table 4.1).

expected to occur gradually, contrary to the expected immediate decrease in current density when methane is produced via direct electron transfer.

Interestingly, current density did not change directly after inhibiting the methanogens with 2-BES (from day 31 onwards) (Figure 4.1). This result suggests that methane is likely not produced via direct electron transfer at -0.7 V vs NHE cathode potential, but rather indirectly via intermediates.

Hydrogen and acetate were detected as intermediates for indirect bioelectrochemical methane production

Between first and second inoculation, hydrogen was measured in the cathode gas phase, while it was absent when the methane-producing biocathode was fully

developed (Figure 4.3). Upon inhibiting methanogens with 2-BES, hydrogen was again produced with a maximum production rate of 0.9 mole e^-/m^2 cathode per day (day 39; Figure 4.3). This shows that hydrogen was an intermediate for indirect bioelectrochemical methane production.

Besides hydrogen, also acetate was detected in the catholyte after inhibiting the methanogens with 2-BES (797 mg/L, day 40), while no formate was detected. The only origin for acetate in the cathode chamber could be bioelectrochemical reduction of CO_2, as CO_2 was the sole carbon source. Since acetate was measured during methanogens inhibition, it is likely that acetate acted as an electron donor for indirect bioelectrochemical methane production. No acetate and formate measurements were performed between days 0 and 31, and therefore it remains unknown whether the biocathode produced acetate or formate together with methane, as was observed by Marshall and co-workers [100].

Effect of cathode potential on mechanisms of electron transfer

To investigate the effect of cathode potential on the mechanisms of electron transfer, the second BES was operated at -0.9 V vs. NHE cathode potential. At this cathode potential, it is expected that methane production occurs mainly indirectly via intermediates hydrogen, acetate or formate, and to a smaller extent via direct electron transfer [168].

Similar to the BES operated at -0.7 V vs. NHE cathode potential, when inhibiting methanogens with 2-BES, current density remained the same for 6 days and decreased slightly with time thereafter (data not shown). Also, hydrogen and acetate were measured after inhibiting the methanogens with 2-BES, while no formate was detected. According to these results, similar electron transfer mechanisms occurred at -0.9 V vs. NHE cathode potential compared to -0.7 V vs. NHE cathode potential.

Because the performance of BES -0.9 V was, unexpectedly, lower than the performance of BES -0.7 V, only trends can be compared and no quantitative conclusions on the effect of cathode potential on the predominant mechanisms can be given at this point.

Mechanisms of bioelectrochemical methane production

Theoretically, methane could be produced bioelectrochemically from CO_2 via direct electron transfer or indirectly via hydrogen, acetate or formate (Figure 4.5, Table 4.2). Bioelectrochemical methane production via direct electron transfer (mechanism 1 in Figure 4.5 and Table 4.2) or indirectly via (bio)electrochemically produced hydrogen (mechanisms 2-6 and 3-6 in Figure 4.5 and Table 4.2) has been reported at cathode potentials ranging between -0.6 V vs. NHE and -1.0 V vs. NHE [23, 168]. However, the role of acetate and formate in methane production at a mixed culture biocathode has been sparsely investigated. Theoretically, acetate and formate can be produced bioelectrochemically from CO_2 via direct electron transfer (mechanisms 4 and 5 in Figure 4.5 and Table 4.2, respectively) or indirectly via (bio)electrochemically produced hydrogen (mechanisms 2-7-8 and 3-7-8 for acetate and mechanisms 5+2-10, 5+3-10, 2-9-10 and 3-9-10 for formate in Figure 4.5 and Table 4.2). Electrochemical reduction of CO_2 to formate is another possible mechanism, but because it is unlikely to occur at a methane-producing biocathode due to the low cathode potentials required ($\leq$ -1.0 V vs. NHE) [48], it is not included as a possible electron transfer mechanism.

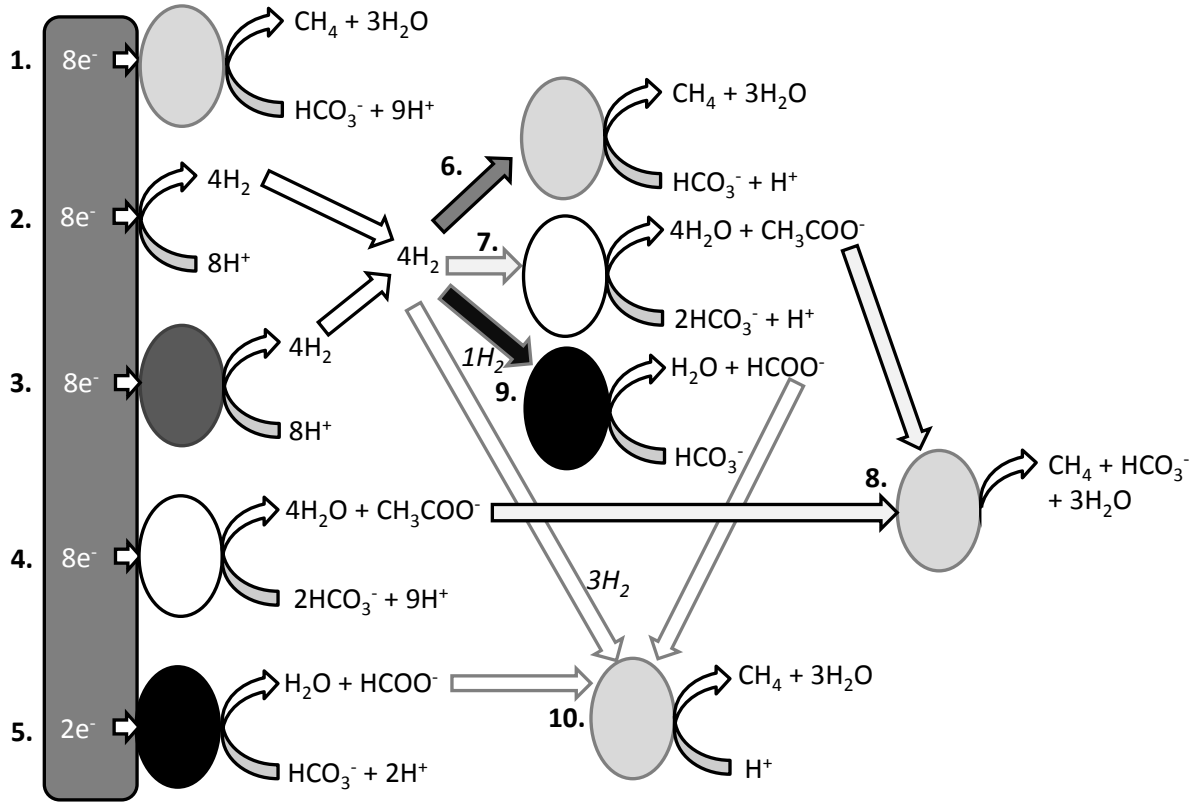

Figure 4.5. Ten possible mechanisms of bioelectrochemical methane production: methane production via direct extracellular electron transfer (the light grey oval indicates the methanogenic archaea) (mechanism 1), indirect methane production via (bio)electrochemically produced hydrogen (the dark grey oval indicates the hydrogen-producing microorganisms) (mechanisms 2-6 and 3-6), indirect methane production via bioelectrochemically produced acetate (the white oval indicates the acetate-producing microorganisms) (mechanisms 4-8, 2-7-8 and 3-7-8), and indirect methane production via bioelectrochemically produced formate (the black oval indicates the formate-producing microorganisms) (mechanisms 5+2-10, 5+3-10, 2-9-10, and 3-9-10).

Bioelectrochemical methane production via direct electron transfer requires the lowest minimum electrical energy input of all mechanisms (11.0 kWh/m^3 CH_4, at standard temperature and pressure, pH 7 for the catholyte, pH 2 for the anolyte and 1 M or 1 bar for all reaction species; mechanisms 1+11 in Table 4.2). Bioelectrochemical production of acetate requires a minimum electrical energy input of 11.3 kWh/m^3 CH_4 (mechanisms 4+11 in Table 4.2), followed by bioelectrochemical production of formate (12.4 kWh/m^3 CH_4; mechanisms 5+11 in Table 4.2) and (bio)electrochemical production of hydrogen (12.5 kWh/m^3 CH_4; mechanisms 2+11 and 3+11 in Table 4.2). At these conditions, it is thus energetically favourable to produce methane via direct electron transfer instead of indirectly via hydrogen, acetate or formate. The energy input for methane formation, however, is highly dependent on catholyte pH and the hydrogen partial pressure, and changing these parameters could be a strategy to decrease the energy input.

This study showed that at cathode potentials -0.7 V vs. NHE and -0.9 V vs. NHE methane is predominantly produced indirectly via hydrogen and acetate (mechanisms 2-6, 3-6, 4-8, 2-7-8, and 3-7-8 in Figure 4.5 and Table 4.2), while formate was not detected. Besides, at cathode potentials -0.7 V vs. NHE and -0.9 V vs. NHE methane production via direct electron transfer hardly occurred, as the current density remained constant directly after inhibiting the methanogens with 2-BES (Figure 4.1). Marshall and co-workers report that at -0.59 V vs. NHE cathode potential, methane was produced via direct electron transfer as well as indirectly via (bio)electrochemically produced hydrogen [100], although no distinction was made between these three mechanisms. The higher cathode potential used in their study might explain the occurrence of the direct electron transfer mechanism. Villano and coworkers report

Table 4.2. An overview of the reaction equations that could occur in the ten possible electron transfer mechanisms of bioelectrochemical methane production.

Mechanism	Reaction equation	Potential (V vs. NHE)[a]	Minimum electrical energy input (kWh/m^3 CH_4)[b]
(Bio)electrochemical reactions cathode			
1	$HCO_3^- + 9H^+ + 8e^- \xrightarrow[electricity]{} CH_4 + 3H_2O$	-0.24	2.1
2 and 3	$2H^+ + 2e^- \xrightarrow[electricity]{} H_2$	-0.41	3.6
4	$2HCO_3^- + 9H^+ + 8e^- \xrightarrow[electricity]{} CH_3COO^- + 4H_2O$	-0.28	2.4
5	$HCO_3^- + 2H^+ + 2e^- \xrightarrow[electricity]{} HCOO^- + H_2O$	-0.41	3.5
Biochemical reactions cathode			
6	$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$		
7	$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$		
8	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$		
9	$HCO_3^- + H_2 \rightarrow HCOO^- + H_2O$		
10	$HCOO^- + 3H_2 + H^+ \rightarrow CH_4 + 2H_2O$		
Oxidation reaction anode			
11	$4H_2 \rightarrow 2O_2 + 8H^+ + 8e^-$	-1.05	8.9

[a] All reported potentials are standard potentials at pH 7 (except for the anode oxidation reaction, pH 3), 298.15 K, 1 bar, and 1 M for all species, using the tabulated values in [159]. Anolyte pH is assumed to be pH 3, as during long-term operation the anolyte acidifies to this value [163].

[b] The electrical energy input is the energy input that is needed for both the cathode plus the anode reaction to occur.

that at cathode potentials equal or lower than -0.75 V vs. NHE methane was predominantly produced via direct electron transfer and indirectly via bioelectrochemically produced hydrogen, although no distinction was made between the two mechanisms [168]. At cathode potentials higher than -0.75 V vs. NHE, methane was predominantly produced indirectly via electrochemically produced hydrogen [168]. Up to now, no study has distinguished between the mechanisms of electrochemical and bioelectrochemical hydrogen production for methane formation. Distinguishing between these mechanisms is an interesting topic for further study, as it may give insights in how to optimize the cathode for methane formation: using a biocathode for bioelectrochemical hydrogen, and consequently methane, production versus using catalytic materials for electrochemical hydrogen production and feeding the hydrogen to methanogens in a post-treatment step [102].

Perspectives

Understanding the mechanisms of electron transfer for bioelectrochemical methane production gives insight in methods to steer the process to improve the performance of a methane-producing BES. At the cathode potentials used in this study, -0.7 V and -0.9 V vs. NHE, methane was primarily produced indirectly via the intermediates hydrogen and acetate. One of the strategies to increase the methane production rate would therefore be to increase the production rates of the intermediates hydrogen and acetate.

The cathode potential determines the energy that is available for bioelectrochemical reactions to occur. Lowering the cathode potential to more negative values (higher cathode overpotential) results in more available energy for the bioelectrochemical reactions to occur, and consequently in higher production rates. However, also the energy input of the BES increases, because a higher applied voltage is required. In this

study, hydrogen and acetate were produced at cathode potentials equal or lower than -0.7 V vs. NHE. The standard cathode potentials for (bio)electrochemical hydrogen and acetate production are -0.41 V vs. NHE (mechanisms 2 and 3 in Table 4.2) and -0.28 V vs. NHE (mechanism 5 in Table 4.2), respectively. Consequently, hydrogen and acetate were produced with a cathode overpotential of at least 0.29 V for hydrogen and 0.42 V for acetate. At -0.7 V vs. NHE cathode potential, maximum reported bioelectrochemical hydrogen production rate is 3 mole e^- eq/m^2 cathode per day [72]. No study so far on bioelectrochemical acetate production was performed at -0.7 V and -0.9V vs. NHE. At -0.59 V vs. NHE cathode potential, maximum reported bioelectrochemical acetate production rate is 1.3 mole e^- eq/m^2 cathode per day [100]. These production rates are similar to the methane production rates in this study. Decreasing the cathode overpotential for these reactions could increase the production rate at these cathode potentials. Cathode overpotentials could be decreased by using cathode materials that lower the cathode overpotential, such as non-noble metals and alloys for the hydrogen evolution reaction [42], by using high-surface area cathode materials, by using a cathode design that allows for good mass transfer of substrates towards and products away from the electrode, and by using an enriched biofilm on the electrode [90]. In this study, undefined mixed cultures were used as inoculum that were not enriched prior to inoculation. The microbial community could be steered towards a higher number of hydrogen-, acetate- and methane-producing microorganisms by enriching the community before inoculation or by adjusting the operational parameters (e.g. pH, temperature and medium composition) to the optimal growth conditions of the favoured microorganisms during BES operation [111]. Another strategy is to operate the BES for a prolonged time, giving microorganisms the opportunity to adjust to the selective conditions, resulting in higher current densities [183]. Also different types of biomass could be screened for the presence of

electrochemically active microorganisms that are able to perform at the optimal operation conditions (e.g. cathode potential) and that could play a role in bioelectrochemical methane formation [103]. Finally, genetic and metabolic engineering could be used as a tool to obtain electrochemically (more) active microorganisms or communities [111].

