

Electricity from wetlands

**Technology assessment of the tubular Plant
Microbial Fuel Cell with an integrated biocathode**

Koen Wetser

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Thesis

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Summary

Sustainable electricity generation by the plant microbial fuel cell

Fossil fuels are currently the main source of electricity production. Combustion of fossil fuels causes air pollution severely affecting human health and nature. This results in an increasing demand for renewable electricity sources. One of the emerging renewable electricity technologies is the plant microbial fuel cell (PMFC) as explained in chapter 1. PMFC generates electricity from the rhizodeposits of living plants. Naturally occurring electrochemically active microorganisms oxidize the rhizodeposits producing electrons at the anode of the PMFC. The electrons flow from the anode, via an external circuit where the electricity is harvested, to the cathode. At the cathode, the electrons reduce oxygen to water. PMFC is based on naturally occurring sustainable and renewable processes without net emissions and competition for arable land or nature. Large scale application of the PMFC is preferred in wetlands because a large waterlogged area is required.

Prior to application, the cathode limitations of the PMFC have to be solved. Oxygen reduction at the cathode is slow, limiting the current and power output of the PMFC. An unsustainable chemical cathode is often used in PMFC research to overcome the cathode limitations. The sustainable oxygen reducing cathode has to be catalyzed when integrated in the PMFC. Most chemical catalyst are expensive and prohibit the commercial use in the PMFC. Oxygen reduction can also be biologically catalyzed by cheap and self-replenishing microorganisms. Next to the biocathode, also a suitable design of the PMFC has to be developed before application in wetlands. A tubular design was previously developed which can be invisibly integrated in wetlands. However, this design still used a chemical cathode and energy intensive pumping. The oxygen reducing biocathode should be integrated in the tubular design and oxygen should be passively supplied in the cathode.

The objective of this thesis is to apply PMFC in wetlands with a sustainable biocathode. First, the biocathode is integrated in a lab scale PMFC. Afterwards, the PMFC is installed in wetlands using an improved tubular design with an integrated biocathode and passive oxygen supply.

Lab scale experiments: integration of the biocathode and electricity localization in the bioanode of the PMFC

In chapter 2, the oxygen reducing biocathode is integrated in a flat plate lab scale PMFC replacing the chemical ferricyanide cathode. The PMFC operated as a completely biocatalyzed system for 151 days. The sustainable PMFC with a biocathode was able to generate more power than the PMFC with a chemical cathode. The long term power generation of the lab scale PMFC improved from 155 mW m^{-2} plant growth area (PGA) to a record of 240 mW m^{-2} PGA. This record was reached due to the higher redox potential of oxygen reduction compared to ferricyanide reduction. Oxygen reduction was effectively catalyzed by microorganisms lowering the voltage losses at the cathode. As a result, the PMFC with a biocathode operated at a 127 mV higher cathode potential than a similar PMFC with a chemical ferricyanide cathode. The long term current generation of both PMFCs was 0.4 A m^{-2} PGA. The current generation was likely limited by the substrate availability in the anode of the PMFC.

In chapter 3, the biocathode is further investigated. This chapter shows that the oxygen reducing biocathode can also catalyze the reversible reaction, water oxidation. Water is the most abundant electron donor available for electrochemical fuel production like the reduction of protons to hydrogen and the reduction of carbon dioxide to hydrocarbons. However, the water oxidation reaction is currently hampering the development of large scale water oxidation technologies. A bioanode containing electrochemically active microorganisms was able to reach a current density of 0.93 A m^{-2} at 0.7 V overpotential with a 22 % Coulombic efficiency linked to water oxidation. An optimized system could be used to produce fuels on a large scale.

The flat plate PMFC of chapter 2 was also used to localize the electricity generation in the PMFC (chapter 4). In this experiment, the anode was partitioned in 30 separate small anodes at different width and depths. The current generation of each anode was analyzed over time and linked to the plant roots. The results show that after a start-up period of 70 days, significantly higher current was generated at anodes close to the plant roots due to rhizodeposition. Besides rhizodeposition (i.e. electron donors), the plant roots also excrete oxygen which is an electron acceptor lowering the current generation of the PMFC. Also oxygen was measured at the anodes close to the plant roots. This likely resulted in internal currents in the PMFC. Current was likely generated

both from living and death roots. The electrons in the PMFC were probably transferred via mediators to locations without roots as mediators were present also at locations without plant roots. These mediators were likely excreted by plants and/or microorganisms in the anode. Electrons were likely not transferred over centimeter distance through conductive microorganism on the plant roots in the PMFC.

Installation of the tubular PMFC with an integrated biocathode in wetlands

After the successful integration of the biocathode in the PMFC, the focus of the research changed to application in wetlands. Two wetlands with an abundant occurrence in the Netherlands were investigated in this research. The first wetland was a *Phragmites australis* dominated fen peat soil, a large perennial grass. The peat soil in this research was collected in national park Alde Feanen in the north of the Netherlands. The second investigated wetland was a *Spartina anglica* dominated salt marsh. *Spartina anglica* is a perennial grass found in coastlines spread over the world. The salt marsh was collected in the Oosterschelde tidal basin in the southwest of the Netherlands.

The first experiment in the wetlands was conducted to investigate the spatial and temporal differences in current and power generation in and between wetlands (chapter 5). PMFCs in the salt marsh were able to generate more than 10 times more power than the same PMFCs in the peat soil (18 vs 1.3 mW m⁻² PGA on a long term). The higher power generation is mainly explained by the high ionic conductivity of the salt marsh and the presence of sulfide which is also oxidized next to the rhizodeposits at the anode of the PMFC. The top layer of the salt marsh generated most power due to the presence of the plants and tidal advection. In the peat soil, there was no significant difference in power generation over depth. Even though, in the top layer more living roots were present. Also the dead roots and organics in peat can be oxidized by the PMFC. In chapter 5, also the maximum current and power output of the wetlands was predicted based on rhizodeposition of the investigated plants and microbial processes in these wetlands. The calculations showed that the potential current generation of PMFC in the salt marsh is 0.21-0.48 A m⁻² PGA and in peat soil 0.15-0.86 A m⁻². In the peat soil, the PMFC is potentially able to generate a power density up to 0.52 W m⁻² PGA.

The second experiment in the wetland was the installation of a tubular PMFC with an in situ started oxygen reducing biocathode and passive oxygen supply into the cathode (chapter 6). The anode was the outside of the tube and placed directly between the plant roots. The oxygen reducing biocathode was located inside the tube. A silicone gas diffusion tube was placed in the cathode compartment to passively supply the required oxygen. The tubular PMFC with biocathode was successfully installed and started in the peat soil reaching a maximum daily average power generation of 22 mW m^{-2} PGA. In the salt marsh, the tubular biocathode PMFC only started while supplying pure oxygen in the gas diffusion tube. Air diffusion did not result in the start-up of the biocathode, likely because the oxygen was directly reduced via internal currents and therefore more oxygen was required. Once started with pure oxygen, the tubular PMFC was able to generate 82 mW m^{-2} PGA which was again higher than the peat soil. Completely biocatalyzed tubular PMFC were installed in both wetlands with natural occurring microorganisms in the anode and cathode. The power generation can be further increased by improving the PMFC design limiting crossover of oxygen and substrate.

Future outlook: application of the PMFC in wetlands

In chapter 5, the potential power generation of the two investigated wetlands was calculated. In chapter 7, these calculations were extended to a worldwide scale. PMFC applied in all wetlands could generate 0.67 to 1.35 TW and could cover 30 to 60 % of the global electricity consumption. 70 % of all the potential power could be generated in the tropics. Worldwide, 1.1 billion people have insufficient access to electricity from which 88 % lives in the tropics (i.e. Sub-Saharan Africa and South Asia). PMFC could be used to reach universal access of electricity in these locations and decrease the amount of premature deaths due to air pollution.

PMFC can be applied with passive or active oxygen supply from the outside air into the silicone tube. The used tubular PMFC with passive oxygen supply can have a maximum length of less than one meter. Active supply of oxygen reduces the net power output of the PMFC, but allowing installation of long tubular PMFC. However, in both cases the material costs should be significantly reduced for economically feasible application at large scale. The costs of the material should be decreased to less than 1 % of the current PMFC costs to have a payback

time of 50 years in the Dutch electricity market for only the tubular PMFC. Further cost reduction is required when also the current collectors, electricity transmission, production and installation costs are included. Application of PMFC in remote locations increases the economic feasibility of the PMFC as the PMFC could be applied independent from the grid reducing the transmission costs and avoiding the regular electricity network charges.

Application of the PMFC in the total area of *Spartina anglica* salt marsh in the Oosterschelde, the location where the plants were collected, could produce a total of 11.6 GWh yr⁻¹. The Oosterschelde could produce the electricity consumption of 8,360 persons and as such produce the electricity need of an average village directly located at the tidal basin. The *Phragmites australis* peat soil in the Alde Feanen national park could produce 2.5 GWh yr⁻¹. The electricity could be directly used for ecotourism purposes, for example for the use of electric boats and a holiday park.

Samenvatting

Duurzame elektriciteit opgewekt door de plant microbiële brandstof cel

Fossiele brandstoffen zijn momenteel de belangrijkste bron voor de opwekking van elektriciteit. De fossiele bronnen moeten vervangen worden door duurzame bronnen omdat de verbranding van fossiele brandstoffen luchtvervuiling veroorzaakt wat slecht is voor de gezondheid en de natuur. Een van de opkomende duurzame bronnen voor elektriciteitsproductie is de plant microbiële brandstof cel (PMBC) zoals uitgelegd in hoofdstuk 1. De PMBC genereert elektriciteit van de organische stof die door de planten in de bodem uitgestoten wordt. Dit zijn onder andere dode wortels en exsudaten. Elektrochemisch actieve micro-organismen die van nature in de bodem voorkomen oxideren de door de planten uitgestoten organische stof. Hierbij produceren de micro-organismen elektronen aan de anode van de PMBC. The elektronen stromen door een extern circuit, waar de elektriciteit wordt geoogst, naar de kathode. In de kathode reduceren de elektronen zuurstof naar water. De PMBC bevat enkel duurzame en hernieuwbare processen zonder schadelijke uitstoot of concurrentie voor de landbouw en de natuur. Elektriciteit kan alleen opgewekt worden in watergebieden als moerassen, draslanden en slikken.

De zuurstofreductie aan de kathode is langzaam en limiteert de elektriciteitsproductie van de PMBC. Deze reactie moet gekatalyseerd worden om meer elektriciteit te kunnen generen. Dit kan met chemische katalysatoren als platina, echter de hoge kosten van deze katalysatoren maken het ongeschikt voor de PMBC. Zuurstofreductie kan ook biologische gekatalyseerd worden door micro-organismen waardoor de PMBC toegepast kan worden met een goedkope biokathode. Voordat de PMBC toegepast kan worden moet er naast de integratie van de biokathode ook een geschikt design worden ontwikkeld. Een buisvormig design heeft de voorkeur. Dit design kan namelijk onzichtbaar in de watergebieden worden geïnstalleerd.

Het doel van deze thesis is de toepassing van de PMBC in watergebieden met een duurzame biokathode. Eerst wordt de biokathode onderzocht in lab schaal experimenten. Daarna wordt de biokathode geïntegreerd in een buisvormige PMBC en toegepast in verschillende watergebieden.

Lab schaal experimenten

In hoofdstuk 2 is de zuurstof reducerende biokathode geïntegreerd in een PMBC met een vlakke plaat als design. Dit systeem draaide voor 151 dagen met zowel een bioanode als een biokathode en genereerde een vermogen van 0.24 W/m^2 , beduidend meer dan de 0.16 W/m^2 die eerder werd geproduceerd zonder biokathode. De PMBC was daardoor niet meer gelimiteerd door de kathode maar door de anode. De biokathode is verder onderzocht in hoofdstuk 3. Dit hoofdstuk laat zien dat de biokathode ook de omgekeerde reactie, van water naar zuurstof, kan katalyseren. In hoofdstuk 4 is de anode in de vlakke plaat PMBC opgedeeld in 30 kleine anodes om meer inzicht te krijgen in de locatie van stroomopwekking. De resultaten laten duidelijk zien dat de meeste stroom wordt opgewekt rondom de wortels van de planten en dat de PMBC dus geïnstalleerd moet worden op een plek met veel wortels.

Toepassing van de PMBC in watergebieden

Voor de toepassing van de PMBC is onderzoek gedaan naar twee verschillende watergebieden, een laagveenmoeras met riet en een slik met slijkgras. De planten en bodem zijn respectievelijk in de Alde Feanen en de Oosterschelde uitgegraven en in het lab geïnstalleerd. Hoofdstuk 5 laat zien dat het slik meer dan tien keer zoveel vermogen levert dan het moeras. Dit komt door de betere geleiding van het zoute water en de aanwezige sulfide wat ook geoxideerd wordt naast de organische stof van de planten. Berekeningen laten zien dat de PMBC theoretisch maximaal 0.52 W/m^2 kan opleveren. In hoofdstuk 6 is de buis PMBC met biokathode geïnstalleerd in de watergebieden. De anode is de buitenkant van de buis en is direct tussen de wortels geplaatst. De biokathode zit aan de binnenkant van de buis. Deze buis genereerde maximaal 0.082 W/m^2 . Een hoger vermogen kan genereerd worden als het design van de buis wordt verbeterd. Hoofdstuk 7 bediscussieert de toepassing van de PMBC in watergebieden. Wanneer de PMBC in alle watergebieden wereldwijd geïnstalleerd zou worden, dan zou daarmee maximaal 60 % van het elektriciteitsverbruik wereldwijd geproduceerd worden. In alleen de Oosterschelde kan de PMBC genoeg energie produceren voor een gemiddeld dorp. Echter de kosten van de buis PMBC moeten drastisch verlaagd worden om het rendabel te maken. De materiaalkosten moeten bijvoorbeeld verlaagd worden tot minder dan 1% van de huidige kosten voor een terugverdientijd van 50 jaar.

Introduction

Chapter 1

Towards application of the plant microbial fuel cell in wetlands

1.1 The search for clean, renewable and sustainable electricity

Currently, 67 % of the global electricity is produced by the combustion of fossil fuels, mainly coal and gas (figure 1)[1]. The combustion of fossil fuels results in a deteriorating change in atmospheric composition. Gaseous pollutants (e.g. SO_2 , NO_x , CO, ozone, volatile organic compounds), persistent organic pollutants (e.g. dioxins), heavy metals (e.g. lead, mercury) and particulate matter are emitted in the air. This air pollution can lead to a severe impact on human health and nature. Human health effects include among others cardiovascular and respiratory diseases, skin irritation, cancer, birth defect and reduced activity of the immune system [2]. The world health organization estimated that 3.7 million people died of outside air pollution in 2012 [3]. This estimate increases to 7 million deaths when including household air pollution due to the use of coal, wood or dung as the primary cooking fuel [3]. Air pollution from the combustion of fossil fuels also results in the greenhouse effect, stratospheric ozone depletion and acid rain [4].

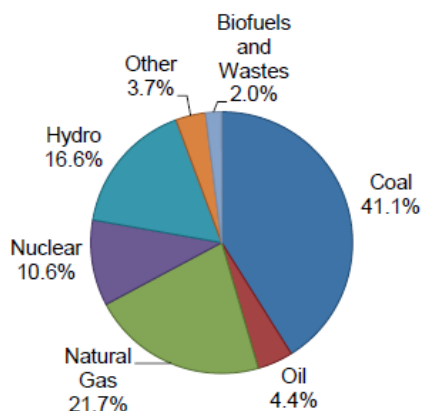
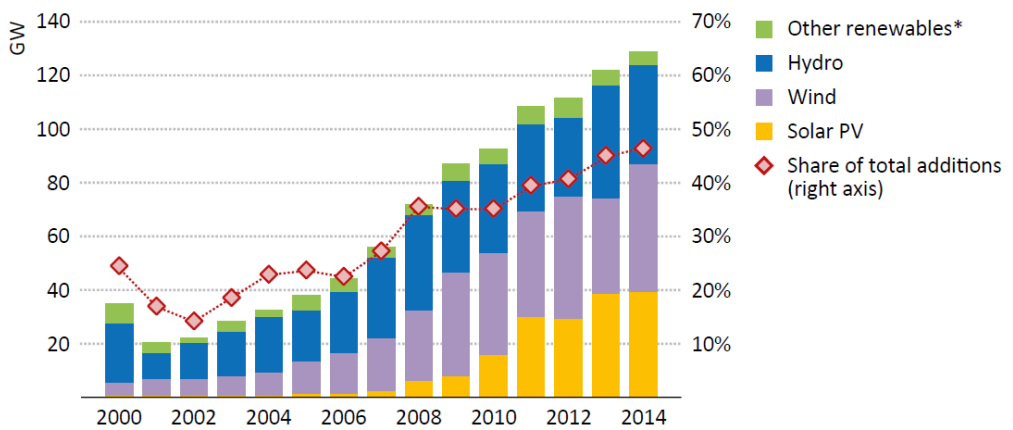


Figure 1: Fuel shares in worldwide electricity generation in 2013 [1]

The severe effects of the combustion of fossil fuels resulted in the development of renewable sources for electricity generation. Nowadays, 22 % of all the electricity in the world is generated by renewable sources mostly hydropower [1]. The market share of renewable electricity is increasing, currently more than 45 % of the power capacity additions are renewable (figure 2)[5]. Hydropower, wind energy and solar energy are the main renewable additions to the electricity market. Also the market share of bioenergy is increasing and is expected to

increase further [6]. However, bioenergy has also drawbacks like deforestation, competition with food production for arable land and emissions during combustion [7, 8].

This thesis is about the plant microbial fuel cell (PMFC) which is an emerging bioenergy technology producing electricity in situ from living plants without harvesting them [9]. PMFC is a sustainable technology because it is renewable, has clean conversions without net emissions and has no competition for arable land or nature [9]. Wetlands are the preferred location for large scale application of the PMFC [9].



* Includes geothermal, marine, bioenergy and concentrating solar power.

Figure 2: Global renewable power capacity additions by type and share of total capacity additions [5]

1.2 Plant microbial fuel cell (PMFC)

1.2.1 Principles of the PMFC

The PMFC, introduced in 2008, generates electricity by oxidizing rhizodeposits from living plants using naturally occurring processes (figure 3)[9-11]. Plants produce organic matter via photosynthesis using energy from sunlight and carbon dioxide. Part of the produced organic matter is released by the plants into the soil in the process called rhizodeposition. Electrochemically active microorganisms oxidize the organic rhizodeposits resulting in the production of electrons, protons and carbon dioxide. The oxidation of organic matter takes place at the anode of the PMFC resulting in the flow of electrons from the anode via

an external circuit to the cathode. At the external circuit the electricity is harvested. Simultaneously with the electrons flowing through the external circuit, ions, ideally the released protons, migrate from the anode to the cathode. At the cathode, the electrons and protons meet, reducing oxygen to water.

The PMFC generates electricity using endless cycles with self-repairing catalysts and plain water as the only product. Therefore, the PMFC is renewable and sustainable. Besides large scale application in wetlands, the PMFC can also be used for small scale application to power for example remote sensors. PMFC can also be integrated in green roofs or rice fields combining the advantages of a green roof or food production with electricity generation [10, 12].