4.4 Acknowledgements

The authors thank Ontwikkelwerkplaats Wageningen and Bert Willemsen for constructing the electrochemical cells, Mauro Garcia Esteban, Gerard P. 't Lam, and Nalan Oya San for their help with constructing the experimental set-up, and Esther M. Hoogstraten, Koen Wetser, Sanne Raes, and Hans Beijleveld for their help with the experimental analysis.

Chapter 5

Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures

This chapter has been published as:

Mieke C. A. A. Van Eerten-Jansen, Annemiek Ter Heijne, Tim I. M. Grootscholten, Kirsten J. J. Steinbusch, Tom H. J. A. Sleutels, Hubertus V. M. Hamelers and Cees J. N. Buisman (2013), *Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures*, ACS Sustainable Chemistry and Engineering 1 (5): 513-518, doi 10.1021/sc300168z.

Abstract

The use of mixed cultures to convert waste biomass into medium chain fatty acids, precursors for renewable fuels or chemicals, is a promising route. To convert waste biomass into medium chain fatty acids, an external electron donor in the form of hydrogen or ethanol needs to be added. This study investigated whether the cathode of a bioelectrochemical system can be used as the electron donor for the conversion of acetate into medium chain fatty acids. We show that medium chain fatty acids were produced in a bioelectrochemical system at -0.9 V vs. NHE cathode potential, without addition of an external mediator. Caproate, butyrate and smaller fractions of caprylate were the main products formed from acetate. *In-situ* produced hydrogen was likely involved as an electron donor for the reduction of acetate. Electron and carbon balances revealed that 45% of the electrons in electric current and acetate, and 31% of the carbon from acetate were recovered in the formed products. This study showed for the first time production of medium chain fatty acids caproate and caprylate from acetate at the cathode of bioelectrochemical systems, and offers new opportunities for application of bioelectrochemical systems.

Keywords: Bioelectrochemical systems, undefined mixed cultures, carboxylic acids, Microbial Electrolysis Cell, biocathodes, MFC, MEC, BES

5.1 Introduction

Biomass is one of the main renewable sources that can be used to replace fossil-based fuels and chemicals with renewable alternatives. Biomass-derived fuels and chemicals and their production should fulfil sustainability criteria, such as decreased air pollution impact [97], net positive energy balance [1], no competition with food production, and lower CO_2 emissions compared to fossil-based production processes [160]. Low-grade waste biomass, such as municipal waste and crop residues, meets these sustainability criteria [160].

Currently, anaerobic digestion is a widely used technology to convert low-grade waste biomass into renewable methane. However, attention is shifting from producing methane towards producing higher-value compounds like alcohols and fatty acids. These can, for example, be biologically produced from volatile fatty acids, key intermediates in anaerobic digestion [54, 65, 84]. Both alcohols and fatty acids are precursors for renewable fuels and chemicals. Steinbusch and co-workers found that medium chain fatty acids caproate (six carbon atoms) and caprylate (eight carbon atoms) could be produced from acetate, hydrogen and/or ethanol by mixed cultures in (fed-)batch bioreactors [150]. Caproate and caprylate have superior physical properties for further processing to fuels and chemicals compared to volatile fatty acids or ethanol, such as a higher hydrophobicity, facilitating separation from the fermentation broth, and a lower oxygen/carbon ratio, resulting in a higher energy density [150]. Recently, it was demonstrated that caproate could be continuously extracted from the fermentation broth via liquid-liquid extraction [2]. Besides using hydrophobicity as driving force, a pH gradient was used as driving force to specifically extract fatty acids by diffusion over a membrane. For medium chain fatty acids production, however, an external electron donor in the form of hydrogen or ethanol needs to be added.

Bioelectrochemical systems (BESs) offer an opportunity for in-situ electron supply

to produce all kinds of products at the cathode [118], like hydrogen [132], copper [157], hydrogen peroxide [133], alkalinity [117], and methane [23, 169]. A BES consists of two electrodes, anode and cathode, with microorganisms growing on one or both electrodes. The microorganisms on the electrode are a cheap, self-regenerating catalyst [31], enabling all kinds of oxidation and reduction reactions that, in absence of the microorganisms, would need a more expensive catalyst to occur. In a previous study, members of our team studied bioelectrochemical production of ethanol from acetate [151]. Using Methyl Viologen (MV) as an electron mediator, besides the main product ethanol (83 mg/L), also butyrate (53 mg/L) was formed. Bioelectrochemical butyrate production was also recently demonstrated using Neutral Red as an electron mediator [24]. At pH 6, the maximum obtained butyrate concentration was 8.8 g/L. To the best of our knowledge, until now, there has been no report of the production of the medium chain fatty acids caproate and caprylate in BES without the use of an electron mediator.

In this study, we therefore investigated whether BESs can be used to produce medium chain fatty acids with a higher length than butyrate from acetate without the use of an electron mediator by supplying electrons or hydrogen in-situ. In this study, two electrochemical cells were compared; the cathode of the first cell was inoculated with a mixed culture, and the second cell was not inoculated and served as a control. As hydrogen possibly plays an important role to drive the production of fuels and chemicals in BESs [118], we operated both cells at a cathode potential of -0.9 V vs. NHE, which is a potential favourable for (bio-)electrochemical hydrogen production [132]. This study shows for the first time that acetate can be reduced to caproate and caprylate by mixed cultures at the cathode of a BES, without the addition of an electron mediator. Acetate was mainly reduced to caproate and butyrate, and also lower concentrations of caprylate were measured.

5.2 Materials and methods

Electrochemical cell

Two flat-plate electrochemical cells (0.56 L) were used as described in [144]. The anodes were made of a platinum-coated titanium mesh (projected surface area 0.025 m^2), and the cathodes were made of graphite felt (projected surface area 0.025 m^2). The anode and cathode compartments (0.28 L each of which 0.03 L was gas headspace) were separated by a cation exchange membrane (Fumasep FKB, Fumatech GmbH, Germany). The cathode headspace was connected to a gas flow meter (Milligascounter®, Ritter, Germany) via an injection port. The anode compartments of both electrochemical cells were connected via a common 10 L anolyte vessel. The electrolytes were recirculated through serpentine flow channels parallel to the electrode in both the anode and cathode compartments. The total catholyte volume (electrochemical cell, tubing and recirculation vessel) was 0.63 L.

Inoculum and electrolytes

One electrochemical cell was inoculated; this electrochemical cell is indicated as BES in this manuscript. The inoculum was obtained from a continuously operating anaerobic fixed film reactor operated at 30°C in which mixed cultures, with *Clostridium kluyveri* assumed to be the predominant microorganism, produced C4-C8 fatty acids from acetate and ethanol [55]. The BES was inoculated with 5 grams of reactor liquid at the start of the experiment (day 0). The biomass concentration of the inoculum was 0.2 g VSS/L at the day of inoculation [55]. As a control, the second electrochemical cell was not inoculated; this electrochemical cell is indicated as "Control".

The anolyte consisted of 100 mM potassium hexacyanoferrate(II) and 50 mM phosphate buffer, and was recirculated at a rate of 10.8 L/h. The catholyte consisted

of 100 mM acetic acid, 3.6 g/L $(NH_4)H_2PO_4$, 0.33 g/L $MgCl_2.6H_2O$, 0.2 g/L $MgSO_4.7H_2O$, 0.5 g/L $CaCl_2.2H_2O$, 0.15 g/L KCl, 4 g/L K_2CO_3, 3.7 g/L NaOH, and trace metals solution and B-vitamins solution as described in [112], and was recirculated at a rate of 10.8 L/h. The anolyte (10 L vessel) was not replenished throughout the experiment and the catholyte was replenished continuously at a rate of 0.16 L/d to ensure sufficient substrate. pH of the catholyte was controlled through a pH controller (Liquisis M CPM 253, Endress + Hauser, Switzerland) at pH 6 using 2 M HCl and 2 M NaOH.

Electrochemical cell operation

To disinfect the reactors both electrochemical cells were flushed with 70% (v/v) ethanol, and thereafter with excess sterilized water. The catholyte of both electrochemical cells was flushed with pure nitrogen (>99.9992%) for at least 30 minutes. The cathodes were operated continuously during the whole experiment.

The electrochemical cells were connected to a power supply (MCP94, Bank Elektronik, Germany) to control the cell voltage. Cell voltage was adjusted to obtain a cathode potential of -0.9 V vs. NHE as described in [163]. The electrochemical cells were operated in a temperature controlled chamber at 30ºC, as this was also the temperature at which the biomass was grown [55].

Analyses

Every 2-3 days, the composition of the catholyte and its headspace was analysed. Gas samples were taken with a 100 µL syringe from the injection port, located between the cathode headspace and the gas flow meter. C2-C8 fatty acids and ethanol concentrations in the catholyte, and hydrogen, methane, nitrogen, oxygen, and carbon dioxide concentrations in the cathode headspace were analysed using gas

chromatography according to [54, 149]. Both the C2-C8 fatty acids and ethanol were measured using a gas chromatograph (HP 5890 series II GC, Germany), with a glass column packed with 10% Fluorad 431 on Supelco-port 100-120 mesh [149]. Gas production was continuously measured with a gas flow meter (Milligascounter®, Ritter, Germany). The total chemical oxygen demand (COD) of the catholyte was measured using the Hach Lange LCK514 cuvette test. The principle of this test is that oxidizable substances react with sulphuric acid-potassium dichromate solution in the presence of silver sulphate as a catalyst at 148°C for 2 hours. Chloride is masked by mercury sulphate. The green coloration of Cr^{3+} was spectrophotometrically analysed at room temperature.

Calculations

We use reduced organics as the generic term for the following products that could be produced from protons or acetate: butyrate, caproate, caprylate, ethanol, hydrogen, and methane. Production of reduced organics ($p_{i,t}$ in mole e^- eq) was calculated according to:

$$production_{i,t} = accumulation_{i,t} + out_{i,t} - in_{i,t}$$

$$p_{i,t} = \left[V_{cat} \cdot (c_{i,t} - c_{i,t-1}) + Q \cdot \frac{(c_{i,t} - c_{i,t-1})}{2} \cdot \Delta t - Q \cdot c_{in,i} \cdot \Delta t\right] \cdot n_{e,i} \qquad (5.1)$$

with subscript i referring to the reduced organic (that is, butyrate, caproate, caprylate, ethanol, hydrogen or methane), V_{cat} the total catholyte volume (0.63 L), c_i the concentration of the reduced organic (mole/L), Q the influent and effluent flow rate (L/s), Δt the time difference between sample time t and previous sample time t-1 (s), $c_{in,i}$ the concentration of the reduced organic in the influent (mole/L), and $n_{e,i}$ the number of electrons contained in the reduced organic (mole/mole; $n_{e,i}$ is 20 for butyrate, 32 for caproate, 44 for caprylate, 12 for ethanol, 2 for hydrogen, 8 for

methane, and 8 for acetate). For each reduced organic, production was calculated according to equation 5.1. Total reduced organics production (in mole e^- eq) was the sum of the production of each reduced organic. The reduced organics (both ethanol and fatty acids) that were present in the inoculated reactor liquid have been subtracted from the calculated production immediately after inoculation.

The electron equivalents for bio-electrochemical reduced organics production at the cathode can originate from two sources: electric current or acetate. Acetate can supply 8 moles of electrons per mole of acetate when it is oxidized to CO_2 and protons. The number of electron equivalents transferred via the current (q_t in mole e^- eq) was calculated according to:

$$q_t = \frac{\int_{t-1}^{t} I dt}{F} \tag{5.2}$$

with I the current (A), and F the Faraday constant (96485 C/mole e^-).

The cathodic electron efficiency ($\eta_{ce,t}$ in %) indicates to what extent electrons from electric current and acetate were recovered in reduced organics, and was calculated according to:

$$\eta_{ce,t} = \left(\frac{p_{i,t}}{q_t + p_{ac,t}}\right) \cdot 100\% \tag{5.3}.$$

The cathodic carbon efficiency ($\eta_{cc,t}$ in %) indicates to what extent carbon from acetate was recovered in reduced organics, and was calculated according to:

$$\eta_{cc,t} = \left(\frac{(p_{i,t}/n_{e,i}) \cdot n_{c,i}}{(p_{ac,t}/n_{e,ac}) \cdot n_{c,ac}}\right) \cdot 100\% \tag{5.4}$$

with $n_{c,i}$ the number of moles of carbon per mole of reduced product (mole C/mole; $n_{c,i}$ is 2 for acetate, 4 for butyrate, 6 for caproate, 8 for caprylate, 2 for ethanol, and 1 for methane).

5.3 Results and discussion

Bioelectrochemical production of caproate and caprylate from acetate

In this study, we show for the first time that ethanol and medium chain fatty acids can be produced by mixed cultures, without the addition of an electron mediator, in the cathode of a bioelectrochemical system (BES). At -0.9 V vs. NHE cathode potential, ethanol and the fatty acids butyrate, caproate, and caprylate were detected in the BES, with caproate as the predominant product (Figure 5.1). The concentration of reduced organics measured immediately after inoculation was corrected for the reduced organics present in the inoculum, indicating that the ethanol and fatty acids were produced as a result of microbial activity in the cathode of the BES. Besides ethanol and fatty acids, hydrogen gas was produced with hydrogen production rates ranging between 5-487 NmL H_2/d (Figure 5.1). The control, on the other hand, did not produce ethanol nor fatty acids and produced only a small fraction of hydrogen. The corresponding current density profiles are shown in Figure 5.2. Current density was on average 1.8±0.6 A/m^2 projected surface area for the BES, about a factor 6 higher than the average current density in the control (0.3±0.2 A/m^2). The maximum production of reduced organics was found after 4 days, and production decreased thereafter (Figure 5.1).

The maximum concentrations of reduced organics were 739 mg/L for caproate, 263 mg/L for butyrate, 36 mg/L for caprylate, and 27 mg/L for ethanol. Previously reported concentrations of bioelectrochemically produced ethanol and butyrate are 83 mg/L for ethanol and 0.053-8.8 g/L for butyrate [24, 151]. In the reported studies, however, an electron mediator was used to facilitate electron transfer. To the best of our knowledge, bioelectrochemical production of caproate and caprylate without the use of an electron mediator has not been reported before.

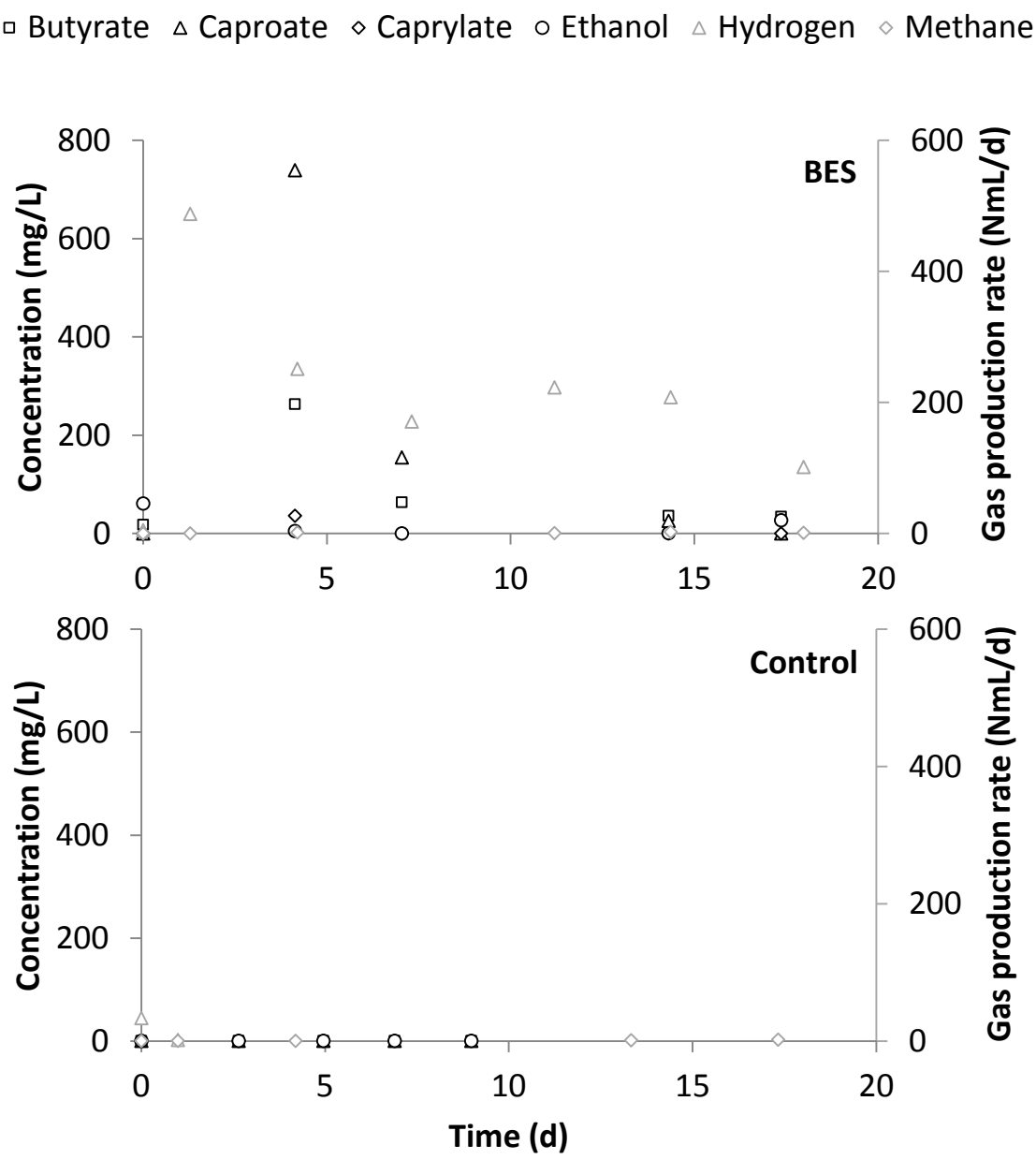

Figure 5.1. Concentration of reduced organics butyrate, caproate, caprylate, and ethanol, and production rates of hydrogen and methane with time at -0.9 V vs. NHE cathode potential (30ºC, pH 6) for the BES and the control.

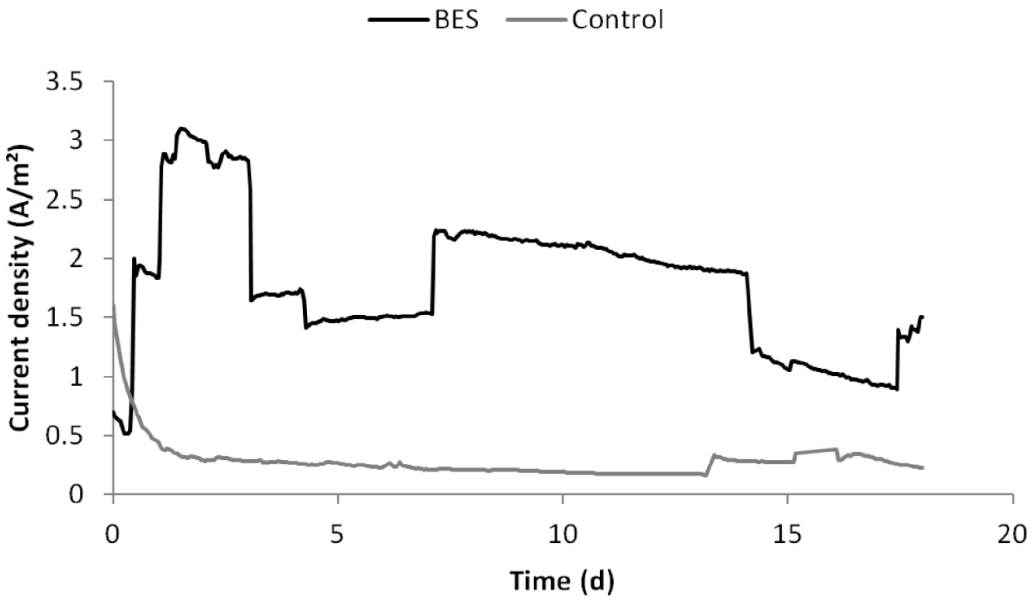

Figure 5.2. Current density with time for both the BES and the control.

In principle, a higher current density would relate to a higher hydrogen production rate. We observed a higher current density in the BES than in the control, and indeed the hydrogen production rate in the BES was higher than in the control. The higher current density and hydrogen production rate in the BES could be a result of bioelectrochemical hydrogen production at the cathode [132].

Cathodic electron and carbon efficiency

Electron and carbon balances have been made after 18 days of operation of the BES. The electrons are available via two electron sources, the converted acetate and the electric current. These electrons were compared to the electrons in the reduced organics (ethanol, butyrate, caproate, caprylate and hydrogen). After 18 days of operation, the average cathodic electron efficiency, the efficiency of capturing electrons from electric current and acetate in reduced organics, was 45% (Figure 5.3). This shows that indeed electrodes can be used for in-situ supply of electrons (or

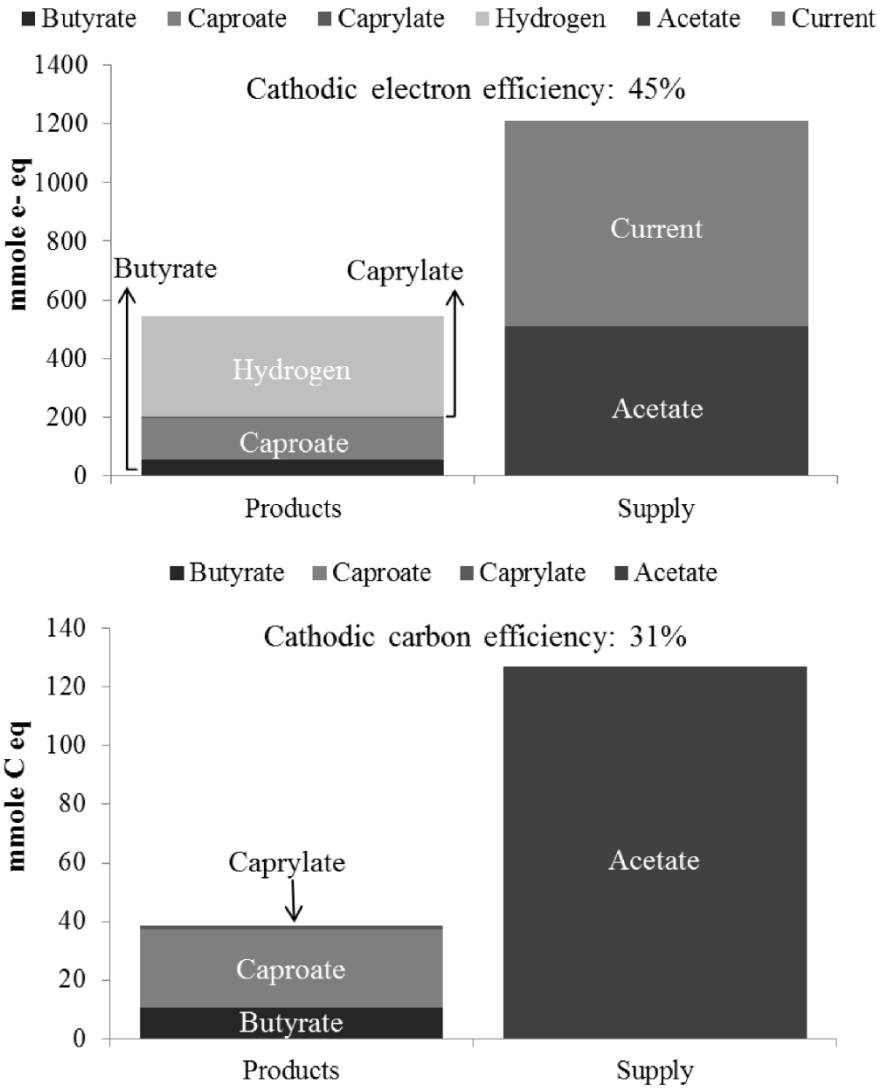

Figure 5.3. Electron and carbon distributions and efficiencies after 18 days of operation. The contribution of ethanol to the cathodic electron and carbon efficiencies was <1%.

hydrogen). The largest part of the electrons was recovered in hydrogen (62%), caproate (26%) and butyrate (10%) (Figure 5.3). For the carbon balance, the carbon in the converted acetate was compared to the carbon in the reduced organics, and was corrected for the organics in the inoculum. After 18 days of operation, the average cathodic carbon efficiency was 31% (Figure 5.3). The largest part of the carbon was

recovered in caproate (69%) and butyrate (27%).

55% of the electrons were not recovered in the products. Possible processes leading to a loss of electrons are diffusion of hydrogen through the membrane to the anode [130], and reduction of other electron acceptors in the catholyte, such as sulphate [29]. The cathodic carbon efficiency being lower than 100% could be the result of carbon leaving the catholyte as another carbon-based product than the measured reduced organics. Production of other carbon-based reduced products than the ones measured (C2-C8 fatty acids and ethanol) could be ruled out as total COD tests matched the total measured carbon-based reduced products in the catholyte. Likely, the unrecovered carbon left the catholyte as CO_2 or HCO_3^-. CO_2 concentrations in the headspace did not show a clear increase in time, with an average concentration of 3.6 ± 1.2%. *Clostridium kluyveri*, assumed to be the predominant microorganism in the inoculum [55], requires CO_2 for growth [161], and might have consumed some of the CO_2.

Mechanisms of reduced organics production

A schematic of the mechanisms of fatty acids production is depicted in Figure 5.4. To produce fatty acids, ethanol is required as electron donor [138, 150]. Microorganisms that produce the ethanol can use either hydrogen (pathway 2) or the cathode directly (pathway 3) as electron donor. The hydrogen required for biological ethanol production (pathway 2) can be produced bioelectrochemically (pathway 4) or electrochemically (pathway 5). Medium chain fatty acids caproate and caprylate can be produced from butyrate (the product in pathway 1) using ethanol (the product of pathways 2 or 3) as electron donor, as described in more detail in [138]. This study cannot decide on the dominant mechanism.

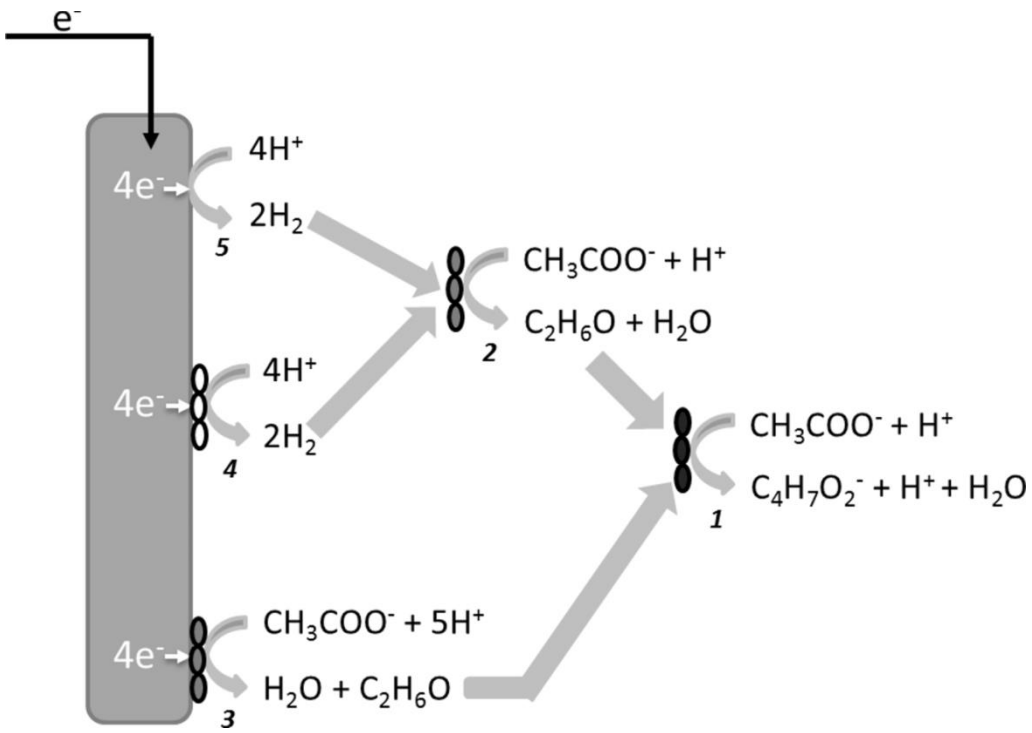

Figure 5.4. A schematic of the mechanisms of fatty acids production. Ethanol is required as electron donor for fatty acids production (pathway 1). Microorganisms that produce the ethanol can use either hydrogen (pathway 2) or the cathode directly as electron donor (pathway 3). The hydrogen required for biological ethanol production from acetate (pathway 2) can be obtained from bioelectrochemical hydrogen production (pathway 4) or electrochemical hydrogen production (pathway 5).