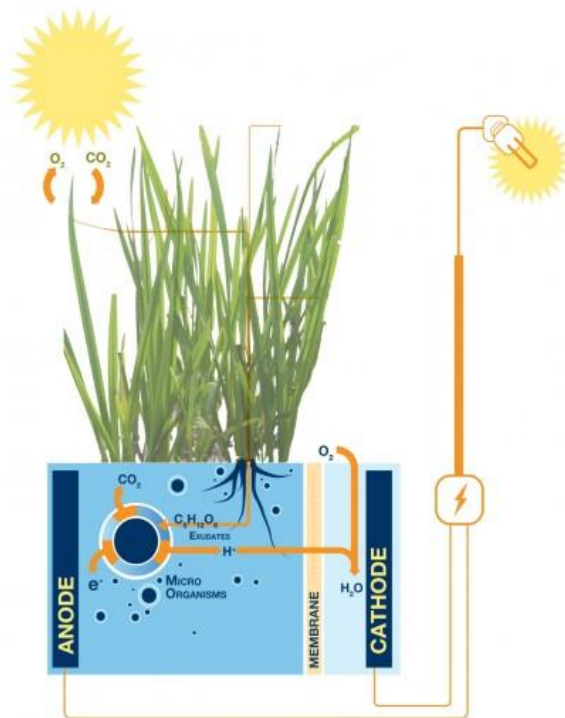


Figure 3: Schematic overview of the PMFC using organic rhizodeposits as fuel and electrochemically active micro-organisms as biocatalyst [9]

1.2.2 Plants release organic matter into the soil

The rhizodeposition of plants is 20 to 60 % of the net fixed carbon [13]. Rhizodeposition can be grouped into two categories, lysates and exudates (figure 4). Lysates are the damaged and sloughed off root cells

of plants and contain up to 30 % of the net fixed carbon [13]. Exudates are the organic matter released by living roots via three different processes, diffusates, secretions and excretions (figure 4). 5-20 % of the net fixed carbon is released into the soil via exudation [13]. More organic matter can be deposited into the soil via controlled release of root border cells. These cells are for example released by the plants because of a pathogen attack or protection against heavy metal toxicity [14]. All organic rhizodeposits, from low molecular weight (LMW) exudates to complete roots, are potential substrates for the microorganisms in the PMFC. Electricity generation is shown with several different plants. The first PMFC experiment was performed with *Glyceria maxima* [9]. Afterwards among others *Spartina anglica*, *Arundinella anomala*, *Arundo donax*, *Canna indica* and *Oryza sativa* (rice) have been studied [11, 15, 16].

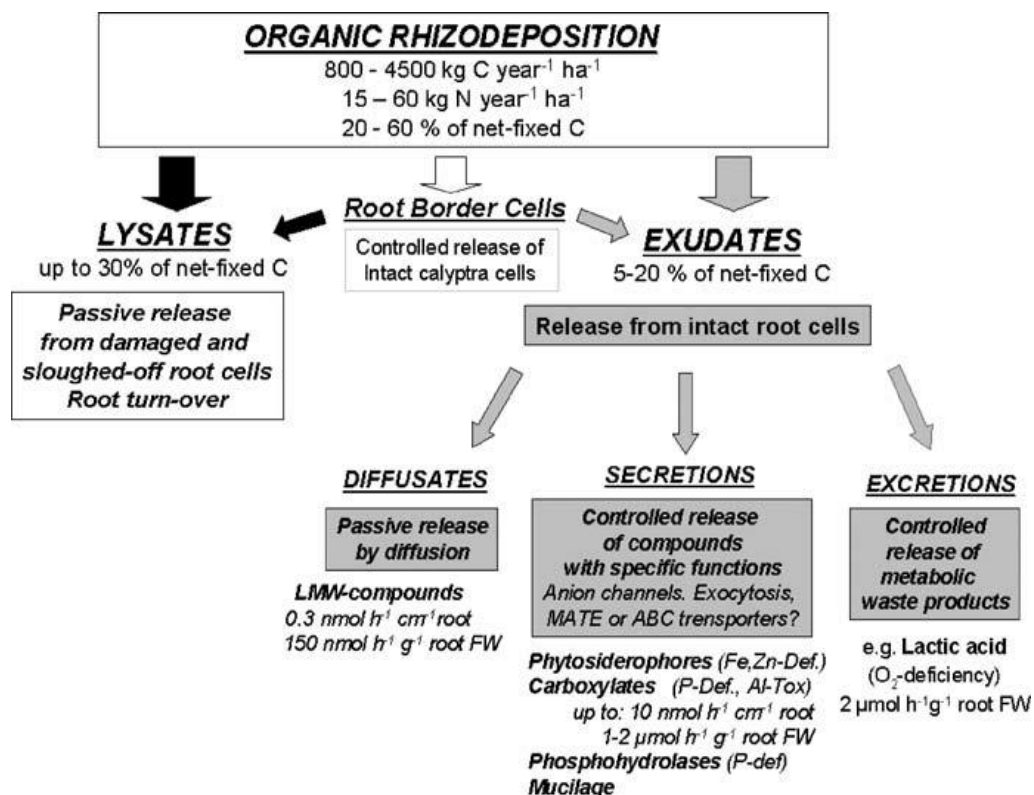


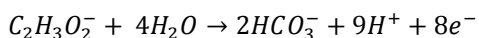
Figure 4: Categorization and release mechanisms of organic rhizodeposition [13]

1.2.3 Electrochemically active bacteria oxidize the organic matter

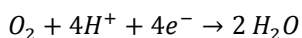
Electrochemically active bacteria (EAB) naturally present in the anode of the PMFC oxidize the organic matter released by the plants [9]. A large diversity of organic matter is available from LMW exudates to more complex organics as cellulose. The complex organics are not suitable for most known EAB, although EAB exist who are also able to oxidize complex organics directly [17]. Consequently, the complex organics in PMFC are first hydrolyzed to LMW organics as acetate [17]. Several studies showed the presence of EAB in the anode of the PMFC [10, 16, 18, 19]. Also microorganisms which are able to hydrolyze cellulose were enriched in the anode of the PMFC [18]. The LMW organics, directly from the exudates of the plants or via hydrolysis, are oxidized by the EAB and are thus the source of electrons in the PMFC. The formation of a biofilm on the anode surface is required for an efficient transfer of electrons to the anode [20]. The EAB are able to transfer the electrons directly via their outer cytochrome electron transfer chains or mediated using natural redox mediators [21].

1.2.4 Oxidation and reduction reaction in the PMFC

Acetate oxidation is the model substrate in PMFC research, because acetate was the main organic found in a rice PMFC [10]. Adding extra acetate resulted in higher power output showing that the EAB in the PMFC were able to oxidize acetate [10]. The microbial oxidation reaction of acetate is:



The theoretical potential of acetate oxidation in PMFC conditions is -0.289 V vs NHE (i.e. -0.499 V vs Ag/AgCl), assuming 0.05 M of acetate and bicarbonate, a neutral pH (10^{-7} M) and a temperature of 298 K [22]. The released electrons from the oxidation of organic matter in the anode flow through an external circuit to the cathode. At the cathode the electrons are reduced, preferably by reduction of oxygen to water:



Oxygen is preferred, because it has a high redox potential, is widely available and the only product is plain water [22]. In PMFC conditions,

the theoretical potential of oxygen reduction is 0.805 V vs NHE (i.e. 0.595 V vs Ag/AgCl), assuming a partial oxygen pressure of 0.2 bar, a neutral pH (10^{-7} M) and a temperature of 298 K [22].

The maximum theoretical cell voltage of the PMFC is 1.094 V assuming acetate oxidation at the anode and oxygen reduction at the cathode. The measured voltage is always lower due to voltage losses at the anode, cathode and membrane. Voltage losses occur due to activation energy, microbial energy for maintenance and growth, ohmic losses [23], alternative electron donors and acceptors [24], ionic losses, transport losses and a possible pH gradient [25]. The main voltage loss in the PMFC is related to the high activation energy of oxygen reduction [26].

1.3 Application of the PMFC in wetlands

1.3.1 Wetlands

PMFC technology requires anaerobic and waterlogged conditions to facilitate the transport of ions between the anode and the cathode and to avoid oxygen intrusion from the air into the anode [9]. For large scale application of PMFC, an enormous area of waterlogged and anaerobic land is required. Wetlands are therefore the ideal natural area for PMFC technology as wetlands are transitional lands between terrestrial and aquatic systems with the water level slightly above, at or near the surface [27] covering 8-10 million square kilometers of land worldwide [28]. The enormous land coverage shows the potential of PMFC application in wetlands. As a result, wetlands can be considered as a new source of electricity.

Wetlands have multiple values and functions. This includes natural biological functions as food chain productivity and habitat, shoreline protection, groundwater recharge, flood and storm water storage, water quality improvement and cultural values [29]. Generating electricity from wetlands by the PMFC gives a potential extra function to wetlands and likely increases the value of wetlands. This may result in a better protection of the wetlands and therefore conservation of the other functions and values.

Six types of wetlands can be classified based on the water regime and nutrient supply: bogs, fens, swamps, temporary lakes, marshes and permanent lakes (figure 5)[30]. The differences in water regime and nutrient supply results in different vegetation and sediments (figure 5).

The water regime, nutrient supply, vegetation and sediment likely all influence the potential power generation of a specific wetland. Next to that, the power generation of the PMFC is also affected by location specific conditions as climate (e.g. solar radiation and temperature) and salinity [15, 31]. The power generation of the different wetlands likely varies and the wetlands suitable for large scale electricity generation should be identified.

A maximum power generation of 1.6 W m^{-2} plant growth area (PGA) was envisaged for the application of PMFC in Western Europe [32]. This value is based on the best known efficiencies of the main processes, with an average solar radiation of 150 W m^{-2} in Western Europe, increased photosynthetic efficiency of 5 %, rhizodeposition of 70 % of the photosynthates and an energy recovery of 60 % in the PMFC. This results in a power generation of 3.2 W m^{-2} PGA for optimized lab PMFCs. In natural conditions 50 % energy harvest is expected (1.6 W m^{-2})[32]. The highest recorded long term power generation (two week average) of PMFC technology before the start of this research in 2012 was 155 mW m^{-2} PGA in the lab [33].

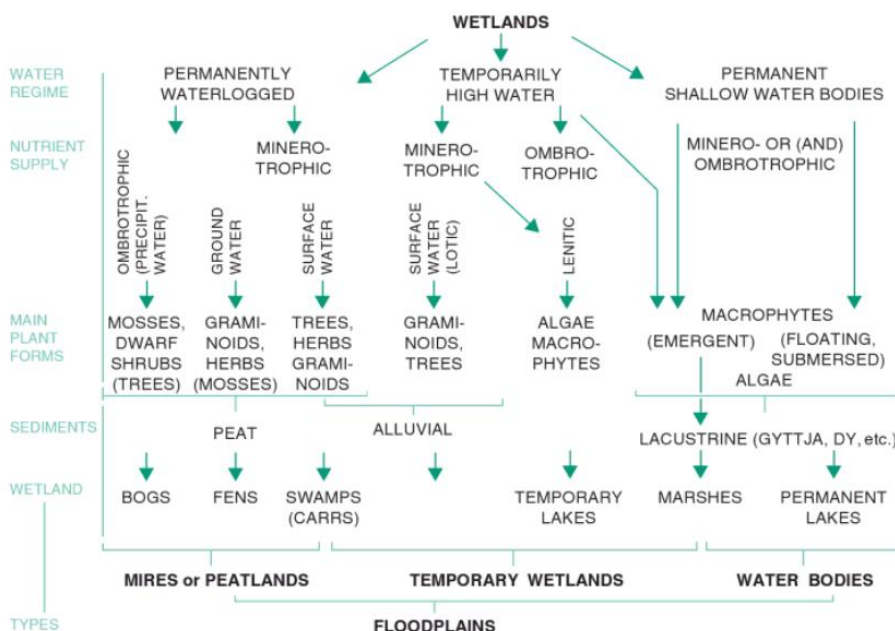


Figure 5: Wetland types in relation to water regime and nutrients supply and the resulting differences in vegetation and sediments [30]

1.3.2 More electron donors present in wetlands besides rhizodeposits

PMFC research is mainly about oxidizing the rhizodeposits of plants in lab scale systems, focusing on for example different plant species and growth medium [15, 33]. In wetlands, also alternative electron donors are present next to the rhizodeposits. These alternative electron donors are located in the sediment or are supplied by an external flux, for example a river. Among others sulfide, organic fertilizer [34], biodegradable dissolved organic matter and dead biomass (e.g. phytoplankton, algae, seeds) are possible alternative electron donors in wetlands. Power can be generated by oxidizing these alternative electron donors in the sediment [35]. These so called sediment microbial fuel cells (SMFC) have been applied among others in marine and river sediments [36-38]. Application of the PMFC in wetlands combines the PMFC with the SMFC oxidizing both the rhizodeposits and electron donors in the sediment and is therefore expected to generate more power than SMFCs and lab scale PMFCs separately. PMFC applied in wetlands is not only beneficial because of the power generation, but the PMFC also reduce the methane emission of the wetlands. The electrochemically active microorganisms outcompete the methanogens for electron donors which results in lower greenhouse gas emissions from the wetlands [39].

1.3.3 Cathode improvement necessary before applying PMFC in wetlands

The main limitation of the PMFC is the cathode. The reduction of oxygen at the cathode is slow [26] and limits the current and power generation of the PMFC [31]. As a result, the oxygen reducing cathode is often replaced by a chemical ferricyanide cathode in PMFC research [40]. The reduction of ferricyanide is irreversible within the PMFC, resulting in the depletion of ferricyanide. The ferricyanide cathode is thus not sustainable and can therefore not be used for application of the PMFC in wetlands. The sustainable oxygen reducing cathode has to be catalyzed to overcome the limitation of the cathode in the PMFC. Platinum is the best know catalyst for oxygen reduction in microbial fuel cells [41]. The high costs of platinum prohibit the commercial use in PMFC. Oxygen reduction can also be catalyzed by microorganisms [42]. Microorganisms are cheap and self-replenishing and are as such very promising for application within the PMFC [43]. Biocathodes are able to reach a

current density of 0.9 A m^{-2} on solid plate graphite cathodes at a fixed cathode potential of 150 mV vs Ag/AgCl. Up to date, the maximum current generation of PMFC is lower, 0.4 A m^{-2} PGA [33]. This indicates that the oxygen reducing biocathode can also be integrated in the PMFC without decreasing the current generation of the PMFC. Oxygen reducing biocathodes are successfully started in large variety of locations, among others in seawater, rice fields and marine and river SMFC [42, 44, 45]. Therefore, it is expected that the oxygen reducing biocathode can also be successfully integrated in natural wetlands were PMFC could be applied.

1.3.4 Wetlands investigated in this thesis

In this thesis, a *Phragmites australis* dominated fen peat soil and a *Spartina anglica* dominated salt marsh are investigated (figure 6). Both wetlands are selected because of their abundant occurrence in the Netherlands [46]. *Phragmites australis* is a common large perennial grass spread out in temperate and tropical zones [46]. *Spartina anglica* is also a common perennial grass. *Spartina anglica* was introduced for coastal protection and is now an invasive species found in coastlines all over the world (e.g. Western Europe, Australia, East Asia and North America) [46]. Both wetlands were dug out in the natural location in the Netherlands and installed in our lab (Plant growth lab, Environmental Technology, Wageningen University). Each lab wetland covers an area of 1.2 by 1.0 m and were 1.0 m in depth and consists of the local plants, sediment and water. The *Phragmites australis* dominated peat soil was collected in national park Alde Feanen in the north of the Netherlands (GPS coordinates N53°.131038 E005°.934536). The *Spartina anglica* dominated salt marsh was collected in the Oosterschelde, a tidal basin in the southwest of the Netherlands (GPS coordinates N51°.446788, E004°.093096).

1.3.5 Designing the wetland PMFC using tubular systems

One of the most important aspects in the PMFC research is the cell design, as the power output can be significantly increased by an improved design [47]. Currently most PMFC research focused on lab scale PMFCs. The design of the first PMFC experiment was a vertical glass tube anode placed in a beaker where the cathode was located [9].



Figure 6: Wetlands investigated in this thesis. Left: Phragmites australis dominated peat soil. Right: Spartina anglica dominated salt marsh. Pictures are taken from the location where the plants were collected (date photo respectively 20/8/13 and 3/5/14)

The design was improved to a flat plate design to minimize the distance between the anode and cathode and as such decrease the transport losses [47]. Both the beaker and the flat plate are not suitable for application in wetlands. PMFC in wetlands should be invisibly applied without excavation of the top soil. Therefore, a tubular PMFC was developed [48]. This tubular design consisted from inside to outside of a cathode, membrane and anode. The anode is placed directly in between the roots. This design has to be improved as a chemical cathode was used with energy intensive pumping of the catholyte [48]. The chemical cathode should be replaced by an oxygen reducing biocathode and oxygen should passively diffuse into the cathode to circumvent extensive energy input for aeration [49].

1.4 Objective of this thesis

The objective of this thesis is to apply PMFCs in wetlands with a sustainable biocathode. The first step is to integrate the biocathode in a lab scale PMFC. After successful integration, the biocathode PMFC is installed in wetlands using an improved tubular design.

1.5 Thesis outline

Chapter 2, 3 and 4 of this thesis focus on lab scale systems, while chapter 5,6 and 7 focus on application of the PMFC in wetlands. In chapter 2, the chemical ferricyanide cathode of the PMFC is replaced by a biological oxygen reducing cathode making PMFC a completely sustainable biotechnology. The new cathode even resulted in a long term record power output of 240 mW m^{-2} PGA. The new record was mainly reached due to the high redox potential of oxygen reduction. The cathode potential was 127 mV higher in the PMFC with a biocathode than the PMFC with a ferricyanide cathode due to effectively catalyzed oxygen reduction by microorganisms.

Chapter 3 only focuses on the biocathode and not on the PMFC. In this chapter, the reversibility of the biocathode is shown, catalyzing water oxidation instead of oxygen reduction. The water oxidation reaction is currently hampering development of large scale water oxidation technologies to reduce protons to hydrogen or carbon dioxide to hydrocarbons. Operating the biocathode as a bioanode resulted in an increased current density and higher oxygen evolution compared to a blank anode.

Chapter 4 clarifies the differences in current generation at different widths and depths in the anode of a lab scale PMFC. Current generation is explained by rhizodeposition, radial oxygen loss and electron transfer mechanisms. Root density is clearly linked to current generation and both living and dead roots are likely able to generate electricity. Also oxygen was measured at the plant roots which likely resulted in internal currents. Electrons were likely transferred in the anode via mediators, which were excreted by the plants or microorganisms. The results show that the PMFC should be applied at locations with a high root density.

Chapter 5 is the first chapter about the application of the PMFC in wetlands. The objective of this research is to clarify the differences in electricity generation between a salt marsh and a peat soil based on experimental data and theoretical calculations. PMFCs applied in a salt marsh generated 10 times more power than similar PMFCs applied in a peat soil. The salt marsh reached a volumetric long term record power output of 2.9 W m^{-3} anode (18 mW m^{-2} PGA). The top layer of the salt marsh generated most power due to the presence of the plants and tidal advection. Theoretical calculation showed that PMFC technology is

potentially able to generate a power density up to $0.52 \text{ W m}^{-2} \text{ PGA}$, which is clearly lower than the earlier envisioned $3.2 \text{ W m}^{-2} \text{ PGA}$.

In chapter 6, PMFCs with oxygen reducing biocathodes were applied in the salt marsh and peat soil using a tubular design which can be integrated invisibly in the wetlands. Oxygen was passively supplied to the cathode via a gas diffusion layer. The start-up of the biocathodes was successful for the peat soil PMFCs. The maximum daily average power generation of the best peat soil PMFC was $22 \text{ mW m}^{-2} \text{ PGA}$. In the salt marsh, the biocathodes only started with pure oxygen diffusion instead of air diffusion, reaching a maximum daily average power generation of $82 \text{ mW m}^{-2} \text{ PGA}$. The design of the PMFCs in both wetlands has to be improved to further increase the power mainly by limiting the crossover of electron donors from the anode to the cathode and electron acceptors from the cathode to the anode.

In the final chapter, Chapter 7, the application of PMFC in wetlands is assessed in relation to power output and design. The worldwide potential electricity generation of the PMFC is estimated. Next, the tubular PMFC design is evaluated including materials, size and maximum costs for economic feasibility. Also, application of the PMFC in the two selected wetlands is analyzed with possible local uses for the generated electricity. Finally, potential applications and possible benefits besides electricity generation to increase the value of the PMFC are discussed.

Part one:

Lab scale experiments

Chapter 2

Electricity generation by a plant microbial fuel cell with an integrated oxygen reducing biocathode

Abstract

In this study we show that a chemical ferricyanide cathode can be replaced by a biological oxygen reducing cathode in a plant microbial fuel cell (PMFC) with a new record power output. A biocathode was successfully integrated in a PMFC and operated for 151 days. Plants growth continued and the power density increased reaching a maximum power output of 679 mW m^{-2} plant growth area (PGA) in a 10 minutes polarization. The two week record average power densities was 240 mW m^{-2} PGA. The new records were reached due to the high redox potential of oxygen reduction which was effectively catalyzed by microorganisms in the cathode. This resulted in a 127 mV higher cathode potential of the PMFC with a biocathode than a PMFC with a ferricyanide cathode. We also found that substrate availability in the anode likely limits the current generation. This work is crucial for PMFC application as it shows that PMFC can be a completely sustainable biotechnology with an improved power output.

Keywords

Plant microbial fuel cell, oxygen reducing biocathode, *Spartina anglica*, bio-electricity

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2.1 Introduction

The threat of climate change, the depletion of fossil fuels, environmental pollution and the growing energy demand increase the urgency for new sustainable and reliable energy sources [50]. Several solar, hydro, wind and bio-energy technologies are already implemented and common in day-to-day life. The market share of bioenergy, such as bioethanol, bioelectricity and biodiesel is increasing [51]. However, bioenergy is not always sustainable. Deforestation and competition with food production for arable land are two occurring disadvantages [7]. The plant microbial fuel cell (PMFC) is an emerging technology which can produce electricity via living plants. PMFC is sustainable because it is renewable, has a clean conversion without emissions and has no competition for arable land or nature [9]. In the PMFC, plants grow in the anode where rhizodeposits are the substrates oxidized by electrochemically active bacteria to generate electricity. Natural wetlands offer a new electricity source in which PMFC can be integrated without extensive excavation of the soil [48]. PMFCs can also be implemented in rice paddy fields combining food and electricity production and so circumventing the competition with food production [10, 11]. In addition, PMFC can be integrated in green roofs, combining the advantageous of green roofs (e.g. insolation, biodiversity) and electricity generation [12]. Even though PMFC is based on photosynthesis, it is expected to deliver electricity 24 hours per day and year-round in case of suitable conditions (e.g. temperature and plant growth) [31]. The theoretical maximum electricity output of a PMFC is 3.2 W m^{-2} plant growth area (PGA) [32], currently a long term performance of 0.155 W m^{-2} PGA is reached [33].

For research purpose often ferricyanide is used in PMFC as final electron acceptor. Ferricyanide is unsustainable as it reacts to ferrocyanide and is not able to react back to ferricyanide within the PMFC. The reduction of ferric- to ferrocyanide is fast resulting in a minimal resistance of the cathode [52]. Ferricyanide is therefore suitable to investigate the limitations of the anode. For sustainable electricity production, ferricyanide cannot be used as final electron acceptor. Oxygen is preferred because it has a high redox potential, is widely available and the product is plain water [22]. Graphite is often used as cathode material in a PMFC. However, the reduction of oxygen on graphite is slow [26] and limits the power output of the PMFC [31]. Electrocatalysts like platinum are able to catalyze the reduction of oxygen. The high costs and the potential poisoning compounds in the

solution make platinum undesired to be applied in the PMFC [53]. The reduction of oxygen can also be catalyzed with mixed culture microorganisms [42]. Microorganisms are cheap, self-replenishing and can be used in combination with the graphite electrodes [43]. Mixed culture microorganisms are able to catalyze the reduction of oxygen by growing a biofilm on graphite electrodes and are able to reach a stable current density of 0.55 A m^{-2} projected electrode surface area (non-porous electrode) at pH 7, 31 °C and a controlled cathode potential of 150 mV vs Ag/AgCl [54].

Oxygen reduction can also be indirectly catalyzed by microorganisms for example by ferrous iron oxidizing microorganisms (*Acidithiobacillus ferrooxidans*). On the electrode surface ferric iron is chemically reduced to ferrous iron. The microorganisms in the catholyte oxidize the ferrous iron back to ferric iron to maintain the concentration of ferric iron in the cathode. Higher current densities (4.4 A m^{-2} projected electrode surface area; porous electrode) are reached with ferric iron reduction. A bipolar membrane is required between the anode and the cathode to maintain the solubility of iron in the cathode (pH < 2.5). [55]

The capacity of catalyzing microorganisms in the cathode of the PMFC in the range $0.55 - 4.4 \text{ A m}^{-2}$ projected surface area exceeds the long term average current density of the best performing PMFC (0.38 A m^{-2} PGA) [33]. Also the potential of oxygen reduction (+0.60 V vs Ag/AgCl (pH 7)) and ferric iron reduction (+0.56 V vs Ag/AgCl) are higher than the potential of ferricyanide reduction (+0.20 V vs Ag/AgCl at pH 7 with 20 mM phosphate buffer) [56].

Replacing the ferricyanide reduction by a biocathode in the PMFC is a crucial step in the development of the technology towards its application. A biocathode in PMFC will make PMFC a sustainable biotechnology without decreasing the current and power output compared to a ferricyanide cathode. Oxygen reducing biocathodes are already combined with a bioanode in sediment microbial fuel cells [57] and single chamber microbial fuel cells [58].

The objective of this study is to show that a ferricyanide cathode can be replaced by an oxygen reducing biocathode in a PMFC without decreasing the power output. This study combines a bioanode and biocathode by integrating an oxygen reducing biocathode in a PMFC. First, the PMFC with a chemical oxygen reducing cathode was started and studied. Afterwards, the cathode was inoculated with microorganisms and an oxygen reducing biocathode was developed. The

biocathode included, besides the microorganisms which catalyzed the oxygen reduction, also phototrophic algae which increased the oxygen concentration in the cathode during illumination. An adaptive control strategy was applied and long term performances and limitations of the biocathode PMFC were revealed.

2.2 Material and methods

2.2.1 Experimental setup

For the experiment a flat porous plate PMFC was constructed using transparent plastic plates (figure 1). The PMFC had one anode compartment with a size of 190 x 30 x 190 mm (l x w x h) and a PGA of 27 cm² (160 x 17 mm). Two cathode compartments of each 190 x 10 x 190 mm were connected to both flat sides of the anode. The anode and cathode were separated by a BPM (fumasep FBM, FuMa-tech GmbH, St. Ingbert, Germany) to maintain the pH gradient between the anode and cathode [55]. The anode was made from three layers of graphite felt (190 x 30 x 50 mm) by stacking several layers of felt (190 x 10 x 50 mm) (10 mm Grade WDF, National Specialty Products Carbon and Graphite Felt, Taiwan) [47]. The three layers were physically separated with plastic rings (D=10 mm) to create a top, middle and bottom anode section. Each section of the anode and both cathode were connected with a golden wire as current collector. In this experiment, the differences in current distribution between the three layers was not analyzed and the three layers were therefore electrically connected together during the entire experiment. Also the cathodes were made of graphite felt. Unlike the anode, the cathodes consisted only of one layer of graphite felt (3 mm, Grade WDF, National Specialty Products Carbon and Graphite Felt, Taiwan).

In the anode the salt water grass species *Spartina anglica* was planted. This grass species was also used in previous PMFC researches [33, 40, 47]. The plants were collected at the Dutch coast one week before the experiment was started at the same location as was used in earlier PMFC research (GPS coordinates N51°.67654 E004°.13656) [40]. In the PMFCs, several stems of *Spartina anglica* were planted with a total weight of 55 grams. Also 86 grams of dead roots were added to the anode of the PMFC which can accelerate the start-up, due to the availability of hydrolysable organic matter.

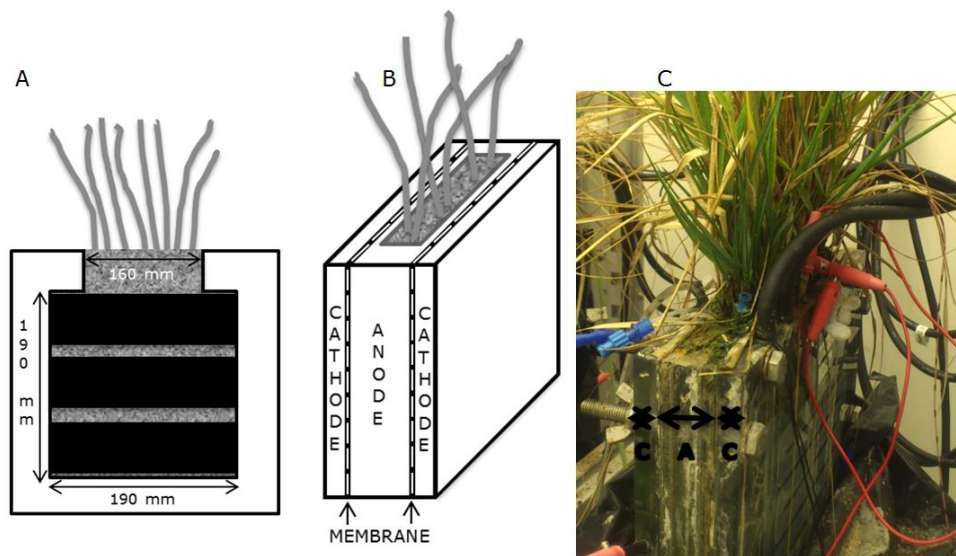


Figure 1: Schematic overview of a cross section of the anode (A), the position of the electrodes in the PMFC (B) and a picture of PMFC (C)

2.2.2 Operation

On day 1 of the experiment the plants were planted in the anode. The plants were constantly kept waterlogged by pumping a nitrate-less, ammonium-rich plant growth medium from a storage tank into the anode (Gilson minipuls 3, Den Haag, The Netherlands, 1 rpm, up to 300 ml day⁻¹). The used medium showed the best performance [33]. The medium was constantly flushed with nitrogen and included micronutrients [40] and 5 g L⁻¹ NaCl. The PMFC was operated in a climate chamber at a temperature of 20 °C and a humidity of 70 % (Microclima 1750, Snijders Scientific, Tilburg, The Netherlands). The light and dark ratio was 14:10 hours and the average PAR light intensity during illumination was $373 \pm 125 \mu\text{mole m}^{-2}\text{s}^{-1}$ ($82 \pm 28 \text{ W.m}^{-2}$) measured at the top, middle and bottom height of the leaves with a light meter (Li-250A, Li-cor, Lincoln, USA). The light intensity on the cathode was $37 \pm 10 \mu\text{mole m}^{-2}\text{s}^{-1}$ ($8 \pm 2 \text{ W.m}^{-2}$).

The PMFC was started with a mixed culture oxygen reducing biocathode and was inoculated with aerobic wastewater from the treatment plant in Bennekom, the Netherlands on day 26 and again on day 182. Aerobic wastewater contains among others bacteria, fungi, algae and protozoa [59]. On day 182 also catholyte from a running

oxygen reducing biocathode at pH 7 was added [54]. The mixed culture biocathode was started at pH 7 (± 0.5) by manually adjusting the pH with NaOH and HCl. The pH was only adjusted to pH 7 until the biocathode started up. The catholyte consisted of phosphate buffer (0.02 mol L^{-1}), macro- and micronutrients [60] and vitamins [55]. The catholyte was constantly recirculated via a one liter bottle through both cathodes (Watson-Marlow 505S, Rotterdam, The Netherlands, 30 rpm, 150 ml min^{-1}) and kept air saturated with pressurized air. From day 292 to day 323 the recirculation and aeration of the cathode was stopped to investigate the oxygenic phototrophic effect in the cathode. On day 323 the recirculation and aeration was started again.

The PMFC was controlled with an external resistance of 1000Ω between the anode and cathode from day 1 until day 182. From day 182 until day 323 the cathodes were controlled with a potentiostat (Vertex, Ivium Technologies, Eindhoven, The Netherlands). The cathodes were controlled with a three electrode set up in which the cathode was the working electrode, the anode the counter electrode and a 3M KCl Ag/AgCl reference electrode in the catholyte the reference electrode. All cathode potentials in this paper are therefore given in mV vs Ag/AgCl. First, the cathodes were controlled at a cathode potential of 150 mV (day 182 – day 238). 150 mV was selected as that resulted in successful start-up [61]. Afterwards, the cathode potential was stepwise increased to 235 mV (day 238 – day 254), to 265 mV (day 254 – day 268), to 285 mV (day 268 – day 292) and to 335 mV (day 292 – day 323). From day 323 the cell voltage was controlled in a two electrode set up at 600 mV until the end of the experiment on day 336.

2.2.3 Measurements

The cell voltage and the anode, cathode and membrane potential were measured and logged similar as in previous PMFC research [33]. The catholyte pH was measured with a pH meter (Liquisys M, Endress+Hauser, Reinach, Switzerland). Oxygen concentration was measured with an oxygen meter (HQ40d, Hach, Düsseldorf, Germany). The presence of microorganisms was confirmed using a light microscope (Eclipse E400, Nikon, Japan).

Chrono amperometry measurements with the potentiostat were used to characterize the long term performance of the PMFC with a three electrode set up, in which the cathode potential was controlled and the current was measured (Vertex, Ivium Technologies, Eindhoven, The

Netherlands). The PMFC and biocathode were characterized with polarization measurements. These polarizations were performed in a two electrode set-up to control the cell voltage. The cell was first set to open cell for 600 seconds. Afterwards the cell voltage was controlled by decreasing the cell voltage from the open cell voltage to a cell voltage of 1 mV and increasing the cell voltage back to the open cell voltage in steps of 100 mV of each 600 seconds. In the end the cell was set to open cell again for 600 seconds. The last measurement of each step (i.e. the last second) is shown in the results.

2.2.4 Calculations

The total resistance of the PMFC (R_{int}) was calculated by adding the resistance of the anode (R_{an}), membrane (R_m) and cathode (R_{cat}) together. The separate resistances were calculated based on the current (I), the measured potential (E) and the theoretical potential (E^{th}) of the anode (eq 1), cathode (eq 2) and membrane (eq 3). The theoretical anode potential is estimated at -0.43 V vs Ag/AgCl which is the theoretical potential of acetate at pH 6 (50 mM acetate pH 7: -0.50 V vs Ag/AgCl; 65 mV voltage drop per pH unit based on Nernst equation). The theoretical cathode potential is the potential of oxygen reduction (0.60 V vs Ag/AgCl, pH 7). The theoretical membrane potential is assumed to be 0 mV.

$$R_{an} = \frac{E_{an} - E_{an}^{th}}{I} \quad (\text{eq 1})$$

$$R_{cat} = \frac{E_{cat}^{th} - E_{cat}}{I} \quad (\text{eq 2})$$

$$R_m = \frac{E_m - E_m^{th}}{I} \quad (\text{eq 3})$$

2.3 Results and discussion

2.3.1 Successful integration of the biocathode in the PMFC

The inoculation with microorganisms on day 26 did not result in a successful start-up of the PMFC (data not shown). Apparently, by applying an external resistance of 1000 Ω it was not possible to develop a biocathode. Possibly due to the unstable cathode potential (data not

shown). The second inoculation (day 182) together with the controlled cathode potential of 150 mV resulted in a successful start-up of the mixed culture PMFC (figure 2). Three days after the second inoculation, the PMFC started to produce a positive current and electric power at a cathode potential of 150 mV.

Controlling the cathode at 150 mV in flat plate MFCs is known to be effective to start-up mixed culture biocathodes in MFCs [61]. The current increased the first days to a daily average current density $0.12 \text{ A m}^{-2} \text{ PGA}$ on day 192. The average anode potential was -300 mV on day 192, resulting in a daily average power density of $54 \text{ mW m}^{-2} \text{ PGA}$. Between day 185 and day 192 the cathode resistance decreased and also the anode resistance decreased (figure 3). The performance of the PMFC improved when the oxygen reducing biocathode was integrated as the current and power density increased while the resistance decreased. During the start-up the pH was controlled at pH 7 (+/- 0.5). Afterwards, the pH of the catholyte stabilized at a pH of 6.5 (+/- 0.5) without adjusting the pH. A pH of 6.5 is suitable for an oxygen reducing biocathode [54].

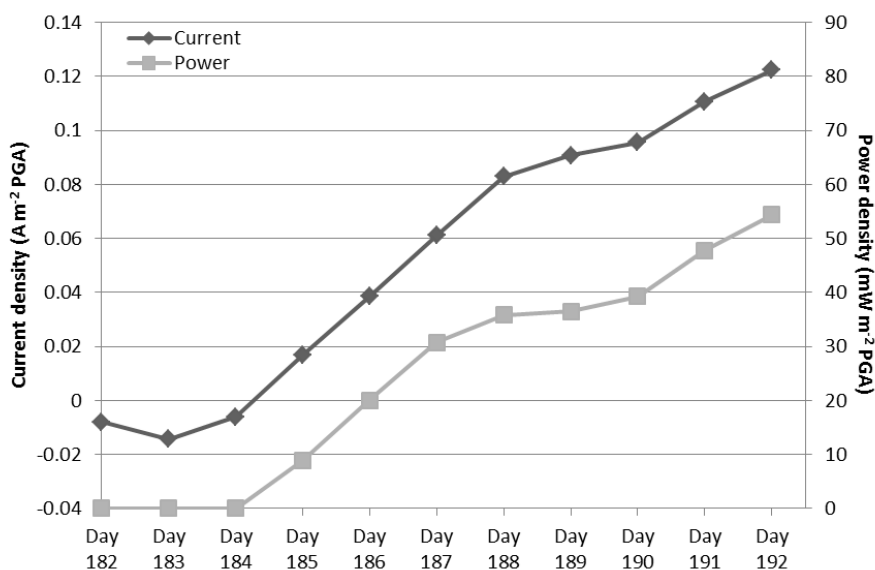


Figure 2: Increase in average daily current and power density of the PMFC after the second inoculation (day 182) of the biocathode at a potentiostatic controlled cathode potential of 150 mV

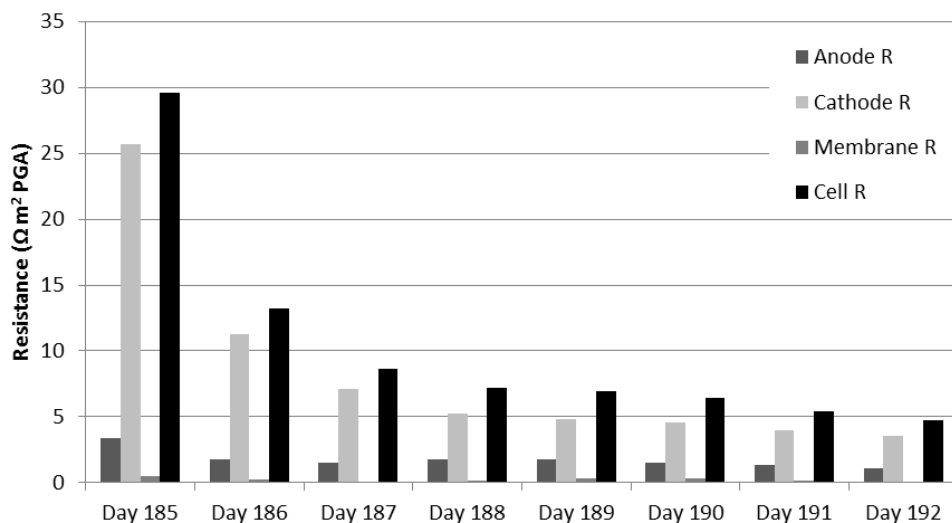


Figure 3: Anode, cathode, membrane and cell resistance during the start-up of the biocathode of the mixed culture PMFC. Both the anode and cathode resistance decreased when the oxygen reduction was improved by the biocathode

The addition of microorganisms increased the onset potential of the cathode (i.e. OCP) and decreased the cathode resistance (figure 4). Light microscopy confirmed the presence of microorganisms, likely bacteria and green algae were detected on the cathode. The onset potential increased from 97 mV during the polarization without microorganisms, to 243 mV on day 195, to 274 mV on day 252 and to 376 mV on day 328. A higher onset potential of the cathode indicates that the catalytic activity of the microorganisms increased [62]. A lower cathode resistance most likely resulted in an higher voltage efficiency. More current is able to flow at the same cathode potential which results in an higher power density.