Most likely, the dominant mechanism of fatty acids production was via hydrogen (pathways 4 and 5). At -0.9 V vs. NHE cathode potential, both electrochemical hydrogen production and bioelectrochemical hydrogen production via hydrogen-producing microorganisms that can use the cathode as electron donor can take place [89, 132].

Direct electron transfer from the cathode to microorganisms for fatty acids production has until now only been demonstrated once [107]. *Sporomusa ovata* was able to directly accept electrons from an electrode potentiostatically controlled at -0.4 V vs. NHE to convert CO_2 to acetate and small amounts of 2-oxobutyrate [107]. Direct

electron transfer from the cathode to microorganisms for longer chain fatty acids production has not been demonstrated. Fatty acids production using the cathode directly as electron donor would offer a considerable advantage compared to production via hydrogen, as the required energy input would theoretically be lower, for instance 0.23 V lower for butyrate production compared to (bio-) electrochemical hydrogen production (E_{cat} = -0.36 V vs. NHE at 30ºC and pH 6) (eq. 5.5).

$$2CH_3COO^- + 6H^+ + 4e^- \rightarrow C_4H_7O_2^- + H^+ + 2H_2O$$

(E_{cat} = -0.13 V vs. NHE) (5.5)

The thermodynamic cathode potential was calculated, using the Gibb's free energies as described in [63], and using 30ºC, pH 6 and 1M for acetate and butyrate.

Further investigation of the mechanisms, and studying the possibility for steering towards a direct mechanism, would therefore be an interesting topic for further study.

Perspectives

This study showed for the first time that caproate and caprylate could be produced at the cathode of a BES. Compared to fermentation processes that use externally added electron donors, supplying electrons *in-situ* in a BES has several advantages: (i) no infrastructure for supply of hydrogen is required, (ii) *in-situ* electron supply at the right cathode potential might result in locally high hydrogen partial pressures, which are favourable for the reaction, and (iii) the electron donor is renewable when renewable electricity is used.

Important elements to be addressed in future studies are (i) continuous production of medium chain fatty acids, (ii) choice of a suitable electron donor at the anode, and (iii) separation of the medium chain fatty acids from the catholyte.

Electron transfer for the bioelectrochemical production of reduced organics can

occur via two mechanisms: via direct extracellular electron transfer or via electron mediators produced by the microorganisms [128]. The production of reduced organics stagnated after 18 days of operation, possibly caused by biomass or electron mediator wash-out due to the continuous flow conditions. Therefore, ways to retain the biomass, such as increasing the solid retention time, or a (fed-)batch system should be further investigated. Alternatively, continuous addition of inoculum could prolong reduced organics production. This principle has already been applied in continuous *n*-butanol fermentation, where the biomass cells were grown in a separate continuous stirred tank reactor and continuously added to the fermentation broth [126]. This resulted in an extension of continuous *n*-butanol production from 7 days to over 2 months [126].

Hexacyanoferrate(II) was used as an electron donor at the anode. Hexacyanoferrate(II) was chosen to prevent the possible crossover of acetate (in case of a bioanode) or oxygen (in case of water oxidation) from anode to cathode. Further work needs to include sustainable alternatives for hexacyanoferrate(II) such as water or organic matter. Using water has the advantage that it is a widely available and cheap resource, however, a higher electrical energy input is needed compared to using domestic wastewater as electron donor [90], and diffusion of the produced oxygen to the cathode [18, 78] could negatively affect organics production. Using domestic wastewater has the advantage that a lower electrical energy input is needed compared to using water as electron donor, however, it will lead to a loss in overall carbon efficiency because part of the acetate is oxidized to CO_2. The feedstock price and the price of electricity will determine what will be the most attractive electron donor in the future.

For implementation of the technology it is important that a renewable source of acetate is used for the production of medium chain fatty acids at the cathode. Acetate

is a key intermediate in anaerobic digestion. In anaerobic digestion acetate is finally converted into methane. Production of medium chain fatty acids instead of methane from acetate has, however, higher energetic and economic value than producing methane from acetate [150]. Sustainable sources that could be anaerobically digested to acetate are low grade wet biomass sources, such as the organic fraction of municipal solid waste [150].

In this study, a mixture of reduced organics was produced, with caproate as the main component. To increase the applicability and economic value of the produced products, concentration and purification is required. Separation steps, however, require an additional energy input. Besides, the almost similar physical properties (*e.g.* charge, hydrophobicity) of C2-C6 fatty acids poses a challenge for selective separation of reduced organics from the fermentation broth. To decrease the energy input for separation, it would be interesting to study if further concentration and selective production of specific reduced organics is feasible. Variability in the feedstock composition, and the use of mixed cultures to deal with this variable feedstock composition, puts challenges on selective production of specific reduced organics [5]. Increasing the production rate, and the concentration of the reduced organics, is probably the key challenge for implementing the technology.

Chapter 6

General discussion

The aim of this thesis was to investigate the principles and perspectives of bioelectrochemical methane production from renewable electricity and CO_2. In this chapter, the present performance of methane-producing BESs is evaluated. The main bottlenecks that limit system's performance as found in this thesis, are discussed. Besides, we show that our envisioned first application is to upgrade CO_2 in biogas of anaerobic digestion to additional methane. Finally, the feasibility of production of higher-value organics, such as medium chain fatty acids, in BES will be discussed.

6.1 Current status of methane consumption

Methane is the main component of natural gas. Fossil-derived natural gas contributed to 21.4% of the 2010 total global primary energy supply (114 EJ/year) [70]. Natural gas is expected to be the fastest-growing fuel [36], estimated to almost double in volume over the 2000–2035 projection period, from 87 EJ in 2000 towards 134-176 EJ in 2035 [67, 69]. Extraction and consumption of fossil-derived natural gas, however, affect the environment and human health. Therefore, there is a societal need to go from a fossil-derived energy system towards a renewable energy system. Our future renewable energy system is believed to be mainly renewable electricity-based [98]. Renewable electricity is, however, produced intermittently, and excess produced electricity needs to be stored not to get lost [57, 98, 113]. A methane-producing BES could convert excess renewable electricity and CO_2 in renewable methane, thereby meeting the societal demand to replace fossil natural gas with renewable methane.

Natural gas is currently used directly for cooking, domestic heating, and for transportation (as compressed natural gas or liquefied natural gas). Natural gas is also used to produce electricity by combustion in gas turbines or steam boilers. Besides using methane for energy production, methane itself is also used for several chemical production processes: for instance, the production of synthesis gas (H_2 and CO) via

steam reforming [7], the production of ammonia or urea via the Haber-Bosch process [137], or the production of methanol via a direct catalytic conversion or using synthesis gas [49]. As an example, about 3-5% of the worldwide natural gas consumption is consumed by the Haber-Bosch process [146].

6.2 Current status of a methane-producing Bioelectrochemical System

The implementation of a methane-producing BES highly depends on its performance. The performance of BESs is often indicated by two key characteristics: the methane production rate and the energy efficiency, which is the efficiency of capturing the added electrical energy into methane (chemical energy).

In this thesis, methane production rates increased 40 fold from 0.12 L CH_4/m^2 cathode per day in 2012 (**Chapter 2**), to 4.6 L CH_4/m^2 per day in 2013 (**Chapter 3**), and to 5.2 L CH_4/m^2 per day in 2014 (**Chapter 4**) (Table 6.1, Figure 6.1A). The methane production rates obtained in this thesis are similar to reported methane production rates, which range from 0.12 to 24 L CH_4/m^2 per day (Table 6.1, Figure 6.1A).

The energy efficiency of a methane-producing BES is the product of coulombic efficiency and voltage efficiency [59]. Coulombic efficiency is the efficiency of capturing the electrons from the electric current in methane. Coulombic efficiency values reported in literature range from 19% [61] to 96% [23] for methane-producing BES (Table 6.1, Figure 6.1B). Voltage efficiency values have only been reported two times in literature, and range from 13.4% [163] to 75% [170]. Energy efficiencies have been only scarcely studied and were reported three times in literature [74, 120, 163], ranging between 3.1% [163] and 30% [120].

It is interesting to notice that, despite the methane-producing BES was at its infancy at the start of this thesis and several studies on the methane-producing BES have been published during the development of this thesis (Table 6.1), performance did not show a clear increase with time (Figure 6.1). There is a large variation in the reported methane production rates and coulombic efficiencies for methane-producing BES. An explanation for this is that there is also a large variation in reactor configurations, electron donors, inocula, and operational conditions used (Table 6.1). Methane-producing BESs differed in (i) the number of compartments: single-chamber versus two-chamber with the anode and cathode compartment being separated, (ii) the type of membrane used in a two-chamber BES, (iii) the design of the reactor, for instance H-shaped reactors versus flat plate reactors, (iv) and the type of electrode material used. The inocula differed in their origin, and whether the inocula was enriched prior to inoculation. Finally, the operational conditions differed in the (i) applied potential, (ii) the electron donor used at the anode, and (iii) batch versus continuous operation.

In the following paragraphs, the main bottlenecks that limit the performance of a methane-producing BES are discussed. Based on the insights obtained in this thesis, methods to improve the performance are discussed.

Table 6.1. Overview of the performance of methane-producing biocathodes that use CO_2 as electron acceptor. Studies in which bioelectrochemical production of methane was reported as an (unwanted) side reaction were not included in the overview. AEM = anion exchange membrane, PEM = proton exchange membrane, CEM = cation exchange membrane, SS = stainless steel.

Operational mode	**Electron donor**	**E_{cat} (V vs. NHE)**	**Inoculum (source)[a]**	**Current density (A/m^2)[b]**	**Methane production rate (L/m^2 per d)[b]**	**CE (%)[c]**	**Ref.**
two-chamber (CEM), batch-fed	n.r.	1.5 V[d] (graphite felt)	Enrichment culture (lab-scale anaerobic reactor fed acetate, butyrate, and propionate)	n.r.	n.r.	n.r.	[109]
two-chamber (AEM), batch-fed	Water	-0.8 V (carbon cloth)	Enrichment culture* (return activated sludge WWTP)	1.8	4.5	96	[23]
single-chamber, batch-fed	Acetate (bio-anode)	-0.90 V (graphite granules)	Enrichment culture (granular anaerobic sludge mesophilic winery digester)	n.r.	n.r.	79	[28]
single-chamber, continuously-fed	Acetate (bio-anode)	-0.83 V (graphite granules)	Enrichment culture (granular anaerobic sludge mesophilic winery digester)	n.r.	n.r.	86	[28]
two-chamber (PEM), batch-fed	Water	-0.9 V (carbon paper)	Enrichment culture (anaerobic sludge packed bed biofilm reactor fed fatty acids)	2.9	9.2	80	[168]
single-chamber, continuously-fed	Acetate (bio-anode)	-0.9 V[d] (SS mesh)	Enrichment culture (effluent acetate-oxidizing bio-anode)	5.8	1.8	n.r.	[120]
single-chamber, continuously-fed	Acetate (bio-anode)	-1.4 V (carbon fibre	Enrichment culture (return activated sludge	4.1	8.7	88	[21]

		sheet)	WWTP)				
two-chamber (CEM), continuously-fed	Hexacyano-ferrate(II)	-0.7 V (graphite felt)	Non-enriched culture (anaerobic sludge UASB treating distillery WW)	0.9	1.4	65	[163]
two-chamber (CEM), continuously-fed	Water	-0.55 V (graphite felt)	Non-enriched culture (anaerobic sludge UASB treating distillery WW)	0.2	0.12	23	[163]
two-chamber (PEM), batch-fed	Water	-0.59 V (graphite granules)	Enrichment culture* (retention basin brewery wastewater)	n.r.	n.r.	55	[100]
single-chamber, batch-fed	Acetate (bio-anode)	-1.25 V[d] (carbon felt)	Non-enriched culture (mesophilic digestive sludge)	n.r.	10	n.r.	[79]
two-chamber (PEM), continuously-fed	Acetate (bio-anode)	-0.93 V (graphite granules)	Non-enriched culture (anaerobic sludge WWTP)	0.1	0.2	79	[170]
two-chamber (CEM), batch-fed	Water	-0.75 V (carbon felt)	Enrichment culture (activated sludge WWTP)	3.4	6.7	89	[75]
two-chamber (CEM), batch-fed	Water	-1.15 V (carbon felt)	Enrichment culture (anaerobic sludge WWTP)	15	24	57	[75]
single-chamber, batch-fed	Water	-1.5 V[d] (carbon paper)	Pure culture *Methanobacterium thermoautotrophicus* strain ΔH	n.r.	1.0	n.r.	[136]
two-chamber (CEM), batch-fed	Hexacyano-ferrate(II)	-0.7 V (graphite felt)	Non-enriched culture* (anaerobic sludge UASB treating distillery WW)	1.6	4.6	99	[165]
two-chamber (CEM), batch-fed	Hexacyano-ferrate(II)	-0.6 V (graphite felt)	Non-enriched culture* (anaerobic sludge UASB treating distillery WW)	0.9	2.7	92[e]	[165]

single-chamber, batch-fed	Acetate (bio-anode)	-1.0 V[d] (carbon paper)	Pure culture *Methanobacterium thermoautotrophicus* strain ΔH	n.r.	2.0	19	[60]
single-chamber, batch-fed	Acetate (bio-anode)	-0.75 V[d] (carbon felt)	Enrichment culture (formation water almost depleted oil-reservoir)	2.6	8.8	98	[81]
two-chamber (CEM), batch-fed	Acetate and sulphide (bio-anode)	-0.7 V (carbon felt)	Enrichment culture* (anaerobic sludge)	n.r.	0.7	57	[74]
two-chamber (CEM), batch-fed	Acetate and sulphide (bio-anode)	-0.75 V (carbon felt)	Enrichment culture* (anaerobic sludge)	n.r.	0.8	51	[74]
two-chamber (Nafion), batch-fed	Water	-0.6 V (platinum coated graphite block)	Enrichment culture (anaerobic sludge WWTP)	0.3	0.8	78	[143]
two-chamber (Nafion), batch-fed	Water	-0.6 V (graphite block)	Enrichment culture (anaerobic sludge WWTP)	0.04	0.12	104	[143]
two-chamber (CEM), continuously-fed	Water	-0.7 V (graphite felt)	Non-enriched culture (anaerobic sludge WWTP)	2.9	5.2	73	[162]

[a] WWTP = Wastewater treatment plant; WW = wastewater
[b] Calculated using the projected cathode surface area.
[c] CE = Cathodic electron efficiency.
[d] Cell voltage instead of cathode potential was reported.
[e] The reported cathodic electron efficiency over a time span of 24 days was 92±29%.
* Microbial community composition is reported.
n.r. = Data was not reported and could not be derived from other reported data.

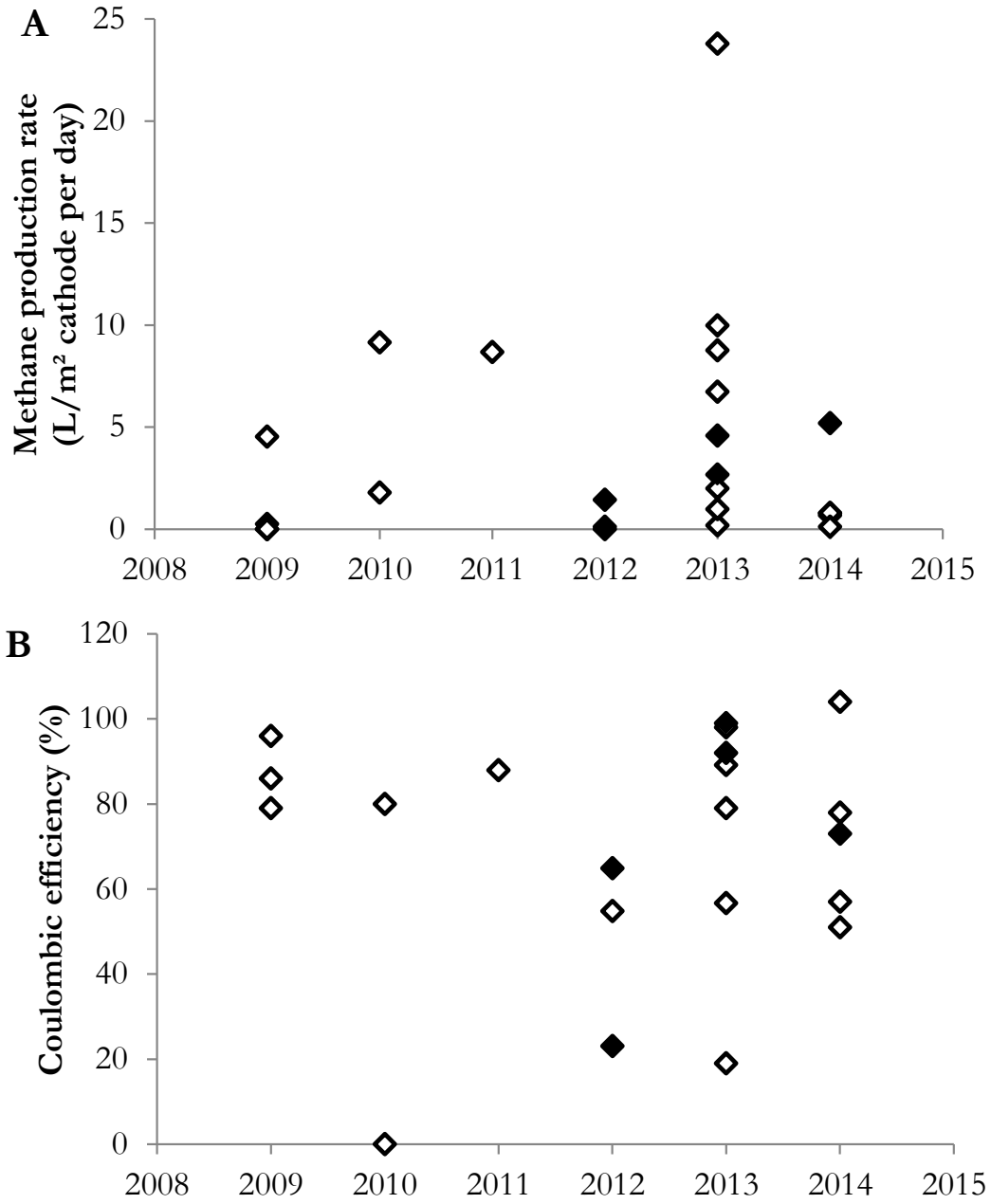

Figure 6.1. Reported methane production rates (A), and coulombic efficiencies (B) for methane-producing BESs with time. Solid symbols are the results presented in this thesis.

6.3 Factors affecting the methane production rate and energy efficiency

In this thesis, causes for energy losses, and possible solutions to prevent or minimize these losses, have been discussed. The main causes for energy losses and their possible solutions will be discussed in more detail in this paragraph.

Cathode design

In **Chapter 2**, an internal resistance analysis was performed to determine the energy losses of a methane-producing BES [163]. The analysis revealed that most energy was lost at the cathode, independent of the type of electron donor used (hexacyanoferrate(II) or water) [163]. An explanation for the high energy losses at the cathode could be (i) mass transfer limitations near or inside the cathode or (ii) limited coverage of biomass on the electrode.

Products (oxygen, methane and protons) could accumulate near or inside the porous graphite felt electrode [163]. This could negatively affect reaction kinetics, and gas accumulation inside the electrode could result in less available effective cathode surface area. In **Chapter 2** and **3** of this thesis, the catholyte was flown parallel to the cathode instead of forced through the cathode, causing limited mixing near the electrode and resulting in mass transfer limitations near and inside the cathode. By directing the catholyte through the cathode, mass transfer limitations could be minimized. Sleutels and co-workers demonstrated for a hydrogen-producing Microbial Electrolysis Cell that current density increased to 11 A/m^2 by forced flow through the electrode compared to 5.1 A/m^2 with parallel flow to the electrode using a 6.5 mm thick graphite felt cathode [145]. To decrease mass transfer limitations, in **Chapter 4** of this thesis, the catholyte was directed through the cathode. This resulted

in the lowest cathode partial resistance achieved in this thesis: 0.24 Ω·m^2 at -0.7 V vs. NHE cathode potential in **Chapter 4** compared to 2.6 Ω·m^2 at -0.55 V vs. NHE in **Chapter 2** and 0.67 Ω·m^2 at -0.6 V vs. NHE in **Chapter 3**.

In **Chapter 3** it was found that the cathode was not fully covered by microorganisms [165], another possible cause for the high energy losses at the cathode. By growing microorganisms on the complete surface of the graphite electrode, its catalytic properties for bioelectrochemical methane production could be improved. Adhesion of microbial cells to the cathode could be improved by changing the hydrophobicity of the cathode [127] or by modifying the cathode surface [186]. The hydrophobicity of microbial cells and the cathode surface influence the adhesion properties of microorganisms to the cathode [127]. Besides the hydrophobicity, microbial cells usually have a negative outer-surface charge, resulting in electrostatic repulsion with negatively charged surfaces, such as the cathode [15, 166]. Addition of positively charged groups or metallic-like nanoparticles to carbon cloth cathodes resulted in a 3-7 fold increase in current density for CO_2 reduction to acetate [186] and could be a strategy to improve the reduction rate of CO_2 to methane.

Microbial community

The effect of the microbial community on performance of a methane-producing BES is scarcely documented. Reported studies used inocula of various sources to grow a methane-producing biocathode, and reported studies differ in whether the inoculum was enriched prior to inoculation, and in their enrichment methods (Table 6.1). The mixed culture microorganisms used in methane-producing biocathodes studies originated, for instance, from anaerobic sludges obtained from a mesophilic winery digester [28], activated sludges from domestic wastewater treatment plants [21, 75], or the effluent of an acetate-oxidizing bio-anode in a BES [23, 120] (Table 6.1).

Enrichment methods prior to inoculation are growing the microbial community with an electrode or hydrogen as electron donor, or by using the effluent of a well-performing anode [21, 75, 100, 120, 168, 170]. Despite the variety in inoculum sources and enrichment methods, *Methanobacterium palustre* was detected as the dominant microorganism in several mixed culture methane-producing biocathodes [23, 74, 100], and also detected in this thesis [165]. *Methanobacterium palustre* is a hydrogenotrophic methanogen isolated from a peat bog (Sippenauer moor, Germany) [185]. *Methanobacterium palustre* is able to use hydrogen as electron donor for the conversion of CO_2 into methane [185], although direct use of the electrode has also been suggested [23]. A mixed culture biocathode with *Methanobacterium palustre* being the dominant microorganism (87% of total cells) outperformed a biocathode consisting of pure culture *Methanobacterium palustre* strain ATCC BAA-1077 [23]. Besides *M. palustre*, also *Methanothermobacter thermoautotrophicus* strain ΔH [61, 136] is able to bioelectrochemically produce methane, and *Methanoregula boonei* and *Methanospirillum hungatei* have been detected in a mixed culture methane-producing biocathode [23]. *Methanothermobacter thermoautotrophicus* (formerly known as *Methanobacterium thermoautotrophicum*) is a thermophilic hydrogenotrophic methanogen able to use hydrogen [96] or the electrode directly [61, 136] as electron donor. *Methanoregula boonei*, isolated from a peat bog (Ithaca, USA), is an acidophilic, obligatory hydrogenotrophic methanogen able to use hydrogen only as electron donor [14]. *Methanospirillum hungatei*, isolated from sewage sludge, is a methanogen able to use hydrogen or formate as electron donor [44]. It is currently unknown whether *Methanoregula boonei* and *Methanospirillum hungatei* can use the electrode directly as electron donor.

We not only investigated the microbial community of a mixed culture methane-producing biocathode, but also discussed the role of the detected community members in methane production (**Chapter 3**) [165]. Besides methanogens, bacteria

seem to play an important role in bioelectrochemical methane production. The biocathode consisted of six phylotypes of bacteria [165]. The phylotypes that were closely related to the bacteria *Desulfovibrio putealis* and *Hydrogenophaga caeni* may have produced hydrogen, which in turn was consumed by the methanogens to produce methane. *Desulfovibrio putealis* and *Hydrogenophaga caeni* have been found in hydrogen-producing biocathodes, and *Desulfovibrio* sp. are known to produce hydrogen at cathode potentials equal or lower than -0.44 V vs. NHE [8, 30, 31]. The phylotypes that were closely related to the bacteria *Hydrogenophaga caeni*, *Methylocystis* sp. and *Acidovorax caeni* may have acted as oxygen scavengers [26, 33, 64, 173], creating the anoxic conditions for the methanogens. For the phylotypes that were closely related to the bacteria *Ottowia thiooxydans* and *Petrimonas sulfuriphila* their role in bioelectrochemical methane production remains unclear [50, 148]. Future research could focus on bioelectrochemical production of methane by *Methanobacterium palustre* in absence and presence of the hydrogen-producing phylotypes *Desulfovibrio putealis* and *Hydrogenophaga caeni* to identify possible syntrophic relations.

The syntrophic relationships between methanogens and hydrogen-producing bacteria as hypothesized in this thesis, have also been hypothesized by Marshall and co-workers. They postulated for a mixed culture methane- and acetate-producing biocathode that phylotypes that were closely related to *Sphingobacteriales* WCHB1 and *Sulfurospirillum* bioelectrochemically produced hydrogen, which was in turn converted to methane by the hydrogenotrophic methanogen *Methanobacterium* [100]. Cheng and co-workers identified in a mixed culture methane-producing biocathode phylotypes that were closely related to *Sedimentibacter hongkongensis*, *Clostridium sticklandii*, *Clostridium aminobutyricum*, and *Caloramator coolhaasii* [23]. Unfortunately, they did not investigate the role of these bacteria in bioelectrochemical methane production, and based on the substrate preferences of the identified bacteria no relationship could be identified.

In addition to microbial communities, the coverage of microorganisms on the electrode will determine the conversion rate and current density [141]. In this thesis, biomass density and coverage of a methane-producing biocathode was reported for the first time using the modified Hartree-Lowry protein analysis linked to microscopy images [165]. The methane-producing biocathode described in **Chapter 3** contained on average 55.6±11.9 g VSS/m^2 projected cathode (n = 6 samples). This VSS density is in the range of reported VSS densities for bio-anodes, being 8-66 g VSS/m^2 projected anode [86]. No clear relationship was observed between biomass density and location at the biocathode, being where the catholyte entered the BES, the centre of the cathode, and where the catholyte left the BES. In this thesis, for the first time the VSS density of a methane-producing biocathode was reported. Currently, biomass density and coverage are mainly studied by using either microscopy techniques, such as Scanning Electron Microscopy, or autofluorescence microscopy, or using biomass quantification techniques, such as protein analysis [23, 75, 100, 168]. Although microscopy images give insight in the biomass coverage of the electrode, only fractions of the electrode are visualized from which the amount of biomass is deducted via statistical image post-processing [141]. Measuring biomass density alone, however, does not give insight in whether this biomass is homogeneously distributed over the electrode or located in clusters, as was observed in this thesis [165]. In this thesis, it was not investigated whether the microorganisms on the electrode were alive. Therefore, it is important to determine whether microorganisms were alive at the moment of measurement. This could be performed by live/dead staining of the microbial cells and consequently visualizing the biofilm with fluorescence microscopy. Combination of these microscopy and biomass quantification techniques are required to give a more detailed insight in the presence, activity and distribution of microbial cells on the electrode surface and in the catholyte.