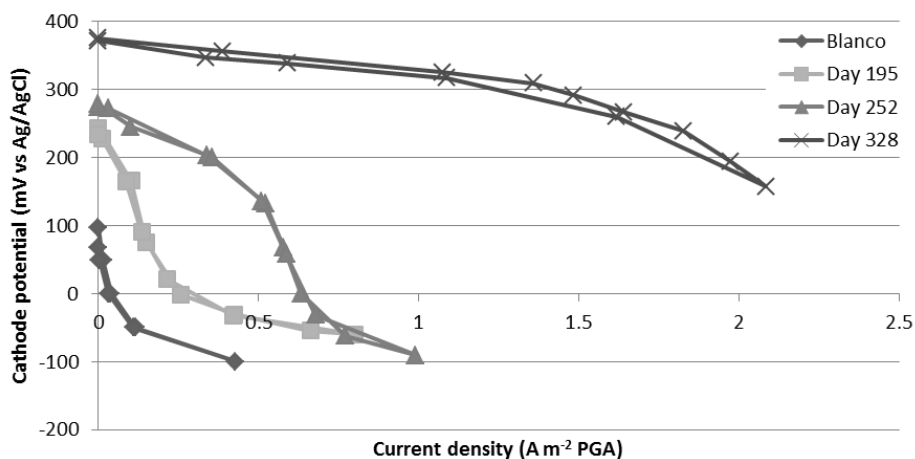


Figure 4: Cathode potential and current during PMFC polarizations. The biocathode increased the onset potential and decreased the resistance of the cathode over time

The highest average daily power density was $367 \text{ mW m}^{-2} \text{ PGA}$ (day 327)(see supporting information). The long term average power density was $240 \text{ mW m}^{-2} \text{ PGA}$ from day 323 to day 336 (two weeks), which is higher than the long term average power density of the best performing PMFC with the same plant species and bioanode configuration, with a ferricyanide cathode ($155 \text{ mW m}^{-2} \text{ PGA}$) [33]. The power density of the PMFC with a biocathode is 54% higher than of the PMFC with a ferricyanide cathode. The current density of the biocathode PMFCs is only 3 % higher ($0.397 \text{ A m}^{-2} \text{ PGA}$ for the PMFC with the biocathode and $0.384 \text{ A m}^{-2} \text{ PGA}$ for the PMFC with a ferricyanide cathode), indicating that the higher power output is mainly due to the higher cell voltage. The cell voltage of the PMFC with biocathode was controlled at 600 mV, while the cell voltage of the PMFC with a ferricyanide cathode was 404 mV. The higher redox potential of oxygen reduction compared to ferricyanide reduction allows an higher cathode potential. However, oxygen reduction on graphite results in high voltage losses. The presence of catalytic microorganisms reduce these voltage losses to a cathode potential higher than a ferricyanide cathode. Therefore, the biocathode PMFC was able to operate at a higher cathode potential than the ferricyanide PMFC.

Ferricyanide reduction under the conditions of the ferricyanide PMFC (i.e. pH 7 with 20 mM phosphate buffer) occurs at a potential of +200 mV vs Ag/AgCl [56]. Also other PMFCs, which were operated with ferricyanide cathodes reached a cathode potential of approximately

+200 mV [33, 52]. The biocathode PMFC operated for 44 days at a cathode potential of more than +300 mV. The considerable time and significant higher cathode potential (>100mV) than theoretical possible with ferricyanide reduction proves that the biocathode PMFC is able to operate at a higher cathode potential for a long time and thus able to produce more energy. The long term power and current densities (two week average) in the biocathode PMFC were achieved at an average cathode potential of 327 mV (sd 25 mV). The increase in cathode potential (127 mV) is the main contribution (65 %) to the higher cell potential. The remaining (35 %) must be due to less voltage losses in the anode and membrane of the PMFC with a biocathode. The difference in voltage losses between the two PMFC could among others related to microbial energy for maintenance and growth and substrate availability (i.e. rhizodeposition of the plants) [47].

The maximum achieved power and current density were respectively 679 mW m⁻² PGA (at a cell potential of 500 mV) and 2.08 A m⁻² PGA (at a cell potential of 1 mV) during a 10 minute polarization on day 328 (figure 5). Biocathodes are able to produce a current density of 0.55 A m⁻² projected electrode surface area (non-porous) [54]. In this setup with a cathode surface of 0.072 m² (porous), the biocathode should be able to reach a current of at least 0.040 A, which is 6.6 A m⁻² PGA. Likely even more cathode surface was available for oxygen reduction because of the nature of the porous electrode. The cathode is thus expected to be able to reach a higher current density than was reached during the polarization. However, the limiting current density of the cathode is not reached, possibly because of the limited substrate availability in the anode.

The results show that a biocathode can be integrated in a PMFC which makes PMFC a completely biocatalyzed system with a bioanode and a biocathode. However, for this experiment an advanced lab system was designed. This design is not suitable for application of the technology in wetlands. For the application, a different design is required (e.g. a tubular design [48]) without the addition of growth medium to the anode and cathode. Further research is needed to start-up a biocathode PMFC in wetlands using the locally available nutrients.

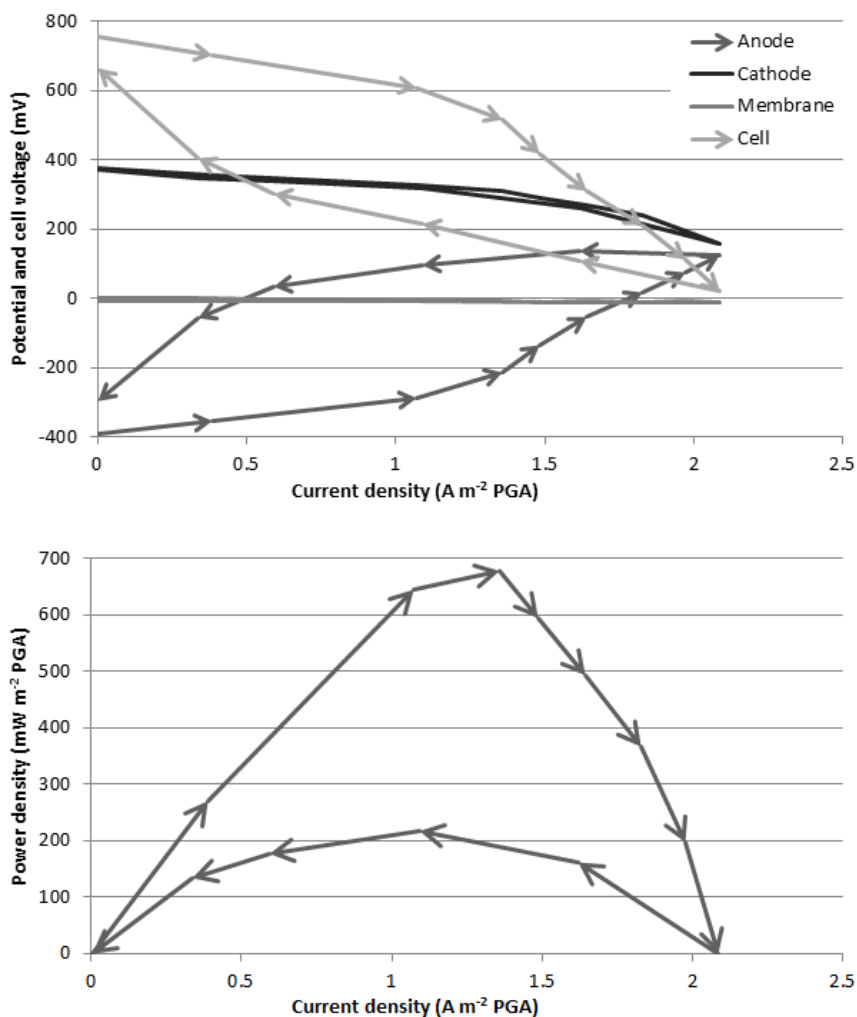


Figure 5: Anode, cathode, membrane potential, cell voltage (top) and power density (bottom) during polarization on day 328. During the polarization the anode resistance build up while the cathode resistance did not build up. The increase in anode resistance (i.e. limited substrate availability) decreased the power output of the PMFC

2.3.2 Plant vitality mixed culture PMFC

The plants in the mixed culture PMFC were vital during the experiment. The PMFC started with 9 stems in the beginning of the experiment. They increased to 25 stems after eight weeks and increased further to over 30 green stems after twenty weeks, creating a dense vegetated PMFC with the tallest shoots over 50 cm. Until the end of the experiment new

shoots started to grow in the PMFC indicating that the plant vitality was good and the oxygen reducing biocathode did not seem to affect the plant growth, though more PMFCs are needed to confirm this. Negative effects were not expected because the catholyte consisted mainly of nutrients and vitamins which are also required for plant growth.

2.3.3 Substrate availability in anode probably limits current density

Both during the short term polarizations (figure 6) as during the long term cathode control experiments (figure 7) the anode resistance increased likely due to limited substrate availability in the anode. The build-up of resistance in the anode of a PMFC was earlier described [52] and was explained as mass transfer resistance (accumulation of protons) or substrate limitations [47]. Figure 7 shows that in 16 hours the anode potential increased from -200 mV to +450 mV. The anode resistance increased from $0.7 \Omega\text{m}^2$ PGA to $1.9 \Omega\text{m}^2$ PGA. If the build-up of resistance would be related to the accumulation of protons, than the pH should drop with 10 units (65 mV per pH unit for 50 mM acetate), which likely did not occur. The build-up of anode resistance was not only related to pH, but probably to substrate limitations and thus more substrate could increase the power output of the PMFC.

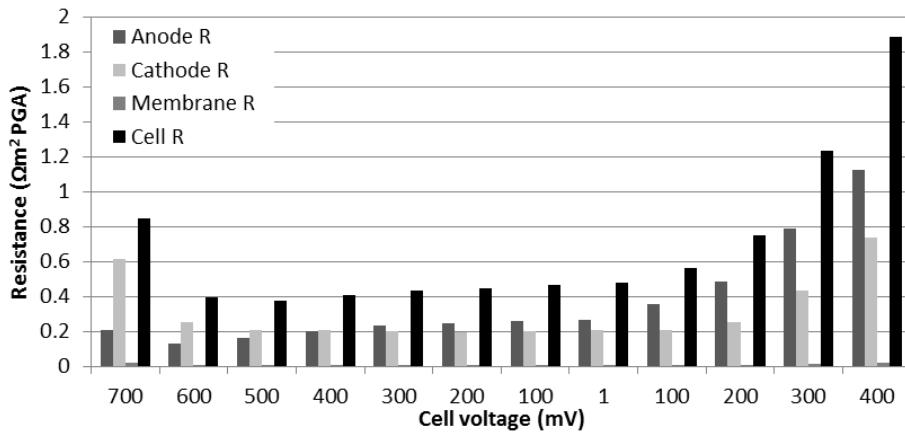


Figure 6: Anode, cathode, membrane and cell internal resistance during polarization on day 328. During the polarization the anode resistance build up while the cathode resistance did not build up

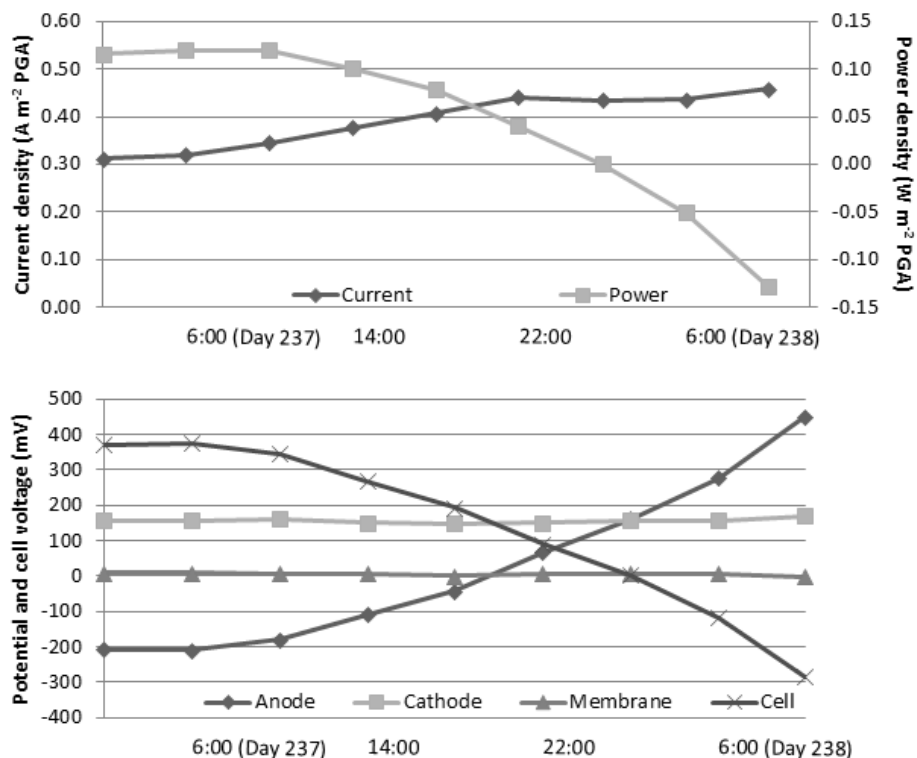


Figure 7: Effect of the increasing current density (top) on the anode potential (bottom), cell voltage (bottom) and power density (top) on day 237 and day 238 when the cathode is controlled at 150 mV. The increased current density resulted in an increased anode potential and a negative cell voltage. An adaptive control strategy is required

The substrate availability per square meter PGA can be increased by deeper anodes as more roots likely results in more rhizodeposition. In this research the anode is 19 cm deep. *Spartina anglica* is known to have roots growing up to 60 cm deep [63]. Increasing the depth of the anode with a factor 3 probably results in more substrate and thus an higher current density and likely a higher power output.

2.3.4 Adaptive control strategy to maximize power output

An adaptive control strategy is required for PMFC because of the needed startup time and controlled potential of the cathode [61, 64] and the dynamic anode potential [33]. The biocathode potential was initially

controlled at 150 mV during the experiment [61]. The cathode is accepting a certain amount of electrons and the potentiostat forces the anode to donate the electrons. When the anode is not able to produce the needed electrons at the desired anode potential (i.e. at least positive cell voltage) the anode potential is increased by the potentiostat to produce more electrons and the anode resistance increases. On day 237-238 the current increased and the anode was likely not able to produce all the electrons at the desired anode potential (figure 7). As a result the oxidation reaction took place at an anode potential higher than the cathode potential, the cell voltage became negative and no electricity was produced.

To maintain a positive cell voltage and a low anode resistance an adaptive control strategy was applied. The requirements for the control strategy were that the controlled cathode potential was high enough to have a positive cell voltage (i.e. anode potential was lower than cathode potential minus membrane potential), while the controlled cathode potential was lower than the onset potential of the cathode. The controlled cathode potential was increased when the anode resistance increased rapidly. The cathode potential had to be increased on day 238 and was set to 235 mV, which was approximately 10 mV lower than the onset potential of the cathode. The cathode continued to improve over time resulting in an increased current of the PMFC. Several times the controlled cathode potential had to be increased when the current increased rapidly to maintain the positive cell voltage (see supporting information figure 1). On day 254 the cathode potential was increased to 265 mV, on day 267 further increased to 285 mV and to 335 mV on day 292. The cathode potential stabilized and to gain more power for a longer period the cell voltage was controlled during the last few weeks of the experiment at 600 mV (day 323 to day 336). A controlled cell voltage avoids the increase in anode resistance and the corresponding decrease in cell voltage and voltage efficiency.

The adaptive control strategy is not only required to maintain the positive cell voltage and the low anode resistance, but also to gain most of the power out of the PMFC. The highest power output is always reached at the lowest internal resistance. During the polarization on day 328, the lowest internal resistance and the highest power were reached at a cell voltage of 500 mV (figure 6). However the lowest anode resistance was reached at cell voltage 600 mV, the anode resistance was thus already increasing. The increasing anode resistance also increases the total resistance. Therefore after some time the lowest

internal resistance is not reached at 500 mV and the cell voltage has to be controlled at a higher voltage to maximize the power output. A possible adaptive control strategy is a maximum power point tracker using fuzzy logic techniques as is often used in PV systems [65]. Hereby, the controller reaches the accurate maximum power point quickly even under the changing conditions of the PMFC.

2.3.5 Phototropic biofilm supplies oxygen in cathode

Besides the oxygen reducing microorganisms also algae were growing within the cathode. A clear film of algae was visible in the cathode compartment. Algae produce oxygen during the day and consume oxygen during the night [66]. The outflow of the cathode compartment of the PMFC contained more oxygen during the day than during the night. On day 268, the oxygen concentration in the outflow was 8.1 mg L^{-1} during the day and 7.1 mg L^{-1} during the night. The oxygen concentration in the inflow was assumed to be constant due to the aeration. The current during the day was higher than during the night and thus more oxygen was reduced during the day. Since, higher oxygen concentration were measured during the day, algae were very likely producing oxygen via photosynthesis and therefore providing oxygen for the cathode. To investigate the ability of the algae to provide oxygen the aeration and pumping of the catholyte was stopped from day 292 to day 323 at a controlled cathode potential of 335 mV. The presence of algae affected the oxygen concentration in the cathode, which resulted in an increased current density of the PMFC during illumination periods (figure 8). A similar effect was observed in earlier investigations [67, 68]. However, even during the dark periods the current is still positive, which indicates that not all the oxygen is reduced in the cathode or consumed by the algae or aerobic microbial activity. Between day 302 and 308 the average power density was 118 mW m^{-2} PGA with peaks up to 165 mW m^{-2} PGA during illumination at an average anode potential of -338 mV. The PMFC with a phototrophic biofilm did not require energy intensive pumping and aeration of the catholyte and was still able to produce electricity. This effect was also observed by Lobato et al. [69]. Also during the dark periods the cathode resistance decreased and the onset potential increased over time (data not shown), as the effect of oxygen concentration on the onset potential is small (e.g. if the oxygen concentration would locally be a factor 20 higher during the day than during the night, the potential would only

increase by 19 mV based on the Nernst equation). This indicates that the reduction of oxygen is catalyzed by the mixed culture microorganisms like bacteria (i.e. reducing charge transfer resistance) and that the algae provided more oxygen during illumination (i.e. reducing possible concentration resistance).

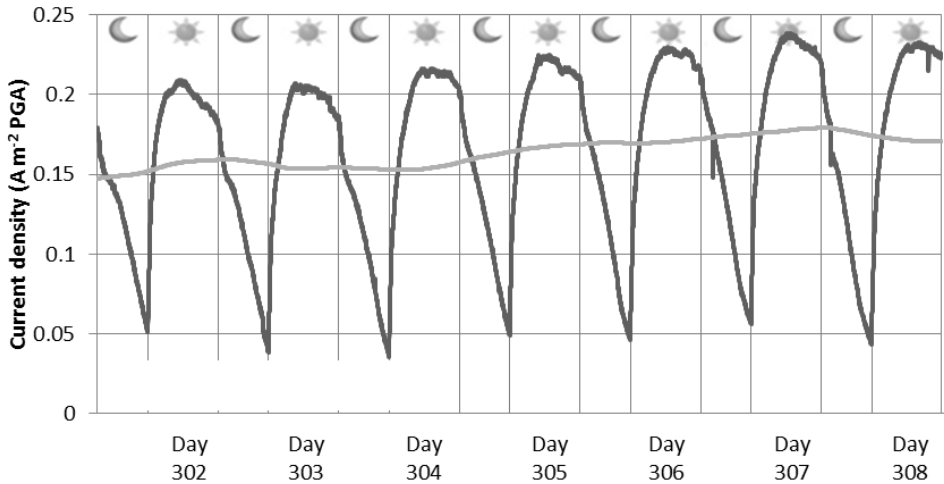


Figure 8: Illumination increased the current density in the PMFC when the active pumping and aeration of the catholyte was stopped due to the presence of algae in the cathode at a controlled cathode potential of 335 mV between day 302 and 308

However, the use of algae is not applicable in PMFC research as the light energy is preferably used for the plants to grow. Large scale application in wetlands would require a certain amount of land and light for the growth of algae. The land available for plants is reduced and as a result also the power output per square meter of land. Stopping the pumping and aeration indicates that the biocathode in the PMFC is able to survive without the recirculation of nutrients and vitamins. The nutrients and vitamins are likely transported from the anode through the membrane. The application of PMFC is preferably without energy intensive aeration and pumping and the nutrients and vitamins in the wetland should support the growth of a biocathode. Passive oxygen supply through a gas diffusion layer should completely avoid the need of aeration and pumping. Oxygen reducing biocathodes are able to function with a gas diffusion layer [49] and the PMFC is also likely able to function with a gas diffusion layer.

2.3.6 Unsuccessful start-up of PMFC with *A. ferrooxidans* biocathode

At the same time as the PMFC with an oxygen reducing biocathode also an experiment with a PMFC with an *A. ferrooxidans* biocathode at pH 2 was started which was not successful. The unsuccessful start-up of the *A. ferrooxidans* PMFC was not related to the biocathode but was solely related to the negative effects of the low pH biocathode on the plant vitality in the anode. The bipolar membrane (BPM) was not able to maintain the pH gradient between the anode and cathode as within a non-planted *A. ferrooxidans* MFC set-up [55]. The pH in the cathode started at 2, within days the pH of the catholyte increased and HCl was added to maintain the low pH. As a result also the pH in the anode started to drop. Already three days after the PMFC was installed, the pH in the anode dropped to 5. Within 35 days, the pH in the anode dropped below 3. Approximately 10 times more protons had to be added to the catholyte than could theoretically been used for the oxygen reduction in the cathode of the PMFC (calculations not shown). Clearly, protons diffused from the cathode through the membrane to the anode. The low pH likely affected the plant vitality. 11 green stems were planted in the anode. After 14 days only five green stems were left and after 61 days the PMFC had no more green stems. PMFC technology should not interfere with plant growth and the experiment was therefore ended. Currently, PMFC application with a low cathode pH is highly unlikely.

2.4 Conclusions

An oxygen reducing biocathode was successfully integrated in a PMFC and operated for 151 days. The two week average power density was 240 mW m^{-2} PGA, which is higher than was achieved with a ferricyanide cathode. This was mainly due to the higher redox potential of oxygen reduction which was effectively catalyzed by microorganisms. As a result, 127 mV higher cathode potential was achieved. Substrate availability in the anode likely limits the current generation of the PMFC. This work shows that PMFC can be a completely sustainable technology with biocatalyst in the anode and cathode, which is an important step for PMFC application.

2.5 Acknowledgement

This project was carried out within the research programme of BioSolar Cells, co-financed by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.” Furthermore, this research is also in part financed by Alliander and Plant-e.

Chapter 3

Bioelectrochemical water oxidation by an electrochemically active biofilm

Abstract

The water oxidation reaction is currently hampering development of large scale water oxidation technologies to reduce protons to hydrogen or carbon dioxide to hydrocarbons. In this communication we present a novel bioelectrochemical water oxidation process driven by a bioanode containing electrochemically active microorganisms. The bioanode reached a current density of 0.93 A m^{-2} at 0.7 V overpotential. Oxygen evolution measurements linked 22 % of Coulombs to a water oxidation reaction process. The actual allied mechanism(s) could be related to reversible enzymes or other microbial-electrochemical pathways. An optimized system could be used to produce fuels on a large scale, since electrochemically active microorganisms can be made from earth-abundant materials in a cheap & self-repairable way.

Key words

Bioelectrochemical water oxidation, electrolysis, electrochemically active microorganisms, hydrogen production

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3.1 Introduction

Water is the primary source of electrons to reduce protons to hydrogen or carbon dioxide to hydrocarbons. The responsible water oxidation reaction is currently hampering development of large scale water oxidation technologies [70]. In pursuit of energy efficient water oxidation catalysts, a diversity of artificial and natural catalysts, including metals, organic molecules, algae and laccases, were investigated [71-76]. Until now, electrochemically active microorganisms, those not relying on photosynthesis, were not assessed for water oxidation [77, 78]. These electrochemically active microorganisms, also called electrotrophs, are able to exchange electrons with electrodes and are as such applied in diverse Bioelectrochemical Systems (BES) [79, 80]. The underlying electron-transfer mechanisms between the microorganisms and electrode rely on direct electron transfer or transfer using artificial or natural electron shuttles [43]. BES typically use fuels derived from organic waste(water), sediments, plant rhizodeposits or substrates from phototrophic biofilms [9, 36, 81, 82]. The produced protons and electrons are used to gain electrical power or chemical products. The advantages of using living microorganisms as biocatalysts include (i) self-repair ability, (ii) constructed from earth-abundant materials, (iii) cheap when made from e.g. wastewater, and (iv) can be implemented in large-scale technologies. The hypothesis for this study was that electrotrophs have mechanisms to support water oxidation as explained further on. Therefore, the aim of this study was to use electrochemically active microorganisms, without active photosynthesis, for bioelectrochemical water oxidation. In this study, a mixed culture oxygen reducing biocathode was started, which was reversed in polarity to perform water oxidation.

3.2 Materials and methods

A flat plate BES with a graphite plate anode and cathode and a projected surface area of 22 cm² was constructed [60]. The anode and cathode compartment were separated by a cation exchange membrane. The volume of each compartment was 33 mL. The BES operated in a climate chamber at a temperature of 303 K. Both the catholyte and anolyte consisted of bacterial growth medium with 20 mM phosphate buffer, macro- and micronutrients and vitamins at pH 7.0 [61]. The cathode

was inoculated with aerobic sludge from the communal wastewater treatment plant in Bennekom, the Netherlands. The cathode (as working electrode) was controlled with a potentiostat (Iviumstat, Netherlands) in a three electrode set up at 0.15 V vs a 3M KCl Ag/AgCl reference electrode (+0.21 V vs NHE). Pressurized air was flushed through the electrolytes to maintain saturated oxygen concentration in the electrode compartments.

After successful start-up of the biocathode, the water oxidation experiments were performed on day 101. The working electrode was three times controlled as anode at 1.3 V vs Ag/AgCl (0.7 V overpotential for water oxidation under applied conditions) for 600 seconds to test whether the biofilm was able to catalyze the reversible reaction of oxygen reduction. Nitrogen gas was flushed before and between the experiments to create anaerobic conditions to be able to measure oxygen evolution (OXY-4 mini, PreSens, Germany). The electrolyte circulation was stopped during and after the 600s at 1.3 V and the oxygen evolution was measured in the electrode compartment. The maximum measured oxygen concentration are used in the results. The BES operated at open circuit (OCV) for 30 to 60 minutes in between the tests. Before the experiment, the macro- and micro nutrients and vitamins were removed by replenishing the electrolytes with 20 mM phosphate buffer at pH 7.0 to avoid (electrochemical) deposition of e.g. manganese, cobalt on the electrode, which do also catalyze water oxidation [83]. The electrolytes were mixed by placing the BES on a flask-shaker at 75 rpm. The experiment were repeated at open circuit conditions (i.e. without controlled electrode potential at 1.3 V) to prove that the increase in oxygen concentration in the electrode compartments was related to the controlled potential. After the water oxidation experiments, the BES was again controlled as cathode (0.15 V vs Ag/AgCl) and pressurized air was flushed in both electrolytes.

To further study the role of the biofilm, the effect of antibiotics was examined on day 109. The water oxidation experimental cycles were repeated after antibiotics were added to the electrolyte (100 mg L⁻¹ vancomycin, inhibitor of gram positive bacteria and 100 mg L⁻¹ kanamycin, inhibitor of gram negative bacteria). These antibiotics do affect microbial growth and/or activity and may cause even cell dead [84]. After applying antibiotics, the biofilm was physically wiped-off with a tissue doped in ethanol and the experimental cycle was again repeated on day 113.

A blank experiment was performed for comparison of the results. The blank consisted of electrodes without the addition of microorganisms. The same cycle of water oxidation measurements was performed on the blank as was performed on the bioanode.

3.3 Results and discussion

3.3.1 Biofilm catalyzes oxygen reduction before and after water oxidation experiments

The oxygen reducing biocathode reached a maximum current density of 0.585 A m^{-2} . Light-microscopic analysis of a sample taken from the by eye visible biofilm confirmed the presence of microorganisms. Evidently, electrochemically active microorganisms within the developed biofilm were responsible for catalysis of the oxygen reduction reaction. The biofilm was responsible for a higher onset potential and lower overpotential attributed to a lower charge transfer resistance [85]. Biocathodes can effectively reduce oxygen at current densities up to 0.9 A m^{-2} at a cathode potential of 0.15 V [86]. The biocathode in this research was thus performing comparable. The biocathode operated at a current density of 0.035 A m^{-2} directly before the water oxidation experiment. The current density is lower than the maximum current density of 0.585 A m^{-2} possibly due to the removal of nutrients and vitamins from the medium.

Directly after the water oxidation experiments, the biocathode was able to reduce oxygen at a current density of 0.006 A m^{-2} . This confirmed that the biofilm was still able to reduce oxygen however at a lower current density than before the water oxidation experiments. This may be due to the influence of the 1.3 V operation on the biocathode, the production of H_2O_2 (as discussed later), the interruption in nutrient, vitamins, electrons and oxygen supply. The current density increased to 0.017 A m^{-2} within 24 hours of biocathode operation, which shows that the biocathode recovered and that the biocathode is thus self-repairing.

3.3.2 Enhanced bioelectrochemical water oxidation

All results of the electrochemical water oxidation experiments with and without electrochemically active microorganisms are shown in table 1 and figure 1. The average current density of the bioanode biofilm was $0.93 (+/- 0.02) \text{ A m}^{-2}$ and the oxygen evolution was $1.03 (+/- 0.08) \text{ mg}$

L^{-1} at a Coulombic efficiency (CE) for oxygen evolution of 22.0 (+/- 1.3) %. The blank graphite electrode reached a lower current density of 0.70 A m^{-2} and a CE for oxygen evolution of 6.1 (+/- 1.7) %. No oxygen evolution was measured during open circuit conditions confirming that the oxygen was produced due to the applied electrode potential. The higher current density at the start of each cycle was due to the capacitive behavior of the bioelectrode. Similar capacity behavior was described in earlier experiments using the same experimental set up [87].

Table 1: Overview of (bio)electrochemical water oxidation performance of the studied electrodes during 600 seconds polarization at 1.3V.

Studied electrode	Average current (A m^{-2})	Maximum oxygen (mg L^{-1})	Coulombic efficiency for oxygen evolution (%)
Blank	0.70 (+/- 0.00)	0.22 (+/- 0.06)	6.1 (+/- 1.7)
Bioanode	0.93 (+/- 0.02)	1.03 (+/- 0.08)	22.0 (+/- 1.3)
Bioanode+ antibiotics	0.97 (+/- 0.01)	0.24 (+/- 0.01)	5.0 (+/- 0.2)
Bioanode removed	0.68 (+/- 0.01)	0.08 (+/- 0.03)	2.4 (+/- 1.0)

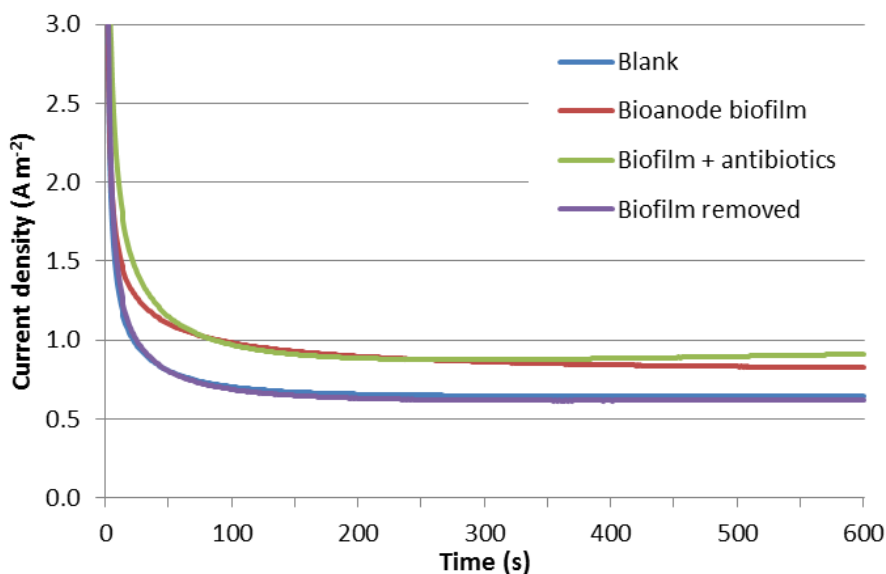


Figure 1: Anodic current density of 3 times 600 seconds polarization at 1.3V of the blank electrode, the developed biofilm, the biofilm with antibiotics and the electrode after biofilm removal

The bioanode showed a 33 % increased current density compared to the blank without biofilm. This increase could be due to increase in water oxidation reactions, oxidation of the biofilm itself (since it's consist of organic matter), and/or other 'side' oxidation reactions from electrolyte compounds entrapped in the biofilm. E.g. small amounts of available chloride ions can be oxidized to $\text{Cl}_2(\text{g})$ under the applied conditions. The fact that oxygen evolution was also enhanced, supports the explanation that the biofilm with electrochemically active microorganisms was beneficial for enhanced water oxidation. The oxygen could also have been formed via anodic electrochemical peroxide formation (according to $2\text{H}_2\text{O} \Rightarrow \text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^-$) which is further decomposed to water and oxygen [74]. To study this, further blank experiments were performed and peroxide formation was measured (Hach, Germany, Test Kit Model HYP-1). It was indeed shown that minor amounts of peroxide were produced (0.2 mg L^{-1}) under the applied potential. Assuming that all this peroxide would decompose to oxygen this would represent 10 % of the measured oxygen evolution. This peroxide may also have been produced within the bioanode experiments. The decomposition of this peroxide can be catalyzed by sole enzymes like laccases and microorganisms reducing peroxide concentrations more rapidly, which supports the beneficial role of the biofilm [88]. Lower peroxide concentrations at the electrode interface will make the peroxide formation reaction thermodynamically more favorable which will enhance the current rate of the water oxidation. This would imply that this bioelectrochemical water oxidation system can (also) be classified as a secondary Microbial Electrochemical Technology [79].

Addition of antibiotics into the bioelectrochemical system resulted in a 77% lower CE for oxygen evolution while current density improved with a few percent. The enhancement of the current can be explained by possible oxidation of released redox active compounds from the microorganisms, like laccases [89]. Since the biofilm was better in water oxidation before the antibiotics, possibly living electrochemically active bacteria and/or intact enzymes were responsible for water oxidation. After removing the biofilm, the water oxidation performance dropped to a comparable rate as the blank electrode. This shows that the developed biofilm was indeed responsible for the observed increase in current and water oxidation process.

3.3.3 Possible biological mechanisms

At this moment several mechanisms can explain the observed bioelectrochemical water oxidation. As discussed earlier, the pathway of water oxidation to peroxide which is further enzymatically decomposed is feasible. Also other biological mechanisms can play a role (see figure 2 for a simplified model). It is known that photosynthetic microorganisms like algae and cyanobacteria can oxidize water when exposed to light. These microorganisms can also be electrochemically active and the underneath electron transfer pathways were recently reviewed [90]. Since our biofilm was not exposed to light it is evident that photosynthesis did not take place. To our best knowledge, there are no microorganisms so far known which can oxidize water without solar energy. However, we can hypothesize that microorganism with a photosynthetic apparatus can oxidize water when the energy needed for this is applied via electrons (or via energetic electron carriers) from an external solid electrode. Therefore these microorganisms may be part of the mechanism. Since the oxygen reducing biocathode was directly reversible it is also possible that similar reversible electron transfer pathways known or expected for oxygen reducing biocathodes were responsible for bioelectrochemical water oxidation [43]. The mechanisms include catalysis by electrode adsorbed extracellular proteins, metal-exopolymer compounds or a direct electron transfer from the electrode to the microorganism [91]. Also the possible role of laccases is again relevant. Laccase enzymes are widespread among fungi and bacteria, have various biological functions and are likely present in the biofilm [92]. Recently it was shown that immobilized laccases on an electrode were responsible for water oxidation [74]. Laccases were already known to catalyze oxygen reduction in microbial fuel cells [93]. Therefore, it may be possible that bacterial laccases (in or outside the microorganisms) were responsible for a reversible process, the actual water oxidation in our experiments [94, 95]. The actual effect of applied antibiotics on laccase is unknown since these may or may not affect laccase activity. For example, it is known that fungal laccase production and activity can be enhanced by application of specific antibiotics; and even laccases were applied to synthesize new antibiotics and therefore can be resilient to certain antibiotics [92, 96, 97]. At this moment we cannot be conclusive on the dominant working principle(s). Though, with this discussion we show that several

directions can be further explored to reveal the working principles and improve the bioelectrochemical catalytic process.