Optimizing methane production via intermediates

A central question in the reduction of CO_2 to methane is if the reduction is via direct electron transfer from the electrode to the methanogen or indirectly via hydrogen. Methane production from CO_2 occurs at a thermodynamic cathode potential of -0.24 V vs. NHE (pH 7 for the catholyte, and 1 M/bar for all components involved in the reaction), while the hydrogen evolution reactions occurs at a thermodynamic cathode potential of -0.41 V vs. NHE. Consequently, methane production via direct electron transfer theoretically requires 11.0 kWh/m^3 methane (at standard temperature and pressure, pH 7 for the catholyte, pH 3 for the anolyte, and 1 M/bar for all components involved in the reaction), whereas indirect methane production via hydrogen requires 12.5 kWh/m^3 methane. In this thesis, for the first time the energy input per m^3 methane was reported for a methane-producing BES using water as electron donor: 73.5 kWh/m^3 methane for a biocathode with 0.21 A/m^2 at -0.55 V vs. NHE cathode potential [163]. This energy input is about 6 times higher than the theoretical energy input. Energy losses at the cathode, methane diffusion towards the anode, and oxygen diffusion towards the cathode, as explained before in more detail, likely caused this high energy input. It is currently, however, unknown whether methane production via direct electron transfer can occur at a sufficient high rate for implementation of the technology.

In **Chapter 4**, it was demonstrated that at cathode potentials equal or lower than -0.7 V vs. NHE, methane was mainly produced via hydrogen and acetate. A strategy to increase methane production rates could be to optimize the production of these intermediates. Currently, it is most energy efficient to produce hydrogen in a separate reactor, either bioelectrochemically or via water electrolysis, and feeding the hydrogen to the methanogens. The theoretical energy input per m^3 methane is lowest for bioelectrochemical hydrogen production, consuming 2.6 kWh/^{3} H_2 [73], being

equivalent to 11.4 kWh/m^3 methane assuming 4 m^3 hydrogen are required per m^3 methane and assuming 10% energy loss for biomass growth (Table 6.2). Hydrogen production via commercial water electrolysis consumes 5.8 kWh/m^3 H_2 [179], being equivalent to 25.5 kWh/m^3 methane (Table 6.2). This energy input includes compression to 30 bar [179]. When methane in a BES would be compressed, an additional energy input of 0.007 V for each 10-fold pressure increase is theoretically required. Hydrogen production via water electrolysis, however, occurs at higher rate, with 4800 A/m^2 for water electrolysis versus 23 A/m^2 for bioelectrochemical hydrogen production [73, 179] (Table 6.2). The results for bioelectrochemical hydrogen production are, however, from an optimized lab-scale study with artificial wastewater [73]. Attempts to reproduce these results in pilot scale with real wastewater are up to today not successful, due to limited hydrolysis of the organic matter, the low temperature of the electrolyte, and hydrogenotrophic methanogenesis [32]. In addition to hydrogen, acetate can be in important intermediate. Bioelectrochemical acetate production has been reported to occur on (modified) carbon-based electrodes [100, 106, 107], and to a smaller extent on stainless steel, nickel, magnetite and iron sulphide [143]. Current densities and energy inputs were, however, not reported [100, 106, 107, 143], and cannot be compared with current densities or energy inputs of methane-producing biocathodes. It is therefore unknown whether acetate can occur at a sufficient high rate for implementation as intermediate for bioelectrochemical methane production.

Table 6.**2.** Comparison of the current status of direct bioelectrochemical methane production with the production of intermediates for indirect bioelectrochemical methane production.

	Current density (A/m^2)	**Energy input (kWh/m^3 H_2 or acetate)**	**Energy input methane equivalent (kWh/m^3 CH_4)**	**Theoretical energy input (kWh/m^3 CH_4)**	**Scale**	**Reference**
Direct bioelectrochemical methane production with water oxidation	0.21	n.a.	73.5	11.0	Lab-scale	[163]
(Bio)electrochemical hydrogen production	23	2.6	11.4[a]	12.5[b]	Lab-scale	[73]
Water electrolysis	4800	5.8	25.5[a]	12.5	Commercial	[179]
Bioelectrochemical acetate production	n.r.	n.r.	n.r.	11.3	Lab-scale	[100, 106, 107, 143]

[a] Assuming 4 m^3 hydrogen are required per m^3 methane and assuming 10% energy loss for biomass growth.

[b] The theoretical energy input is calculated using water as electron donor, while the results reported here are using acetate as electron donor. The theoretical energy input using acetate as electron donor is -0.35 kWh/m^3 CH_4, meaning that no energy is consumed but 0.35 kWh is released upon production of 1 m^3 methane.

n.a. = not applicable

n.r. = not reported

Gas diffusion through the membrane

In **Chapter 2**, we identified cross-over of products through the membrane as an important energy loss. Methane possibly diffused from the cathode through the membrane towards the anode [163]. Besides, oxygen as the product of water oxidation at the anode, could diffuse from the anode towards the cathode [163]. Once at the cathode, it could lead to a decrease in cathodic electron efficiency via two ways: (i) oxidizing methane or (ii) be reduced to water directly at the cathode, consuming electrons that could account for 37% of the measured current density of 0.25 A/m^2 [163]. Oxygen diffusion towards the cathode, however, seemed not to influence the methanogens [163], despite the methanogens being strict anaerobes. Likely, the oxygen reacted immediately away when it was present in the cathode. At increasing current densities losses due to oxygen diffusion become relatively smaller, because oxygen diffusion through the membrane mainly depends on the oxygen concentration gradient and less on the rate of formation [130]. Although the oxygen concentration gradient increases with increasing rate of formation, at higher current densities the oxygen concentration gradient will approach its maximum and the amount of oxygen diffused will remain the same [130]. However, the relative contribution of oxygen diffusion on current density decreases, since at higher current densities more methane is produced than oxygen is diffused through the membrane [130]. To illustrate this, we calculate what would be the maximum current density that could be attributed to oxygen diffusion when we have a maximum flux of oxygen through the membrane. When we assume the anolyte is fully saturated with oxygen, while no oxygen is present in the catholyte, oxygen diffusion through the membrane theoretically equals a current density of 0.15 A/m^2 (the oxygen diffusion coefficient was assumed to be $5 \cdot 10^{-11}$ m^2/s, based on Nafion 117 data from [139]). For implementation of a methane-producing BES, higher current densities will be necessary, at least 100-fold higher than

the theoretic current density due to oxygen diffusion. Therefore, oxygen diffusion through the membrane is not envisioned to be a problem. Therefore, it is not necessary to invest in measures to mitigate oxygen diffusion from the anode to the cathode.

6.4 Implementation of a methane-producing Bioelectrochemical System

Envisioned first application of a methane-producing Bioelectrochemical System: upgrading CO_2 in biogas of anaerobic digestion to additional methane

As discussed in **Chapter 2**, a first application of a methane-producing BES could be to upgrade CO_2 in biogas of anaerobic digestion to additional methane. Upgrading of CO_2 in biogas is needed for the biogas to meet national gas grid requirements. Biogas produced in anaerobic digestion has methane partial pressures of 0.50-0.60 atm, while natural gas in the gas grid needs to have methane partial pressures higher than 0.80 atm. The upgrading could be done *in-situ*, placing the electrodes inside the digester [135], or *ex-situ*, upgrading the biogas outside the digester, either in a separate biomethanation reactor or in a BES [95, 102] (Table 6.3).

Several lab-test have been performed to study the effect of electrolysis-enhanced anaerobic digestion. By placing electrodes inside the digester biogas could be upgraded *in-situ*. Electrolysis-enhanced anaerobic digestion results in stabilization of the digestion process [34, 135] and increased biogas production [154]. Stabilization and increased biogas production could be a result of micro-oxic zones, the supply of additional electrons or hydrogen, and biomass retention on electrodes. Micro-oxic zones at the anode enhanced biomass hydrolysis [154, 167] and acetoclastic

Table 6.3. Comparison of *in-situ* (placing the electrodes inside the digester) and *ex-situ* biogas upgrading technologies (upgrading the biogas outside the digester, either in a separate biomethanation reactor ("*ex-situ*" in table) or in a BES ("Methane-producing BES (*ex-situ*)" in table). Since all technologies are still under development, future performances are uncertain. Therefore, the current performance is displayed.

	In-situ	***Ex-situ***	**Methane-producing BES (*ex-situ*)**
Electron donor	Electricity	Hydrogen	Electricity
1 or 2 stage process	1	2	2
Scale	Lab-scale	Pilot-scale	Lab-scale
Gas grid quality ($pCH_4 \geq 0.80$ atm)?	No	Yes	Unknown
Energy efficiency	+	+/-	-

methanogenesis [187]. The additional electrons supplied by the cathode, mainly in the form of hydrogen, resulted in increased methane production [34, 154], exceeding the additional methane production one would expect based on the added electrons. Electrochemical reactions at the cathode alone could not explain the increased methane production, but improved syntrophic interactions of the microbial community at the electrode [135] and biomass retention on the electrodes [34] were pointed out to explain the high methane production and energy efficiency. Electrolysis-enhanced anaerobic digestion has up to now not led to methane partial pressures of the produced biogas meeting gas grid quality: 0.58-0.80 atm methane for electrolysis-enhanced anaerobic digestion [135, 154] versus at least 0.80 atm methane to be able to inject the obtained biogas in the gas grid (Table 6.3). At the current stage of development, *in-situ* biogas upgrading processes could improve the methane yield, but a biogas quality that meets gas grid requirements can currently not be guaranteed.

The methane content of the biogas could also be increased via post-treatment of the biogas in a separate reactor (*ex-situ*). An electron donor is added, often in the form

of hydrogen, that supplies the electrons for CO_2 reduction to methane via, for instance, biomethanation [99]. *Ex-situ* upgrading of biogas using hydrogen as electron donor has been reported for, for instance, upgrading untreated biogas of a brewery wastewater digester by pure culture *Methanothermobacter thermoautotrophicus* [102], and upgrading synthetic biogas (60% H_2, 25% CH_4 and 15% CO_2) by mixed cultures [95]. Reported methane partial pressures of the produced biogas ranged between 0.61 atm [102] and 0.95 atm [95] (Table 6.3). At the current stage of development, *ex-situ* biogas upgrading processes could produce a biogas quality that meets gas grid requirements.

An alternative technology to upgrade biogas *ex-situ* is the methane-producing BES, with the electrons being supplied by the electrode directly or indirectly in the form of hydrogen. In this thesis, it was not investigated whether we could produce a biogas that meets gas grid requirements. Since methane is produced from CO_2, and carbonic acid ($H_2CO_3 = CO_2 + H_2O$, pKa = 6.3) is a weak acid, the methane partial pressure of the gas phase depends on the pH of the catholyte. Carbonic acid, however, is not only a substrate, but also a buffer. Therefore, studying the optimum carbonic acid concentration in combination with catholyte pH to meet gas grid requirements, but also to have optimum substrate availability and buffering capacity of the catholyte, is a necessary field of further study.

Availability of the inputs electricity, water and CO_2

For bioelectrochemical methane production, amongst others, (renewable) electrical energy, water and CO_2 are required. Ideally, these inputs need to be available in large quantities, and independent of space and time. The source of these inputs determines whether a methane-producing BES can be implemented and whether it is a truly sustainable technology.

The first input, renewable electrical energy, could be produced in large quantities by

for instance harvesting solar energy using photovoltaics, wind energy using wind turbines, or reversed electrodialysis using the energy from mixing salt and fresh water. Global renewable electricity production was 2.9 EJ/year in 2010 [70]. If this renewable electricity would be converted into methane in a BES at 100% energy efficiency, and assuming an 80% methane content in natural gas, this renewable electricity is equivalent to $7.2 \cdot 10^{10}$ m^3 methane/year or 3.1% of the global natural gas consumption. Renewable electricity production is expected to rise both in amount and in the share of the total primary energy supply [69]. Some of the renewable electricity sources, such as solar and wind energy, are time dependent, resulting in production not always meeting demand. A methane-producing BES could use the renewable electricity that is produced in excess to produce methane, offering the attractive possibility to store excess renewable electricity in the form of methane, that can be easily integrated in the current infrastructure.

The second input, water, is only required in small quantities: 1.5 L water per m^3 methane (assuming 2 moles of water are required per mole of methane), being a factor 1000 lower than for growth of maize and consequent anaerobic digestion of the maize into methane (assuming 625 L water is required for the production of 1 kg maize [124], and assuming a methane yield of 0.5 L CH_4/kg maize [171]). A wide variety of water resources could be used as electron donor in a methane-producing BES. However, to minimize energy losses, preferably water resources with a higher conductivity should be used, such as wastewaters or sea water. Water supply is not envisioned to be a limitation, due to the low water requirement, and the variety of water resources that could be used as electron donor.

The last input, CO_2, could ideally be supplied in large quantities by removing it from air. The concentration of CO_2 in air is, however, very low: 390 ppm in 2010 [105]. Removing CO_2 from air via for instance scrubbing or via bipolar membrane

electrodialysis [37], however, requires a large energy input. For instance, removing CO_2 from air using a counter current packed column with a KOH solution (resulting in K_2CO_3) was estimated to consume 70 kJ per mole CO_2 captured [172]. Recovery of CO_2 and regeneration of KOH from K_2CO_3 was estimated to consume 360 kJ per mole CO_2 captured, so in total 430 kJ/mole CO_2 was consumed [172]. A system that captures CO_2 with CaO (resulting in $CaCO_3$) at high temperature requires 320 kJ/mole CO_2 recovered [172]. Removing CO_2 from air via bipolar membrane electrodialysis is estimated to consume 200 kJ/mole CO_2. The energy density of methane is 890.4 kJ/mole methane [120]. Assuming all CO_2 is converted into methane, recovering CO_2 from air is estimated to consume about 25-50% of the energy contained in methane. Therefore, using CO_2 from more concentrated, stationary sources seems to be more relevant for implementation of a methane-producing BES. More concentrated CO_2 sources that could be used directly and in large quantities for bioelectrochemical methane production are CO_2 from for instance the urea industry (100% CO_2 in flue gas; 113 Mt CO_2 in 2002), from the cement industry (20% CO_2 in flue gas; 932 Mt CO_2 in 2002), or from fermentative processes (100% CO_2 in flue gas; 17.6 Mt CO_2 in 2002) [104]. Combining all these CO_2 sources (317 Mt pure CO_2 in 2002), and assuming all CO_2 is converted bioelectrochemically into methane, about $1.6 \cdot 10^{11}$ m^3 methane/year can be produced, which is equivalent to 7% of the 2010 global natural gas consumption (assuming 80% methane in natural gas) [70]. Our envisioned first application of a methane-producing BES is upgrading of CO_2 in biogas of anaerobic digestion (25-45% CO_2 in biogas). In the Netherlands, it is estimated that about $1.5 \cdot 10^9$ m^3 biogas could be produced per year via co-digestion [11]. When upgrading all CO_2 in this biogas to additional methane (assuming 35% CO_2 in biogas), annually, $5.3 \cdot 10^9$ m^3 methane could be produced, which is equivalent to 1% of the Dutch 2007 gas consumption [11].