3.4 Conclusions & Outlook

This study showed that a biofilm containing electrochemically active bacteria was beneficial for water oxidation. The bioanode reached a current density of 0.93 A m^{-2} at 0.7 V overpotential and 22 % Coulombic efficiency linked to water oxidation. The allied mechanism(s) could be related to reversible enzymes or other microbial-electrochemical pathways. By combining bioelectrochemical water oxidation with a biocathode producing H_2 or organics, a complete biocatalyzed system could be developed (figure 2). An optimized system can be applied on a large scale since electrochemically active microorganisms can be made from earth-abundant materials in a cheap & self-repairable way.

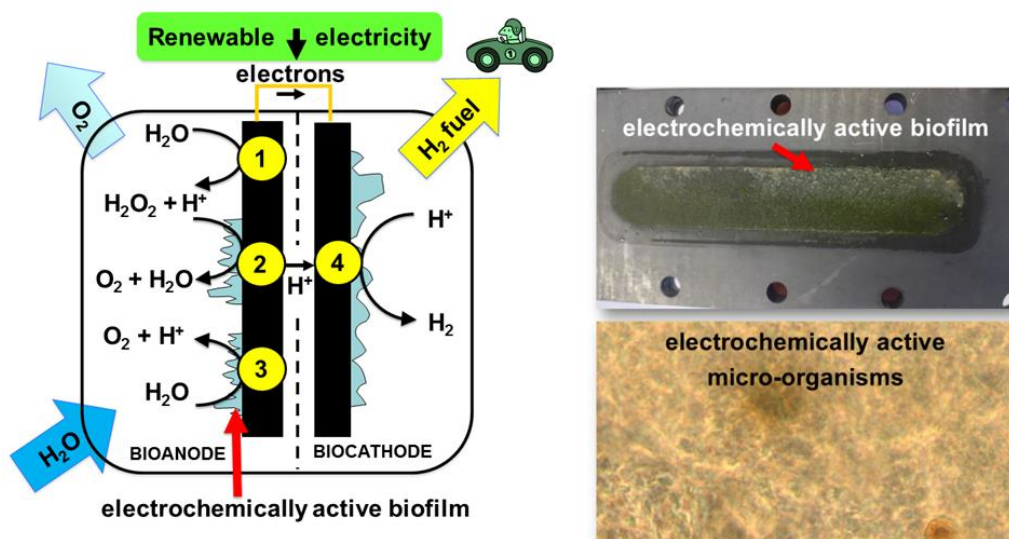


Figure 2: Concept of completely biocatalyzed water electrolysis system for H_2 fuel production. Water is flowing into the bioanode. The electrochemically active biofilm is responsible for bioelectrochemical water oxidation at the anode releasing electrons, protons & oxygen. Hereby water can be oxidized to peroxide (#1) which can be decomposed by catalases from microorganisms (#2). Also, the electrochemically active biofilm can be directly responsible for the oxidation of water (#3) according to several explained mechanisms in

commutation text. Energy is added to the electron flow which are used the reduce protons by the electrochemically active biofilm at the biocathode (#4). The photos (right) show the by eye visible electrochemically active biofilm and microorganisms visible under a light-microscope.

3.5 Acknowledgements

This project was carried out within the research programme of BioSolar Cells, co-financed by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. Furthermore, this research is financially supported by Alliander and Plant-e.

Chapter 4

Localization of electricity generation and electron transfer mechanisms in partitioned bioanode of *Spartina anglica* Plant Microbial Fuel Cell

Abstract

The anode of the Plant Microbial Fuel Cell (PMFC) is dynamic due to changing rhizodeposition, radial oxygen loss, microbial composition and external environmental conditions. In this study, the current generation in a lab scale *Spartina anglica* PMFC is localized at 4 cm² scale. Differences in current in relation to location and time are revealed by a flat porous-plate PMFC with 30 small anodes at different width and depth. The current generation is explained by rhizodeposition, radial oxygen loss and electron transfer mechanisms. The results show that after a start-up period of 70 days significantly higher current is generated at anodes close to plant roots due to rhizodeposition. Current can likely be generated both from living and death roots. Also oxygen was present at the anodes close to the plant roots, probably resulting in internal currents. Electrons are not transferred over centimeter distance through conductive microorganism on the plant roots in the PMFC. Electrons may be transferred via mediators as mediators were present likely originating from plants or microorganism. Current generation is linked to root density and therefore the PMFC should be installed at locations in the rhizosphere with most roots.

Keywords

Spartina anglica, plant microbial fuel cell, electron transfer mechanism, rhizodeposition, radial oxygen loss, partitioned bioanode

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Localization of electricity generation and electron transfer mechanisms
in partitioned bioanode of *Spartina anglica* Plant Microbial Fuel Cell

4.1 Introduction

The Plant Microbial Fuel Cell (PMFC) generates electricity from living plants [9]. Electrochemically active bacteria (EAB) oxidize organic rhizodeposits from the plants in the anode of the PMFC. The released electrons flow to the cathode via an external circuit where the electricity is harvested. At the cathode, oxygen is reduced to water. PMFC is investigated at lab-scale, on green roofs [12], rice paddy fields [10] and other wetlands [98, 99]. The maximum long term power generation of the PMFC achieved at lab-scale is 240 mW m^{-2} plant growth area (PGA) [100].

Plants release 20 to 60 % of the net fixed carbon into the soil as rhizodeposition [13]. Rhizodeposition contains low molecular weight (LMW) organic exudates and high molecular weight (HMW) organic lysates. Exudation is 5-20% of the net fixed carbon and is passively or controlled released into the soil via diffusates, secretions and excretions [13]. Lysates are passive release of damaged and sloughed off root cells and contain up to 30 % of the net fixed carbon [13]. Next to the passive release of root border cells, plants are also able to release these cells controlled as protection for example against a pathogen attack or heavy metal toxicity [14]. Both the LMW exudates as the HMW lysates are potential substrates in the PMFC. The HMW lysates as cellulose are possibly not directly suitable for EAB and are hydrolyzed to LMW organics before being oxidized by the EAB [17]. The presence of the EAB in the anode of the PMFC was shown by several studies [10, 16, 18, 19]. Also microorganism known to hydrolyze cellulose were present in the anode of the PMFC [18]. These insights support the theory that PMFC generates electricity by oxidation of organics directly from the exudates or via hydrolysis.

Plants do not only release electron donors, but also release the electron acceptor oxygen. Plants grow via aerobic respiration and plants thus need oxygen to grow. Aquatic plants with the roots in anaerobic waterlogged conditions, including those studied in PMFCs, transport the required oxygen for aerobic respiration from the air to the roots via their aerenchyma [101]. Besides providing oxygen for aerobic respiration, plant do also release oxygen for detoxification of phytotoxins [102] and efficient nutrient acquisition [103]. The radial oxygen loss by the roots results in oxic zones around the roots [104]. The released oxygen can be reduced while oxidizing organic matter. The electrons from these organic rhizodeposits are thus lost for the PMFC. The current generation

in the PMFC is depending on the availability of organic rhizodeposits and can be reduced due to the presence of oxygen.

Whether electricity in the PMFC is generated by oxidizing the exudates from the living roots and/or oxidizing the LMW hydrolyzed from the sloughed of (dead) roots is unknown. Also the quantitative decrease in current generation due to oxygen is not clear. A PMFC model (with *Glyceria maxima* as model plant) predicted that current was generated only from oxidizing the dead roots and not from the living roots. The living roots were calculated to release more electron acceptors (i.e. oxygen) than electron donors (i.e. exudates). Dead roots do not release oxygen and were therefore predicted to be responsible for the current generation [105]. In contrast to the model, other PMFC research showed that current was generated by living roots [47]. In that research, two PMFCs with a top, middle and bottom anode sections were constructed. In one PMFC most current was generated at the top layer with most roots, while in the other PMFC, most current was generated in the bottom layer with only one living root [47]. This living root likely had a hotspot [47], a location with enhanced exudation and microbial activity [106].

The location of rhizodeposition is not automatically also the location of current generation in the PMFC. The electrons produced by the EAB could be transported to a different location by mediators or conductive biofilms. An example of redox mediators are humic substances which are able to shuttle electrons [107]. Bacteria are capable of producing redox mediators [108]. These mediators can also be used by other bacteria and as such enhance electron transfer [109]. Also plant roots exudates many valuable compounds facilitating interaction between roots and microorganisms [110]. Likely, these exudates also contain redox mediators like siderophores [111]. Conductive biofilms increase the current density of microbial fuel cells due to a lower resistance in electron flow through the biofilm [112]. In marine sediments, bacteria are discovered which are able to transport electrons over centimeter wide zones [113]. Electron transport via a redox mediator or conductive bacteria could therefore result in current generation at a different location than rhizodeposition.

The objective of this study is to localize the current generation in the anode of the PMFC and to clarify the differences in relation to rhizodeposits, radial oxygen loss, mediators and conductivity of microorganisms on the plant roots. Local current generation is

investigate in a flat plate PMFC with 30 small anodes at different width and depth.

4.2 Materials and methods

4.2.1 Experimental setup

The flat porous-plate PMFC was constructed using several plastic plates and consisted from anode to cathode of: a transparent end plate, a 7 mm thick anode compartment (figure 1), a 5 mm thick light blue plate with 30 square holes (of 20 x 20 mm) to place the anodes, a cation exchange membrane (fumasep FKB, FuMa-tech GmbH, St. Ingbert, Germany) and a cathode compartment. The graphite felt (PGF, CGT Carbon GmbH, Asbach, Germany) anodes had a size of 20 x 20 x 12 mm each and were made from 6 layers of felt (20 x 20 x 3 mm) squeezed together to make sure that the anode remained in the right place and would not connect to other anodes. The total size of the anodes was 0.144 L. The anode compartment had a total size of 0.364 L. Each anode had a golden wire current collector. Also the cathode was made from graphite felt (190 x 190 x 3 mm) with a golden wire current collector. Ferricyanide was reduced in the cathode in this experiment (0.05 M $\text{Fe}(\text{CN})_6^{3-}$ with 0.02 M phosphate buffer at pH 7).

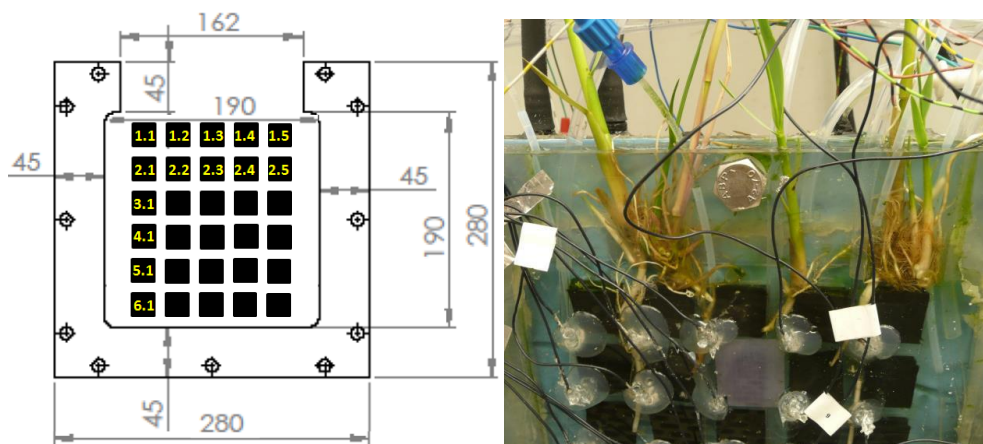


Figure 1: Left: Schematic overview of the anode compartment of the PMFC with the 30 anodes. Right: Photo of the setup directly after installation

In the anode, 3 plants of the salt water grass *Spartina anglica* were planted with a total of 7 stems (figure 1). The total fresh weight was 17 gram. *Spartina anglica* is a model specie for PMFC research [33, 47, 100]. The plants were collected in the Oosterschelde, the Netherlands (GPS coordinates N51°.446788, E004°.093096).

4.2.2 Operation

The plants were planted in the anode on day 1 of the experiment. The plant were kept waterlogged by pumping a nitrate-less, ammonium-rich plant growth medium from a storage tank into the anode (Minipuls 3, Gilson, Den Haag, The Netherlands, 0.2 rpm). This medium included 5 g L⁻¹ NaCl and showed the best performance in previous research [33]. The PMFC was operated in a climate chamber with a light:dark ratio of 14:10, a temperature of 20 °C and a humidity of 70 % (Microclima 1750, Snijders Scientific, Tilburg, The Netherlands) [100]. Catholyte was recirculated from a one liter bottle into the cathode (505S, Watson-Marlow, Falmouth, United Kingdom, 10 rpm).

The 30 anodes of the PMFC were controlled at 0.05 V vs a 3 M KCl Ag/AgCl reference electrode (by a MultiWE32 connected to a Vertex, both Ivium Technologies, Eindhoven, The Netherlands). All potentials are therefore reported vs Ag/AgCl.

4.2.3 Measurements

The cell voltage and the anode, cathode and membrane potential were measured and logged identically as in previous PMFC research [33]. The catholyte pH was measured with a pH meter (Liquisys M, Endress+Hauser, Reinach, Switzerland). The 30 anodes were characterized by polarization and cyclic voltammetry by controlling the anode potential at different potentials. For the polarization, the PMFC was first set to open cell. After that the anode potential was gradually increased from open cell voltage (OCV) to 250 mV and decreased back to OCV. Each step was 600 seconds. Polarizations were mainly used for the maximum current generation of the PMFC. Therefore, current generation at the last second at 250 mV was used for the results. The CV scan started at an anode potential of 50 mV, decreased to -400 mV and increased back to 50 mV at a scan rate of 10 mV s⁻¹.

The ohmic resistance between different anodes was measured by electrochemical impedance spectroscopy (Iviumstat, Ivium

Technologies, Eindhoven, The Netherlands). The anodes were controlled at OCV between the measured anodes after which the voltage difference between the anodes was increased and decreased by 10 mV (i.e. the amplitude). The voltage fluctuation was done at 26 frequencies from 1.000.000 to 10 Hz. The ohmic resistance was derived from the intersection of the data with the x-axis in the Nyquist plot. This resistance can be attributed to a series resistance for ohmic transport in the electrolyte and other contact effects [85]. Impedance data was validated by duplicate measurements and monitoring the sin-waves during the measurement. The finiteness criteria was excluded since data was only used until the intercept of the x-axis of the Nyquist plot [114]. A similar method was applied to measure centimeter-long electron conduction through marine sediments with gold electrodes [115]. We performed the method on anode 1.2-1.3, anode 1.3-1.4 and anode 1.4-15. These anodes were clearly connected via many roots. Also anodes without roots were analyzed. Anodes with no connections were selected for the measurements: anode 2.1-2.2, anode 2.2-2.3, anode 2.3-2.4 and anode 2.4-2.5. These anodes were close to the anodes in the top layer and therefore the other conditions like ionic conductivity were similar.

Oxygen was measured with a planar optode at an fluorescent chemical optical sensor foil close to anode 2.3 (figure 1). The oxygen concentration was measured on the sensor foil with a digital camera combined with imaging technology (Visisens, PreSens, Regensburg, Germany). Plant vitality was analyzed based on the amount of green shoots and the length of the green shoots. Root density was visually analyzed from pictures of the roots.

The results of anode 6.5 are not used in the analysis because the current density of this anode was too high due to oxidation of the current collector. Fixing the current collector solved the problem. However, this was solved at the end of the experiment. Anode 3.5 had a connection problem during the polarization at day 48 and therefore operated at open cell. These results are therefore not used.

4.3 Results and discussion

4.3.1 Current generation during start-up period likely caused by damaged plants due to installation

At the first days of the experiment, the current generation of the top layer was higher than the current generation in the other layers (data not shown). The higher current generation in the top layer is likely caused by the installation of the plants which were installed only in the top layer (figure 1). The plants were transplanted to a different location and likely parts of the roots were damaged or sloughed off due to the installation. These roots can still leak organic exudates [116] and lysis of these sloughed off roots could form substrate hotspots [117], while not excreting oxygen into the rhizosphere. Therefore, these phenomena explain the increased current output the first four days. Afterwards, the current in the top layer dropped likely because the exudates from the sloughed off roots were oxidized and a similar current was generated in all layers. This is likely due to the start-up period of lab scale PMFC which is usually 50 to 80 days [9, 33, 48]. Differences in current generation between layers are therefore expected to occur after the start-up period.

The polarization on day 48 showed no significant differences in maximum current generation between the different layers (figure 2). Only anode 1.3 generated a slightly higher current than the other anodes. This is likely caused by the lower vitality of the plant (i.e. shoot was no longer green) close to anode 1.3 (figure 3) which may resulted in no or less radial oxygen loss, but still leaking exudates, similar as during the first few days. Current could also have been generated by hydrolyzing the dead plant root [105]. The higher current density of anode 1.3 compared to the other anodes was also visible in the long term data (data not shown). On day 12, anode 1.3 generated 5 % more current than the second best anode (anode 1.4). This percentage gradually increased to 20 % on day 35 (vs the second best anode, anode 2.5).

	Column					Av	SD
	1	2	3	4	5		
Layer 1	4.5	5.4	8.9	7.1	5.4	6.3	1.8
2	7.1	6.3	7.1	8.0	4.5	6.6	1.4
3	7.1	6.3	8.0	7.1	error	7.1	0.7
4	5.4	5.4	7.1	6.3	6.3	6.1	0.7
5	6.3	6.3	7.1	4.5	3.6	5.5	1.5
6	6.3	8.0	7.1	4.5	error	6.5	1.5
Av	6.1	6.3	7.6	6.3	4.9		
SD	1.0	1.0	0.7	1.5	1.2		



Figure 2: Left: Current generation in mA m^{-2} PGA of the 30 anodes at an anode potential of 250 mV during the polarization on day 48. Also the average (Av) and standard deviation (SD) of the layers and columns are shown. Right: Photo of the roots on day 38

4.3.2 Current generation increases with increasing root density

The current generation of the PMFC started to increase after a 70 day start-up period. The current generation of anode 1.2, 1.3 and 1.4 increased (figure 3). From day 106 also the current generation of anode 1.1, 1.5 and all anodes in layer 2 increased (figure 3). A maximum long term current generation of 90 mA m^{-2} PGA was achieved as total of all anodes (except anode 5.6 which had a technical problem) from day 102 to day 115. The top layer contained most roots and generated more than three times the current of the layers without roots (figure 4). Also some roots were present in the second layer. The current generation in the second layer was almost twice the current of the layers with no roots. Some roots were present around anode 3.1 and 3.5 (figure 4). Also at these anodes, the current generation was slightly higher than in the anodes without roots (figure 4).