Type of electron donor used

In this thesis, water was used as electron donor. In BES research, organics from wastewater, in artificial wastewater often represented by acetate, is another widely used electron donor. Using water has the advantage that it is a widely available and cheap resource. Besides, its use results in a net CO_2 capture in methane, and consequently in additional methane yield per unit of biomass or land area, as is explained in **Chapter 2**. Methane-producing BES that use acetate as electron donor and that recycle the CO_2 produced at the anode as substrate at the cathode, produce the same mix of methane and CO_2 compared to existing biomass conversion technologies, such as anaerobic digestion. When oxidizing acetate, 8 moles of electrons and 2 moles of CO_2 are produced per mole of acetate, the latter being present in the form of bicarbonate at pH 7 as used in this thesis (Figure 6.2). The formed 8 moles of electrons are sufficient to reduce only 1 mole of bicarbonate into methane at the cathode (Figure 6.2). Overall, using acetate as electron donor, per mole of acetate 1 mole of bicarbonate and 1 mole of methane is produced (Figure 6.2), being the same final products as obtained with anaerobic digestion. Finally, using water as electron donor results in a process independent of biomass. Consequently, the process becomes independent of water, nutrients, and land area, all components that are already scarce or are expected to become scarce in the future [108]. A disadvantage of using water is, however, the need of an almost three times higher electrical energy input compared to using domestic wastewater as electron donor: 629.8 kJ/mole CH_4 for water versus 224.0 kJ/mole CH_4 for acetate (assuming all electrons end up in methane; based on the tabulated values in [159] at standard temperature and pressure, pH 7 and 1 M or 1 bar for all components involved in the reaction). Besides, diffusion of the produced oxygen to the cathode could negatively affect methane production [18, 78]. In **Chapter 2**, it was calculated that oxygen diffusion can account for losses of up to

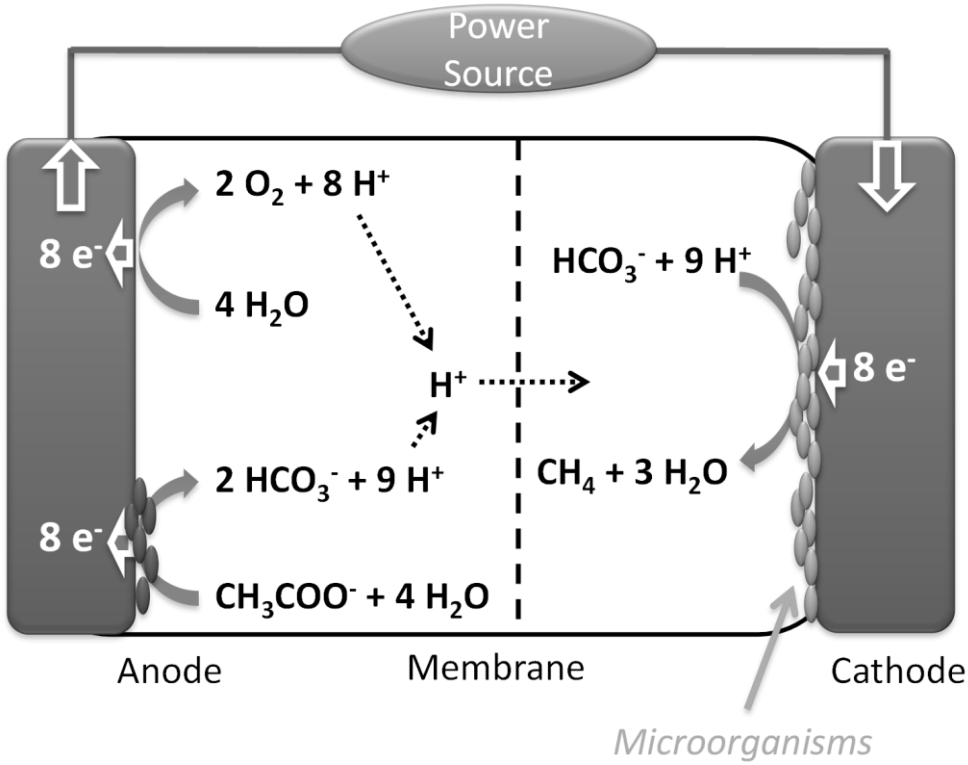

Figure 6.2. Comparison of water (top reaction at the anode) and acetate (bottom reaction at the anode) as electron donor for a methane-producing BES.

0.9 A/m^2 cathode or 12 L CH_4/m^3 reactor per day, which are significant losses at low current densities and methane production rates. The feedstock price and the price of electricity will determine what will be the most attractive electron donor in the future.

Estimation of the economic potential of a methane-producing Bioelectrochemical System

For implementation of a methane-producing BES its use should result in revenue. In this paragraph, we analyse the economic potential of a methane-producing BES. The products and services in a methane-producing BES that could result in revenue are production of methane and production of pure oxygen (Table 6.4). The reported revenue, however, is highly dependent on the quality of the productBesides methane,

oxygen could be recovered as an additional product. Pure oxygen (>99.999% (v/v)) has about a 20 times higher economic value then methane: about €5000,-/ton oxygen (Table 6.4). Recovery of oxygen is thus an interesting additional product of a methane-producing BES [35]. A service that possibly could result in additional revenue is storing excess renewable electricity to produce methane. At this stage, however, it cannot be determined whether consumption of excess electricity will result in revenue and how much this revenue will be. Methane has, however, a low economic value compared to, for instance, fatty acids, ethanol or succinate (Table 6.4), which could also be produced bioelectrochemically [24, 75, 100, 106, 107, 109, 140, 147, 151, 164, 184].

Table 6.4. Economic value of the products and services that could be produced in a BES.

Component	Economic value (€/ton)	Reference
Methane (natural gas)[a]	230[a]	[175]
Pure oxygen (99.999% (v/v))[b]	5200	Linde Gas BV
Acetic acid	500	[174, 178]
Caproic acid	8800	[181]
Ethanol	750-1500	[176]
Succinic acid	2200-6600	[177, 180]

[a] Prices mentioned are for natural gas. In the calculations it was assumed that natural gas has an energy density of 11.8 kWh/kg (personal communication Maarten van Riet).

[b] €75,- for 10.8 m^3 99.999% (v/v) O_2 (personal communication with Linde Gas BV, The Netherlands, 19th June 2013).

6.5 Production of higher-value organics than methane in Bioelectrochemical Systems

Besides converting CO_2 and electricity into methane, a BES could also be used to produce higher-value organics, such as fatty acids and alcohols (Table 6.4). Currently, fatty acids and alcohols are produced by fermenting (low-grade) organic biomass (*e.g.* [3, 55, 134, 150]). For medium chain fatty acids production, however, an external electron donor in the form of hydrogen and/or ethanol needs to be added [3, 55, 150]. A BES could provide the electrons *in-situ*, either as the electrode directly or indirectly via hydrogen. Advantages of *in-situ* electron supply are: (i) no infrastructure for the supply of hydrogen is required, and (ii) the electron donor is renewable when renewable electricity is used to power the BES.

In this thesis, acetate was converted into medium chain fatty acids caproate and caprylate at -0.9 V vs. NHE cathode potential [164]. Besides caproate and caprylate, also butyrate, ethanol and hydrogen were formed [164]. Fatty acids were likely produced indirectly via bioelectrochemically produced hydrogen [164].

Medium chain fatty acids can be used in the chemical industry as building blocks for biodiesel or kerosine-like products [46, 125], polymers, plasticizers, paint additives, and lubricants [182]. Compared to methane, caproate has almost 40 times higher economic value (Table 6.4). This high economic value, however, is for pure caproate, while in this thesis, a mixture of fatty acids was obtained at low concentrations. For instance, maximum concentration for caproate was 739 mg/L [164], while the solubility is 11 g/L at 20°C. Selective production of single compounds at high concentrations is a challenge: none of the reported studies produced only a single product, or produced products at high concentrations, for instance, approaching the maximum solubility [24, 75, 100, 106, 107, 109, 140, 147, 151, 164, 184]. Caproate

production could be steered by adjusting the hydraulic retention time [56], or adding bicarbonate to the catholyte [56] or CO_2 to the cathode headspace [5]. Caproate concentrations of up to 12.0 g/L have been reported for mixed culture acetate and ethanol fermentation, with the caproate not inhibiting the microbial community for 69 days [56]. This caproate concentration is higher than the solubility product of caproate, facilitating separation from the fermentation broth. Another method is inline extraction of caproate from the fermentation broth using a membrane [2]. Caproate concentrations of 97% were achieved, and caproate was produced at 1.5 g/L reactor per day [2]; a strategy also possible in BESs.

As described in **Chapter 5**, production of fatty acids stopped after 18 days, likely due to biomass wash-out due to the continuous flow conditions [164]. Several methods to retain biomass inside the BES were mentioned: increasing the solid retention time, using a fed-batch mode of operation, or the continuous addition of inoculum that was grown in a separate bioreactor [164].

Research on the production of higher-value organics than methane in BES increases, with 9 publications in the past 4 years [24, 100, 106, 107, 141, 147, 151, 164, 184]. Time will tell whether production of higher-value organics will become an application of BESs.

Summary

Nowadays, most of our energy and fuels are produced from fossil resources. Fossil resources are, however, finite and their use results in emissions that affect the environment and human health. For reasons of energy security and environmental sustainability, there is therefore a need to produce energy and fuels from renewable resources. However, currently several challenges need to be overcome before renewable resources can be implemented on a large scale for the production of renewable energy and fuels. At the moment, all the renewable resources can be converted into electricity. However, renewable electricity is often produced intermittently. Therefore, excess renewable electricity, when supply does not meet demand, needs to be stored not to get lost. On the other hand, fuels can currently only be produced directly from biomass. There are, however, discussions about whether sufficient biomass can be produced in a sustainable way to cover the global demand.

A methane-producing Bioelectrochemical system (BES) is a novel technology to store excess renewable electricity in the form of methane, independent of biomass. Key principle of the methane-producing BES is the use of microorganisms as catalysts for the reduction of CO_2 and electricity into methane. At the start of this thesis, the methane-producing BES was at its infancy, and for implementation of the technology a more thorough understanding of the technology was needed. Therefore, the aim of this thesis was to investigate the principles and perspectives of bioelectrochemical methane production from CO_2. Focus was on the main bottlenecks limiting system's performance.

In **Chapter 2**, the performance of a flat-plate methane-producing BES that was operated for 188 days was studied. The methane production rate and energy efficiency

were investigated with time to elucidate the main bottlenecks limiting system's performance. Using water as electron donor at the anode, methane production rate was 0.12 mL CH_4/m^2 cathode per day and overall energy efficiency was 3.1% at -0.55 V vs. Normal Hydrogen Electrode (NHE) cathode potential during continuous operation. Analysis of the internal resistance showed that in the short term, cathode and anode losses were dominant, but with time also pH gradient and transport losses became important.

Since the cathode energy losses were dominant, in **Chapter 3**, the microbial community that catalyses the reduction of CO_2 into methane was studied. The microbial community was dominated by three phylotypes of methanogenic archaea, being closely related to *Methanobacterium palustre* and *Methanobacterium aarhusense*, and six phylotypes of bacteria. Besides methanogenic archaea, the bacteria seemed to be associated with methane production, producing hydrogen as intermediate. Biomass density varied greatly with part of the electrode being covered by a thick biofilm, whereas only clusters of biomass were found on other parts of the electrode.

Based on the microbial community it seemed that methane was produced indirectly using hydrogen as electron donor. Therefore, the electron transfer mechanisms of bioelectrochemical methane production were investigated in **Chapter 4**. Understanding the electron transfer mechanisms could give insight in methods to steer the process towards higher rate. A mixed culture methane-producing biocathode was developed that produced 5.2 L methane/m^2 cathode per day at -0.7 V vs. NHE cathode potential. To elucidate the formation of intermediates, methanogenic archaea in the biocathode were inhibited with 2-bromethanesulfonate. Methane was primarily produced indirectly using hydrogen and acetate as electron donor, whereas methane production via direct electron transfer hardly occurred.

Besides producing methane, a BES could also be used to produce higher value

organics, such as medium chain fatty acids. Currently, medium chain fatty acids are produced by fermenting (low-grade) organic biomass using an external electron donor, such as hydrogen and/or ethanol. A BES could provide the electrons *in-situ*, either as the electrode directly or indirectly via hydrogen. In **Chapter 5**, medium chain fatty acids production in a BES at -0.9 V vs. NHE cathode potential was demonstrated, without addition of an external electron mediator. Caproate (six carbon atoms), butyrate (four carbon atoms), and smaller fractions of caprylate (eight carbon atoms) were the main products formed from acetate (two carbon atoms). *In-situ* produced hydrogen was likely electron donor for the reduction of acetate. Electron and carbon balances revealed that 45% of the electrons in electric current and acetate, and 31% of the carbon in acetate were recovered in the formed products.

In **Chapter 6**, the present performance of methane-producing BESs was evaluated. Analysis of the performances reported in literature did not reveal an increase with time. Based on the main bottlenecks that limit system's performance as found in this thesis, methods to increase performance were discussed. Besides, we showed that our envisioned first application is to upgrade CO_2 in biogas of anaerobic digestion to additional methane. Finally, the feasibility of production of higher-value organics, such as medium chain fatty acids, in BES was discussed.

Samenvatting

Momenteel wordt het merendeel van onze energie en brandstoffen gemaakt van fossiele bronnen. Fossiele bronnen zijn echter eindig en het gebruik ervan resulteert in emissies die een negatieve invloed hebben op humane gezondheid en het milieu. Om redenen van energiezekerheid en duurzaamheid is er daarom de behoefte om energie en brandstoffen van duurzame bronnen te produceren. Voordat dit echter op grote schaal mogelijk is, moeten er eerst verschillende uitdagingen worden opgelost. Momenteel kan van alle duurzame bronnen elektriciteit gemaakt worden. Duurzame elektriciteitsproductie fluctueert echter vaak en overtollig geproduceerde elektriciteit, wanneer vraag en aanbod niet overeenkomen, moet worden opgeslagen om niet verloren te gaan. Daarbovenop kunnen brandstoffen op dit moment alleen worden gemaakt van biomassa. Er zijn echter discussies of er wel voldoende biomassa op een duurzame manier geproduceerd kan worden om aan de wereldvraag te voldoen.