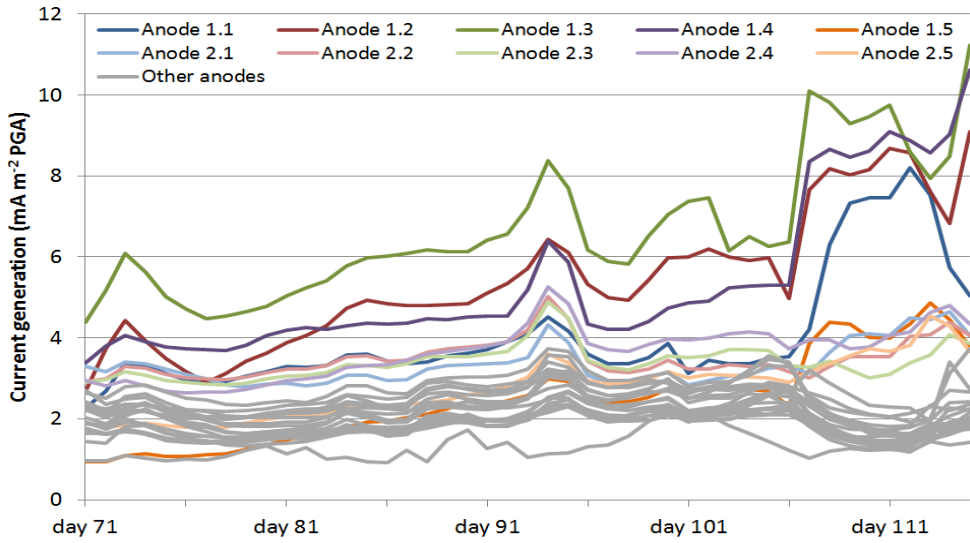


Figure 3: Daily average current generation in mA m^{-2} PGA of each anode from day 71 to day 115. Anodes in the top two layers are highlighted with different colors

		Column						
		1	2	3	4	5	Av	SD
Layer	1	5.5	7.3	8.4	7.6	3.7	6.5	1.9
	2	3.7	3.6	3.5	4.1	3.5	3.7	0.3
	3	2.6	2.1	2.2	2.7	2.6	2.4	0.2
	4	2.3	1.8	2.1	1.8	1.7	2.0	0.3
	5	1.9	2.0	1.8	2.0	1.7	1.9	0.1
	6	2.3	1.8	2.3	1.5	error	2.0	0.4
Av		3.1	3.1	3.4	3.3	2.6		
SD		1.3	2.1	2.5	2.3	0.9		



Figure 4: Long term current generation in mA m^{-2} PGA, Left: Average current generation of each of the 30 anodes from day 102 to day 115 at an anode potential of 50 mV. Right: Photo of the shoots and roots of the plants on day 130. Numbers show the layers of anodes

The total current of 90 mA m^{-2} PGA was lower than found in previous research. Fully developed flat plate PMFCs generate long term maximum currents up to 400 mA m^{-2} PGA [33, 100]. The maximum current in both experiments was reached after more than 300 days of operation. The lower current generation in this research is explained by the presence of roots only in the two top layers of the PMFC (figure 4). The PMFC was not fully developed. A longer operation time would likely have resulted in more roots. The results show that current generation is related to root density and therefore the current is expected to increase further when more roots are present in the anode.

The polarization of day 113 shows a significant higher maximum current generation in the two top layers (figure 5). These layers were responsible for most current generation similar as in the long term data (figure 4). The maximum current generation of the anodes in the first layer increased on average from 6.3 mA m^{-2} PGA on day 48 (figure 2) to 19.5 mA m^{-2} PGA on day 113. The presence of more developed plants and roots resulted in a more than three times higher current generation in the top layer. Also in the second layer, the current generation increased by more than two times compared to day 48. The significant increase in current in the top layers is likely due the rhizodeposition of the plants and the local utilization by electrochemically active microorganisms on the anodes around the plant roots. In the bottom four layers, the current generation is only slightly higher than the current generation on day 48 (figure 2). This confirms that over time, current is linked to root presence and that a higher root density results in a higher current generation. The higher current generation is likely also linked to a better development of the electrochemical microbial community around the plant roots [18]. This could also explain the start-up period observed in PMFCs. The electrochemically active microorganisms are able to outcompete other microbial processes for electron donors due to the anode overpotential [105]. More roots and the resulting higher substrate availability in the top layer likely results in a more development electrochemical microbial community in the top layer than in the other layers with less available substrate. The maximum current is thus generated in the part of the rhizosphere where most roots are present due to more substrate which allows besides a higher current generation also a more developed electrochemically active microorganism. Application, i.e. installation, of the PMFC is therefore preferred at the location in the rhizosphere with most roots.

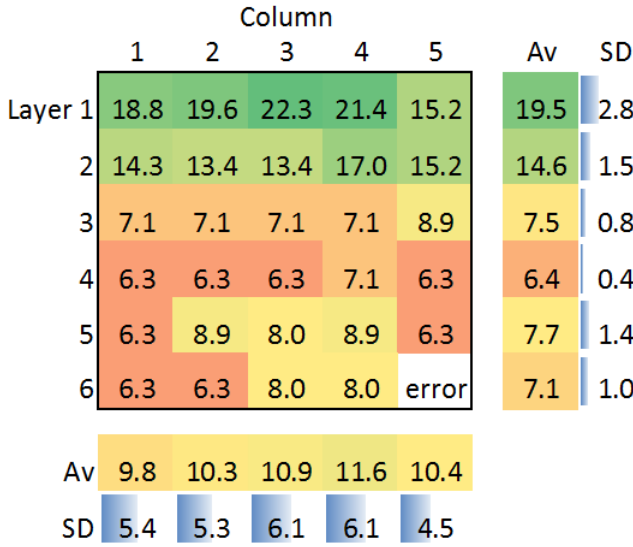


Figure 5: Current generation in mA m^{-2} PGA of the 30 anodes at an anode potential of 250 mV during the polarization on day 113

4.3.3 Current is also generated at anodes with radial oxygen loss

Radial oxygen loss results in oxic zones around the roots of *Spartina anglica* [104] which in theory results into internal currents and thus a loss of electron donors. A higher oxygen concentration was measured directly around the plant roots (i.e. the rhizosphere-zone) with a planar optode in this research. This measurement was only performed on one plant root which confirmed that radial oxygen loss does occur at plant roots of the anode of the PMFC. Since the spatial and temporal radial oxygen loss data are not available, it is not possible to evaluate the quantitative effect of oxygen on the current generation at different locations.

The anodes with roots were affected by the presence of oxygen as evaluated based on the overpotential and cyclic voltammetry (CV). The overpotential is clearly linked to the maximum current density for all layers except the top layer (figure 6). The overpotential is in this case the fixed anode potential (i.e. 250 mV) minus the OCV anode potential. The anode potential at OCV depends on the theoretical anode potential plus losses due thermodynamics (e.g. temperature and concentration differences) and side reactions [118]. The thermodynamic losses in this study possibly vary because of higher concentrations of substrate in the top layer with plants which resulted in higher current generation at the

top layers as shown in the previous section. Also, different microbial populations, pH and electron transfer mechanisms may explain differences in overpotential [21]. Side reactions, mainly oxygen reduction in the bioanode due to radial oxygen loss, result in mixed anode potential. Therefore, the anode potential at OCV lies between the redox potential of organic substrate oxidation and oxygen reduction [118]. This explains that in the presence of oxygen, the anode potential at OCV is higher than in the absence of oxygen. The current and overpotential, thus also the OCV anode potential, were nearly identical in layer 3 to 6 (figure 6). Also in layer 2, the current, overpotential and OCV anode potential were nearly the same in all five anodes. The similar OCV was likely caused by a comparable conditions including concentration of substrate, pH and oxygen resulting in a similar mixed potential. Only in the top layer the current generation and the overpotential clearly varied (figure 6). This shows that in the top layer the conditions were different from place to place. The lower overpotential and thus higher anode OCV of anode 1.1 and anode 1.2 was due to the mixed potential likely caused by the presence of oxygen at these anodes. These anodes were able to generate a higher current than anode 1.5 and all anodes in the second layer, while having a higher OCV and thus likely more oxygen. Therefore, at these layers also higher concentrations of substrate were likely present.

The presence of oxygen is supported by the CV scan on day 133 (figure 7). The CV was not recorded on the same day as the polarization and the results of the CV can therefore not directly be linked to the polarization due to the dynamic anode of the PMFC [33]. However, also in the CV the presence of oxygen is expected as all anodes in the top layer had a higher cathodic (i.e. more negative) current generation at negative anode potentials compared to the anodes in other layers. The anodes in the top layer were thus able to reduce more electron acceptors than the other anodes. The electron acceptor present in the top layer was likely oxygen. The other layers had likely less oxygen and therefore a smaller cathodic current [61]. Particularly anode 1.1 and anode 1.2 had likely a higher concentration of oxygen, which was also shown in figure 6.

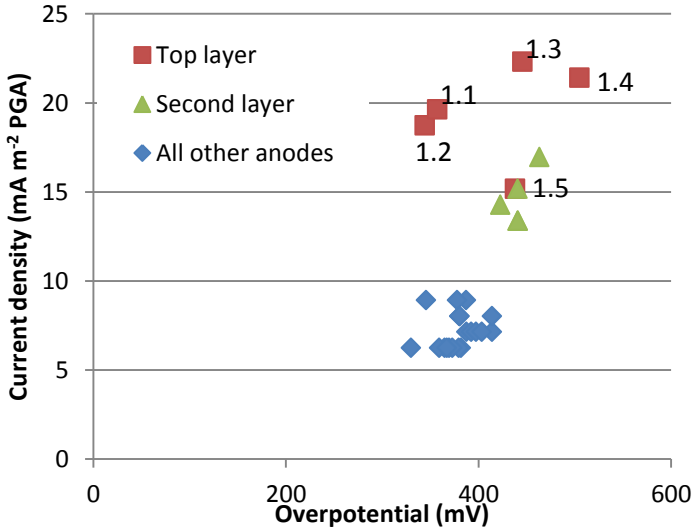


Figure 6: Correlation between overpotential and current density in mA m⁻² PGA during the polarization on day 113. Data of maximum current density, at an anode potential of 250 mV, is shown.

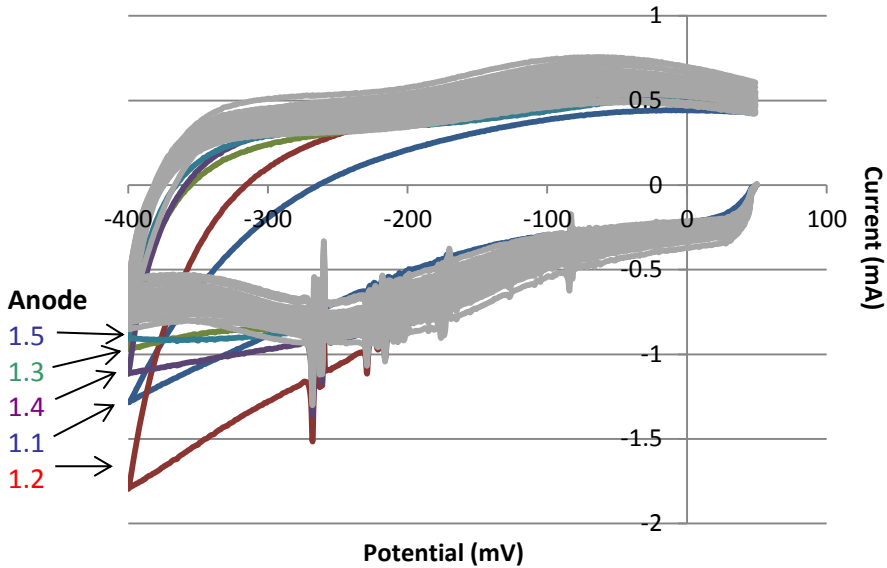


Figure 7: Cyclic voltammetry of the 30 anodes on day 133. Anodes in top layer are highlighted, all other anodes (grey) had comparable currents and are therefore not separately highlighted. Scan started at an anode potential of 50 mV, decreased to -400 mV and increased back to 50 mV at a scan rate of 10 mV s⁻¹. Current is presented in mA per anode.

From figure 6 and 7 can be concluded that most oxygen was present in the top layer. The top layer had most roots and thus likely most radial oxygen loss. The oxygen in the top layer could also be related to oxygen diffusion from the outside air or the phototrophic biofilm which was visible on top of the anode compartment. The top layer was also able to generate the highest current (figure 5). At the same anode likely both oxygen and organic substrate were present at the same time, which probably results in internal currents due to biological or (bio)electrochemical oxygen reduction while oxidizing the substrate from the plants. However, not all the organic substrate or oxygen reacts in these internal currents. The presence of organic substrate and oxygen at the same anode is because exudation and radial oxygen loss also occur at the same location. Both exudates [119] and oxygen [104] are mainly excreted at the root tips of the plants. Although both are also excreted at the rest of the roots [119, 120]. The presence of oxygen likely reduced the current generation of the PMFC, but still current was generated in the PMFC, likely both from living and dead roots. Close to the anodes in the top layer some dead roots were likely present which can generate electricity [98]. However, the roots at the second layer were clearly growing and thus alive. Also at the second layer current was generated by oxidizing the rhizodeposits close to these living roots. Since living plant roots release root border cells [14] and exudates [119] at locations close to each other (i.e. mainly at the root tip), it is not possible to discriminate between these two sources of electron donors at the 4 cm² bioanode scale.

4.3.4 Electron transfer mechanisms in the PMFC

A redox couple is visible on the CV scan of the bioanodes of the PMFC on day 133 (figure 7). The midpoint potential of the redox couple is -176 mV. The redox couple could be related to the redox potential of (electrochemically active) bacterial species which is often similar as the redox potential in this PMFC [21]. Several organic and inorganic redox compounds could be involved in the electron transfer [121]. However, a CV scan of the anolyte sampled from the PMFC showed also a redox couple on a clean graphite felt electrode. Although, the redox couple is less visible in the anolyte (figure 8) than on the anodes of the PMFC (figure 7) the mid-point potential is similar. The redox couple was not visible on the fresh medium before it entered the anode of the PMFC (figure 8). This shows that a redox mediator was formed in the PMFC.

Redox mediators present in the PMFC could be excreted by microorganism or plants [111]. Electrons are possibly mediated via siderophores excreted by plants [111]. Nearly 500 compounds are identified as siderophores with a large range of mid-point potentials [122]. A possible microbial produced mediator is phenazine [109] which has a redox potential (-240 mV) close to the redox potential found at the CV scan [21]. Mediators likely transferred electrons towards the anodes which were not directly connected to roots. This is especially interesting for application as the current generation of the PMFC installed in wetlands may be increased due to mediators transferring electrons from the wetlands towards the anode. For example, by humic acids naturally present in wetlands [107].

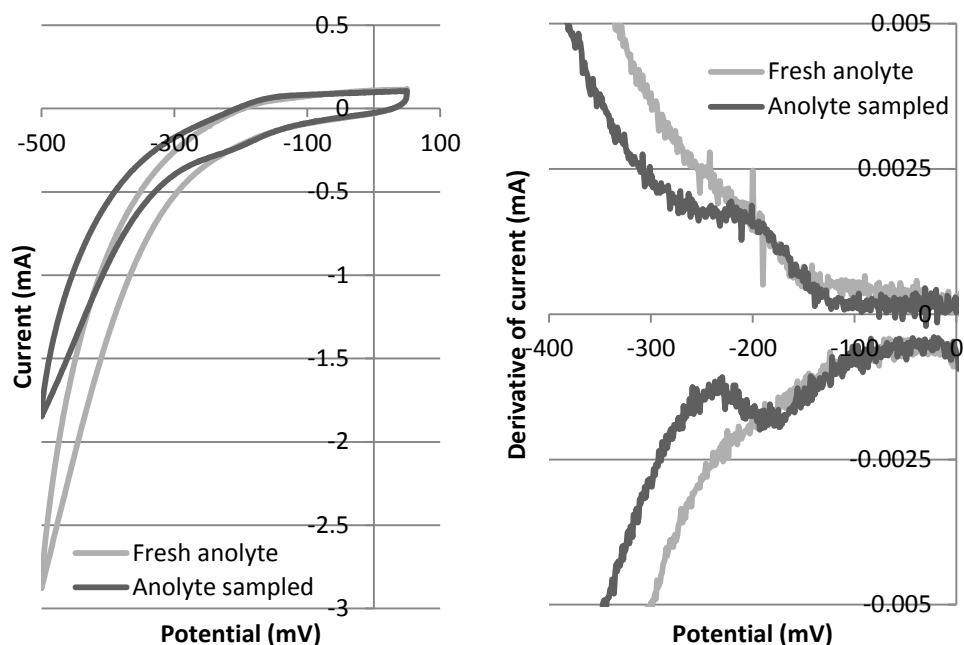


Figure 8: Cyclic voltammetry of the anolyte sampled from the PMFC and the fresh anolyte. Left: Potential vs current. Right: Potential vs derivative of current to highlight the redox couple. Scan was performed with clean graphite felt electrodes (3 mm thick, 10 cm²). Scan started at an anode potential of 50 mV, decreased to -500 mV and increased back to 50 mV at a scan rate of 10 mV s⁻¹

Besides mediators, electrons could also directly be transferred to an anode via conductive microorganism on the plant roots [112]. However, this was likely not occurring over centimeter distance in the PMFC as disconnecting one anode in the top layer did not result in a direct increase in current in the neighboring anodes (data not shown). This was confirmed by impedance spectroscopy, which showed the same ohmic resistance between anodes with roots as anodes without roots. Anodes in the top layer with roots had an average ohmic resistance of $16.5 (+/- 2.2) \Omega$ which was similar as the anodes without roots ($16.6 (+/- 2.0) \Omega$).

4.4 Conclusions

Most current was generated close to the plant roots in the PMFC. After the start up period, the current of the top layer increased by more than three times due to the presence of the roots. The current generation of the top layer was also more than three times higher than in layers without roots. The current generation of the second layer with some roots was almost twice the current of the layers without roots. The significant increase in current in the top layers was due to the rhizodeposition of the plants. The current was likely generated both from living and dead plants. Likely both oxygen and organic substrate were present at the same time, which probably resulted in internal currents and thus the loss of electron donors. Naturally produced mediators were seemingly present in the PMFC transferring electrons. Electrons were likely not transferred over centimeter distance through conductive microorganism on the roots in the PMFC. The results clearly show that the PMFC should be installed at the location in the rhizosphere with most roots to generate the highest current and power.

4.5 Acknowledgement

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Part two:

Plant microbial fuel cells applied in wetlands

Chapter 5

Plant Microbial Fuel Cell applied in Wetlands: spatial, temporal and potential electricity generation of *Spartina anglica* salt marshes and *Phragmites australis* peat soils

Abstract

The plant microbial fuel cell (PMFC) has to be applied in wetlands to be able to generate electricity on a large scale. The objective of this PMFC application research is to clarify the differences in electricity generation between a *Spartina anglica* salt marsh and *Phragmites australis* peat soil based on experimental data and theoretical calculated potential. PMFC in salt marsh generated more than 10 times more power than the same PMFC in peat soil (18 vs 1.3 mW m⁻² plant growth area). The salt marsh reached a record power output for PMFC technology per cubic meter anode: 2.9 W m⁻³. Most power is generated in the top layer of the salt marsh due to the presence of the plants and the tidal advection. The potential current generation for the salt marsh is 0.21-0.48 A m⁻² and for peat soil 0.15-0.86 A m⁻². PMFC technology is potentially able to generate a power density up to 0.52 W m⁻², which is more than what is generated for biomass combustion or anaerobic digestion using the same plant growth area.