Een methaan-producerende Bioelektrochemisch Systeem (BES) is een nieuwe technologie om overtollige duurzame elektriciteit op te slaan als methaan, onafhankelijk van biomassa. Hoofdprincipe van de methaan-producerende BES is het gebruik van micro-organismen als katalysator voor de reductie van CO_2 en elektriciteit naar methaan. Aan het begin van dit proefschrift stond de methaan-producerende BES nog in de kinderschoenen. Voor toepassing van de technologie was een grondige verkenning van de technologie nodig. Daarom was het doel van dit proefschrift om de principes en perspectieven van de methaan-producerende BES te onderzoeken. De focus was om de belangrijkste energieverliezen in kaart te brengen.

In **Hoofdstuk 2** bestudeerden we de prestatie van een platte plaat methaan-producerende BES die voor 188 dagen opereerde. We onderzochten de methaan

productiesnelheid en energie efficiëntie gedurende het experiment om zo de belangrijkste energieverliezen in kaart te brengen. Met water als elektronen bron aan de anode, produceerde de reactor 0.12 mL CH_4/m^2 kathode per dag met een energie efficiëntie van 3.1% bij -0.55 V vs. Standaard Waterstof Elektrode (SWE) kathode potentiaal gedurende continue operatie. Een interne weerstanden analyse liet zien dat op korte termijn de grootste energieverliezen plaats vonden bij de kathode en anode, maar op langere termijn ook door transport verliezen en de opbouw van een pH gradiënt.

Aangezien de grootste energieverliezen plaats vonden bij de kathode, bestudeerden we in **Hoofdstuk 3** de microbiële populatie die CO_2 reduceert in methaan. De microbiële populatie werd gedomineerd door drie phylotypes methanogenen, gerelateerd aan *Methanobacterium palustre* en *Methanobacterium aarhusense*, en zes phylotypes bacteriën. Naast de methanogenen, leken de bacteriën betrokken te zijn bij methaan productie als producenten van waterstof als elektronen donor. De biomassa dichtheid varieerde sterk: een deel van de elektrode was bedekt met een dikke biofilm, terwijl andere delen slechts clusters biomassa bevatten.

Om aan te tonen dat methaan weldegelijk geproduceerd wordt met (microbieel geproduceerd) waterstof als elektronen donor, bestudeerden we in **Hoofdstuk 4** de elektronen overdracht mechanismen. Met begrip van deze mechanismen zou het mogelijk kunnen zijn om het proces te sturen naar hogere methaan productie snelheden. Een methaan-producerende biokathode, bestaand uit gemengde culturen, was ontwikkelt, die 5.2 L CH_4/m^2 kathode per dag produceerde bij -0.7 V vs. SWE. Om microbiële waterstofproductie aan te tonen, werden de methanogenen geïnhibeerd met 2-bromoethaansulfonaat. Methaan werd voornamelijk geproduceerd middels waterstof als elektronen donor, terwijl methaan productie via directe elektronen overdracht nauwelijks plaats vond.

Naast methaan kan een BES ook gebruikt worden voor het produceren van hoogwaardigere stoffen, zoals middellange vetzuren. Momenteel worden deze vetzuren geproduceerd door (laagwaardig) organisch materiaal te fermenteren met een toegevoegde elektronen donor, zoals waterstof en/of ethanol. Een BES zou *in-situ* elektronen kunnen doneren voor deze fermentaties, ofwel direct via de elektrode ofwel via waterstof. In **Hoofdstuk 5** demonstreren we de productie van middellange vetzuren in een BES bij -0.9 V vs. SWE zonder toevoeging van een elektronen mediator. Caproaat (zes koolstofatomen lang), butyraat (vier koolstofatomen lang) en kleine fracties van caprylaat (acht koolstofatomen lang) werden geproduceerd van acetaat (twee koolstofatomen lang). *In-situ* geproduceerd waterstof was waarschijnlijk de elektronenbron voor de reductie van acetaat. Elektronen en koolstofbalansen wezen uit dat 45% van de elektronen in stroom en acetaat en 31% van de koolstof in acetaat werden teruggevonden in de gevormde producten.

In **Hoofdstuk 6** evalueerden we de prestaties van gepubliceerde studies over de methaan-producerende BES. De prestatie van de methaan-producerende BES liet geen stijgende lijn met tijd zien. Op basis van de opgedane kennis over de belangrijkste energieverliezen van een methaan-producerende BES, zoals bestudeerd in dit proefschrift, bediscussiëren we methoden om de prestatie te verhogen. Daarnaast bespreken we een eerste toepassing van de methaan-producerende BES zoals wij die voor ogen hebben: het omzetten van CO_2 in biogas van anaerobe vergisting in extra methaan. Tot slot bediscussiëren we de toepassing van de productie van hoogwaardigere componenten in een BES, zoals middellange vetzuren.

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List of publications

Jan W. Swarts, Ruben C. Kolfschoten, **Mieke C. A. A. Jansen**, Anja E. M. Janssen, Remko M. Boom (2010), *Effect of diffusion on enzyme activity in a microreactor*, Chemical Engineering Journal 162 (1): 301-306, doi 10.1016/j.cej.2010.04.040.

Mieke C. A. A. Van Eerten-Jansen, Annemiek Ter Heijne, Cees J. N. Buisman, Hubertus V. M. Hamelers (2012), *Microbial Electrolysis Cells for production of methane from CO_2: long-term performance and perspectives*, International Journal of Energy Research 36 (6): 809-819, doi 10.1002/er.1954 (Chapter 2 – this thesis).

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Mieke C. A. A. Van Eerten-Jansen, Nina C. Jansen, Caroline M. Plugge, Vinnie de Wilde, Cees J. N. Buisman, Annemiek Ter Heijne (2014), *Analysis of the mechanisms of bioelectrochemical methane production by mixed cultures*, Journal of Chemical Technology and Biotechnology, doi 10.1002/jctb.4413 (Chapter 4 – this thesis).

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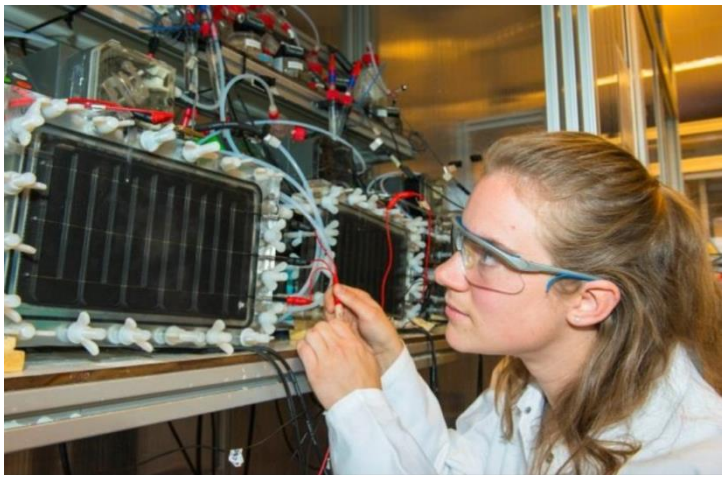
Curriculum vitae



Mieke van Eerten-Jansen (1985) was born in Nijmegen, The Netherlands. She obtained her BSc in Food Technology (Wageningen University) in 2006. During her BSc she did a minor thesis on developing fibrillar protein aggregate structures for meat analogues. She pursued an MSc in Food Technology (Wageningen University) and specialized in Food Process Engineering. During her MSc, she did a major thesis on fast enzymatic reactions in microreactors, and an internship on the stability of cream liqueur (Massey University, New Zealand). During her MSc (graduated in 2008), she became interested in making food processes more sustainable, but this was still of limited importance for food industry and science. Still being in New Zealand, she got a job at the recruitment department of Wageningen University. There, she came across research on renewable energy and wastewater treatment technologies and became interested. To increase her knowledge on these topics, she started a PhD (2009) of which the results are presented in this thesis. Currently, Mieke is working as lecturer and researcher "Biobased Product Development" at HAS Applied University in Den Bosch, The Netherlands.

Acknowledgements/Dankwoord

Gedurende 4.5 jaar heb ik heel veel inspirerende mensen mogen ontmoeten die direct, danwel indirect, een bijdrage hebben geleverd aan het werk wat nu voor je ligt. Het zijn teveel mensen om allemaal persoonlijk te noemen, maar eenieder heel erg bedankt voor je hulp, aanmoediging en/of vriendschap!

During the past 4.5 years I was honoured to have met many inspiring persons that directly or indirectly helped me completing this thesis. I cannot personally thank everyone, but I want to thank you all for your help, encouragement, and friendship!

Dank je! Thank you! Gracias! Ameseginallehu! Teşekkürler! Mercie!

Toch wil ik enkele personen in het bijzonder noemen.

Allereerst mijn begeleiders. Het begon allemaal bij Bert Hamelers, mijn begeleider gedurende de eerste 2 jaar van dit onderzoek. Bert, na een inspirerend mini-college door jou (mijn "sollicitatie") was ik overtuigd dat dit het onderzoek was waaraan ik wilde werken. Je bent een creatief en kritisch persoon waar ik veel van heb geleerd. Annemiek ter Heijne, je was al betrokken bij mijn onderzoek voor Bert naar Wetsus ging en hebt zijn rol overgenomen als mijn begeleider. Ik wil je heel erg bedanken voor je kritische blik en het corrigeren van m'n stellige schrijven, maar ook voor de vele gesprekken over het leven "thuis". Ik vind het een eer je eerste promovendus te mogen zijn en ik hoop dat je net zo tevreden terug kijkt op dit traject als ik. Cees Buisman, bedankt voor je kritische vragen, de discussies over mijn onderzoek en je visie over hoe dit onderzoek past in de praktijk.

Dan de mensen die achter de schermen bergen werk verzetten: het secretariaat en

de technische en analytische staf. Liesbeth Kesaulya, ik kon op elk moment met wat voor vraag bij jou terecht komen en altijd wist jij het antwoord (en voerde het direct uit). Heel erg bedankt! Patricia Vermeer, Romana Zigova en Linda van Geelen, heel erg bedankt voor de vele hand en spandiensten. Anita van de Weerd en Gea van Beuningen, bedankt voor het in het gareel houden van het financiële plaatje. Gea, jij in het bijzonder bedankt voor de fijne gesprekken. Vinnie en Bert, wat voor problemen ik ook had met m'n reactoren, jullie hadden vaak praktische oplossingen. Het doen van metingen ging op rolletjes door de inzet van de analytische staf: ik kon altijd bij jullie terecht. Ook de Ontwikkelwerkplaats wil ik bedanken voor het vele repareren en aanpassen van m'n reactoren.

Aan het eind van m'n proefschrift kon ik rekenen op microbiële expertise, een kritische blik en een persoonlijk praatje van Caroline Plugge en Fons Stams. Caroline en Fons, mede door jullie inzet is dit proefschrift verbeterd.

I was lucky to have many students/researchers that have helped me with this thesis: Anna Veldhoen, Nebiyu Merdekios Madalcho, Nico Claassens, Saskia Moll, Mauro Garcia Esteban, Gerard 't Lam, Nalan Oya San, and Nina Jansen. It was a pleasure working with you and getting to know you. Your hard work resulted in two publications.

Then I want to thank my colleagues at Environmental Technology, Wetsus, and LeAF. I keep good memories of the (long) coffee and lunch breaks, the lunch walks, the puzzling (and getting into the Resource with "text"), the many drinks (of which the Ice Skate Drink is forever on my mind), the department trips, the Hexathlon at the WeDay, the Veluweloop, running the Wageningen "mountain" and rewarding ourselves with beer afterwards, the Canada trip, socializing at conferences, the Nude-dinner parties, the dinners and birthdays, the wedding parties, being a paranimph, BHV trainings ("geen beeld en geluid"), my trip to Ruud in Madrid and Magdalena in Poland, and probably much more moments that I cannot recall at the moment. Miriam en Nora, jullie in het bijzonder bedankt voor alle gezelligheid. Fijn dat jullie mijn paranimfen willen zijn.

Voor de nodige ontspanning werd gezorgd door m'n gang genoten van Dijkgraaf 2C, de Mutsen, de Elzendaal College-groep, Carola & Roel en "Roeland's vrienden". Bedankt voor de vele etentjes, dagjes uit, gezelligheid en altijd een luisterend oor. Anne Wientjes, in het bijzonder bedankt voor de gezellige lunchwandelingen. Ik ben trots met zoveel lieve mensen omringd te zijn.

Ook de mensen van Kennisnetwerk Milieu, Onderwijsinstituut Milieuwetenschappen/Environmental Sciences, Rugbyclub Wageningen en mijn nieuwe collega's bij HAS Den Bosch wil ik bedanken voor de gezelligheid en mentale/fysieke ontspanning.

Mensen waarop ik al kon rekenen nog voor ik m'n promotie begon is mijn (schoon)familie. Ook al was het moeilijk om te begrijpen wat ik nu precies deed, jullie steunden me onvoorwaardelijk en ik vond bij jullie altijd een luisterend oor. Pap en mam, door jullie toewijding en steun heb ik het kunnen schoppen tot Dr. Mieke. Nu ik zelf moeder ben, neem ik hier een voorbeeld aan. Verder wil ik m'n (schoon)ouders bedanken voor het oppassen op Koen aan het eind van m'n thesis.

Tot slot wil ik Roeland bedanken. Je hebt me onvoorwaardelijk gesteund en me de nodige afleiding gegeven. Samen hebben we veel mooie momenten meegemaakt gedurende m'n promotietraject: we zijn getrouwd, waardoor nu ook jouw achternaam bij m'n publicaties genoemd wordt, hebben veel gereisd en zijn nu trotse ouders van Koen. *Ik ben ontzettend wies met oe!*

Mieke

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The SENSE Research School declares that **Ms Mieke van Eerten-Jansen** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 67.5 ECTS, including the following activities:

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- *Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures.* 9th European Congress of Chemical Engineering & 2nd European Congress of Applied Biotechnology, 21-25 April 2013, Den Haag, The Netherlands

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Ellen van der Starre-Hornman and Marian Hornman-Voesten are gratefully acknowledged for designing the cover of this thesis.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.