Keywords:

Plant microbial fuel cell, salt marsh, peat soil, *Phragmites australis*, *Spartina anglica*, bio-electricity

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5.1 Introduction

Sustainable and reliable energy generation is required to cope with the growing energy demand, the depletion of fossil fuels and environmental pollution. This energy generation is related to natural energy sources as solar energy, wind energy and bio-energy. One of the emerging technologies is the Plant Microbial Fuel Cell (PMFC) which generates electricity from living plants [9]. A PMFC is a bioelectrochemical system in which rhizodeposits of the plants are oxidized by electrochemically active bacteria in the anode. These rhizodeposits consist of exudates, lysates and secretions [123]. The released electrons flow through an external circuit, harvesting electrical power, to the cathode where oxygen is reduced to water. The PMFC generates continuous electricity and is based on renewable natural processes.

Theoretically, a maximum electricity generation of 1.6 to 3.2 W m⁻² plant growth area (PGA) was envisaged for respectively a natural-sediment and high-tech implementations [32]. These theoretical maxima were based on best known efficiencies of the main process steps. Currently, a maximum power of 0.24 W m⁻² PGA is achieved [100]. PMFC can only be applied in waterlogged conditions to maintain anaerobic anode conditions and to facilitate ion transportation [9]. PMFC is already investigated on green roofs, combining the advantage of green roofs with electricity production [12], and rice paddy fields, combining food production and electricity production [10]. Recently, PMFC were commercially applied at 100 m² PGA scale to power LEDs [124].

Wetlands are the ideal natural area for PMFC. Worldwide, 8-10 million square kilometers of land is covered by wetlands [28]. The enormous area of wetlands reveals the potential of PMFC and wetlands can therefore be considered as a new source of electricity. PMFC research focused mainly on lab scale systems, comparing for example different plant species [15], growth medium [33] and cathodes [100]. The effect of different wetlands on the performance of the PMFC is not thoroughly analyzed. Each wetland has its own specific conditions on among others climate (e.g. solar radiations and temperature), salinity, plant species and nutrient availability. These factors influence plant growth and exudation which will most likely affect the electricity generation of the PMFC. However, electricity generation in PMFC is not only related to rhizodeposits of the plants, but also to the presence of alternative electron donors [33]. Possible alternative electron donors are

sulfide, organic fertilizer [34], biodegradable dissolved organic matter and dead biomass (e.g. phytoplankton, algae, seeds).

Microbial fuel cells have also been applied in many types of sediment, oxidizing electron donors in the sediment. These sedimentary microbial fuel cells (SMFC) were initially developed by Reimers et al., who showed that marine sediments can continuously generate electricity by oxidizing organic matter [35], mainly from phytoplankton detritus and sulfide [36]. Marine SMFC do not deplete and have been used to power a meteorological buoy [37]. Although marine SMFC are the only SMFC who are practically applied [125], also SMFC have been analyzed in other types of sediment and wetlands (e.g. river sediments [38], rice paddy fields [10] and forested wetland [99]). Applying PMFC in a wetland rich with organic carbon and other alternative electron donors, likely results in a higher power output than what is achieved in SMFC and PMFC systems separately. Understanding and quantifying the availability of electron donors from the plants and other sources is important to estimate the potential of wetlands for PMFC technology.

Next to alternative electron donors also alternative electron acceptors are present in wetlands. Alternative electron acceptor are mainly oxygen (due to diffusion from air and excretions by the plants), carbon dioxide, nitrate and sulfate [18]. The presence and usage of alternative electron acceptors lowers the coulombic efficiency of the PMFC and also results in a lower voltage efficiency due to mixed potentials in the anode. The total amount of converted electron donors and the coulombic and voltage efficiency in wetlands determines the electricity generation of PMFC. Therefore not every wetland will generate the same amount of electricity and thus results from one wetland cannot be directly applied to another wetland.

The objective of this PMFC application research is to clarify the differences in electricity generation between a *Spartina anglica* salt marsh and *Phragmites australis* peat soil. At different times and depths, current generation was analyzed as well as substrate availability (i.e. electron donors) of the PMFCs. With these spatial and temporal analyses, we are able to quantify the different sources of electron donors and to gain more insight where to install PMFC to reach the maximum electricity production. Multiple PMFCs were installed in two different laboratorial wetlands. Both wetlands are widespread over the Netherlands and were collected in contrasting areas. The first wetland is a salt marsh with *S. anglica* plants. *S. anglica* is the model plant of PMFC research and spread out through a large part of the coastline in the

Netherlands and coastlines in other parts of the world (e.g. Western Europe, Australia, East Asia and North America) [46]. The second wetland is peat soil with *P. australis*. *P. australis* is spread out in temperate and tropical zones including the entire Netherlands [46]. Based on the gained insights, a theoretical model was developed to predict the maximum current and power output of PMFC in these wetlands based on the specific locations.

5.2 Materials and methods

5.2.1 Experimental setup

All experiments were performed in a salt marsh wetland and peat soil wetland in our lab. Both wetlands were collected in the natural locations, by digging out the entire wetland including the soil, water and plants. The wetlands were installed in polypropylene pallet boxes with an area of 1.2 m by 1.0 m and a depth of 1.0 m. The salt marsh was collected close to Krabbendijke in the Oosterschelde, the Netherlands (GPS coordinates N51°.446788, E004°.093096) and consisted of marine sediment, seawater and *S. anglica* plants. The fen peat soil was collected in the national park the Alde Feanen, the Netherlands (GPS coordinates N53°.131038 E005°.934536) and consisted of peat soil, ground water and *P. australis* plants.

In each wetland, 9 PMFC were installed at different depths, respectively 3 PMFC at 10 cm, 3 at 30 cm and 3 at 50 cm below the sedimentary surface level (Figure 1). Each PMFC was made of a PVC tube (75 mm diameter) which was placed vertically in the wetlands. At the bottom side of the tube a PVC cap was glued on the tube with a cation exchange membrane (62 mm diameter, fumasep FKB, FuMa-tech GmbH, St. Ingbert, Germany). The anode of each PMFC was located in the wetland, directly under the membrane and consisted of graphite felt (62 mm diameter, 6 mm thickness, Grade WDF, National Specialty Products Carbon and Graphite Felt, Taiwan) with a golden wire as current collector. The inside of the tube was the cathode with a graphite felt (62 mm diameter, 6 mm thickness) and golden wire current collector directly on the membrane. The cathodes consisted of 500 cm³ 50 mol m⁻³ ferricyanide with 20 mol m⁻³ phosphate buffer. The reduction of ferric- to ferrocyanide is fast resulting in a minimal resistance of the cathode. Ferricyanide is therefore suitable to investigate the limitations of the anode. For sustainable electricity production, ferricyanide cannot

be used as final electron acceptor. Anode and cathode were electronically connected via a resistance box. 3M KCl Ag/AgCl (+205 mV vs NHE) reference electrodes were placed at all anodes and cathodes to measure the anode, cathode and membrane potential. Therefore, all anode and cathode potential in this paper are shown in mV vs Ag/AgCl.

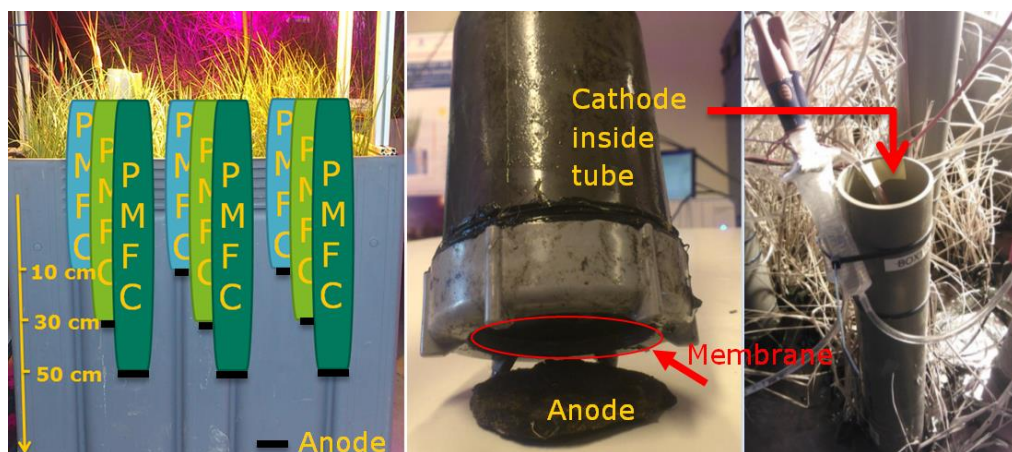


Figure 1: Left: 9 PMFC at different depths, 3 at 10 cm, 3 at 30 cm and 3 at 50 cm below the surface level. Each vertical tube PMFC consisted of a graphite felt anode, which is located in the wetland. The membrane is located directly above the anode. A graphite felt cathode is located inside the tube. Middle: The bottom part of the tube. Right: Picture of tubes installed in wetland

5.2.2 Operation

The complete setup, including the wetlands and PMFC, were installed more than 200 days before the experiment started to allow roots to grow through the anode felt. The PMFC were operated at 1000 Ω during this period. The *S. anglica* plants died after the winter period and new plants were placed in the marine sediment 45 days before the experiment. The water level in the salt marsh was pumped in and out the box to simulate the tidal effect and fluctuated every six hours from approximately 5 cm below the surface level to approximately 5 cm above the surface level and back. The water level of the peat soil was kept at the same height with a float valve approximately 5 cm below the surface level. The temperature in the lab was approximately 25 °C. Each box was illuminated with 1200 W light consisting of two Philips MASTER GreenPower CG T 400 W lightbulbs and one Philips MASTER HPI-T Plus

400 W lightbulb. The light:dark ratio was 14:10 hours with an average PAR light intensity during illumination of $487 (+/- 351) \mu\text{mole m}^{-2} \text{s}^{-1}$ measured at the top, middle and base of the plants with a light meter (Li-250A, Li-cor, Lincoln, USA).

5.2.3 Measurements

The wetlands were analyzed with long term polarization by manually changing the external resistance in steps of at least 24 hours. This period was selecting because it was expected that the PMFCs would operate stably after this period and the performance of the cells could be analyzed per external resistance. The used external resistance and time per step are shown in table 1. The average of the last hour per step is shown in the results.

The cell voltage and the anode, cathode and membrane potential were measured and logged similar as in previous PMFC research [33]. The pH (Liquisys M, Endress+Hauser, Reinach, Switzerland) and conductivity (Cond 315i, WTW, Weilheim, Germany) were measured. Sulfide concentration was measured with Dr. Lange (LCK653) at the end of the experiment. The sulfide concentration was measured close to every PMFC at the same depth. At the end of the experiments, the cells were taken out of the wetlands and roots growing through the anode felt was analyzed. Plant vitality was visually evaluated during the experiment based on the presence and growth of green leaves .

Table 1: Operation time and applied external resistance during each step in the long and short term polarization

External Resistance	Time	External Resistance	Time	External Resistance	Time
16000 Ω	66 h	500 Ω	67 h	1000 Ω	46 h
8000 Ω	29 h	250 Ω	24 h	2000 Ω	27 h
4000 Ω	24 h	100 Ω	28 h	4000 Ω	25 h
2000 Ω	24 h	250 Ω	25 h	8000 Ω	25 h
1000 Ω	25 h	500 Ω	43 h	16000 Ω	25 h

5.2.4 Calculations

All resistances, voltages, currents and power output are calculated as the average of all cells per wetland unless stated otherwise. Two cells are partly excluded for data analyses. One cell at 30 cm is excluded,

because several times the cell short circuited resulting in a cell potential of 0. The data of one cell at 50 cm is only partly analyzed, because of very high (approximately 400 mV) membrane voltage, likely due to an air bubble in the reference electrode. This cell is only used for the current and power calculations. The plant growth area (PGA) is calculated based on the surface area of the anode felt (30 cm²). In this experiment, a vertical tube is placed on top of the anode. However, the anode surface area would be covered by plants when applying the PMFC invisibly in wetlands. Therefore the PGA is considered as the projected anode surface.

The total internal resistance of the PMFCs was calculated as the sum of the anode (R_{an}), cathode (R_{cat}) and membrane resistance (R_m). The anode resistance (eq 1) was based on the theoretical anode potential. For the salt marsh the theoretical anode potential was estimated at -700 mV, the theoretical potential of sulfide oxidation. For the peat soil the theoretical anode potential was estimated at -430 mV, the potential of acetate oxidation at pH 5.8, the pH of the peat soil (50 mol m⁻³ acetate). The cathode resistance (eq 2) was calculated based on the open cell potential of the cathode. The anode, cathode and membrane (eq 3) resistance were calculated according to the following formulas using the current (i) and the measured potential (E).

$$R_{an} = \frac{E_{an} - E_{an}^{th}}{i} \quad (\text{eq 1})$$

$$R_{cat} = \frac{E_{cat}^{OCV} - E_{cat}}{i} \quad (\text{eq 2})$$

$$R_m = \frac{E_m}{i} \quad (\text{eq 3})$$

The theoretical current generation was calculated based on the available carbon. The yearly carbon availability (in mol m⁻² y⁻¹) was calculated from the productivity of the plants and the external flux. Multiplying the total carbon by the faraday constant and the 4 electrons per carbon atom when oxidizing acetate gives the amount of Coulombs per year. The current generation in A m⁻² is calculated by dividing the coulombs by the amount of seconds per year. Acetate oxidation was selected because acetate was found as the major organic exudate in a rice PMFC and addition of acetate resulted in higher power output showing that acetate was oxidized by the electrochemically active microorganisms

[10]. For oxygen the same calculations is used with 4 electrons per oxygen atom when reducing oxygen to water.

5.3 Results and discussion

5.3.1 *The anode mainly limits current density in both wetland PMFCs*

The PMFCs were installed in the wetlands more than 200 days prior to the experiment and operated at 1000 Ω to allow roots to grow through the anode felt [9]. During the start-up period, *S. anglica* plants died. Therefore, fresh plants were collected and placed in the wetlands 45 days before the experiment. All results are based on the operation during a long term polarization. A long term polarization was selected to determine the maximum power of the PMFCs in the wetlands. Short term polarization overestimate the maximum power and do not explain long term results [52]. The external resistance was gradually decreased from 16000 Ω to 100 Ω and gradually increased back to 16 k Ω in steps of at least 24 hours. During the long term polarization, the anode resistance is the main resistance in both wetlands on average more than 75 % of the total internal resistance in both wetlands (figure 2). The anode therefore limits the current and power density in the wetland PMFCs. The anode is located in the wetland and thus the differences in current and power density between wetlands can be mainly ascribed to processes in the wetland and not to the membrane or cathode. The cathode and membrane resistance (i.e. ionic, pH and transport resistance [25]) are low, together on average less than 25 % of the total internal resistance (figure 2). The cathode resistance increased at the end of the polarization, likely due to depletion of ferricyanide in the catholyte.

5.3.2 *Salt marsh PMFC generates more power than peat soil PMFC due to sulfide oxidation*

The salt marsh reached a two week average power density of 18 mW m⁻² PGA, while the peat soil reached an average power density of 1.3 mW m⁻² PGA. The long term power generation of the salt marsh with *S. anglica* was more than 10 times higher than of the peat soil with *P. australis*. The power generation of the PMFCs in both wetlands is visualized per layer at 10, 30 and 50 cm depth in figure 3. Both the current density and cell voltage were higher for the salt marsh than for

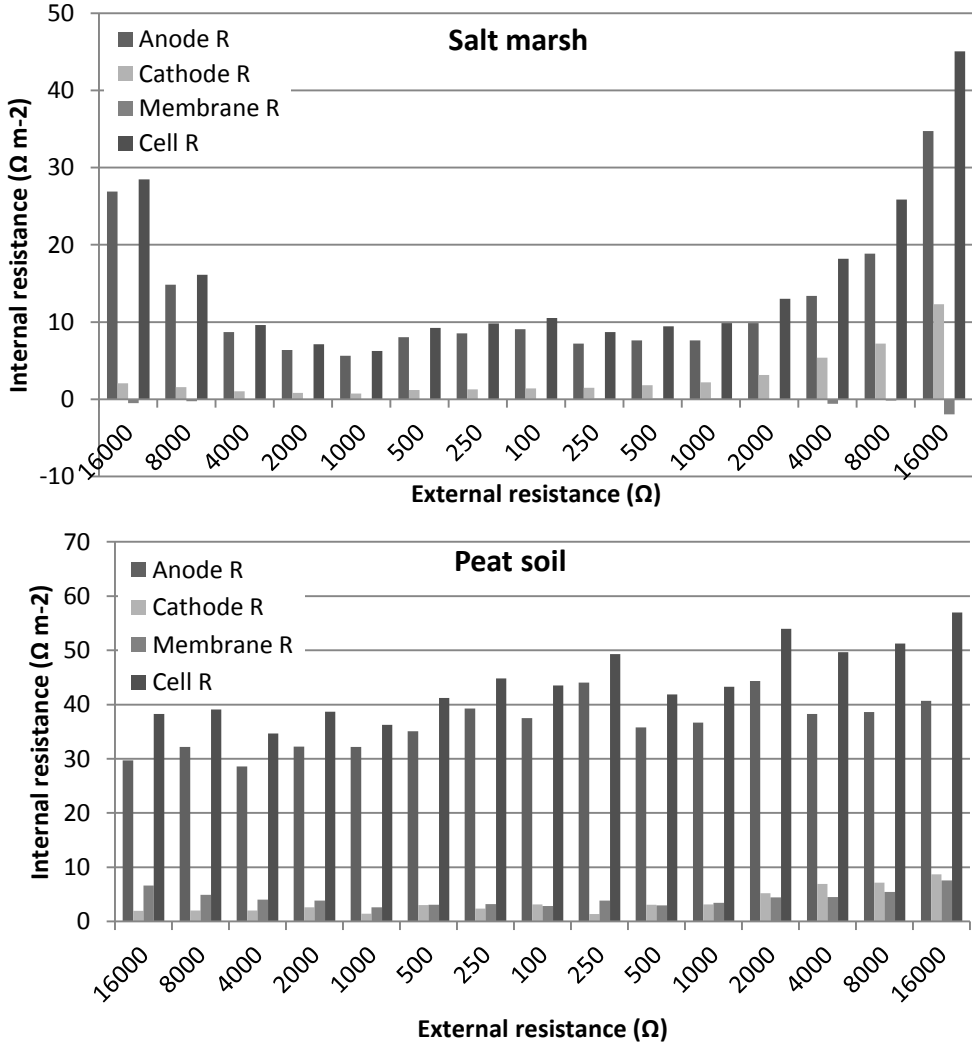


Figure 2: Average anode, cathode, membrane and total internal resistance of the salt marsh with *S. anglica* and peat soil with *P. australis* during the long term polarization

the peat soil, on average respectively 83 mA m^{-2} PGA and 214 mV for the salt marsh versus 14 mA m^{-2} PGA and 93 mV for the peat soil. The PMFC were operated at a changing external resistance. Likely a higher two week average power density is generated when the cells would be operated at the maximum power point for two weeks. The maximum power of the salt marsh was achieved at an external resistance of 1000Ω and on average (of all 9 cells) 34 mW m^{-2} PGA was generated. In the peat soil, the maximum power was achieved at an external resistance of

16 k Ω and on average 3.1 mW m⁻² PGA was generated. The higher power output of the salt marsh is explained by the high conductivity and the high concentration of sulfide (162 mg L⁻¹) (table 2), which was also oxidized next to the present organic matter. The oxidation of sulfide in marine sediment is fast as it is limited by mass transfer [36]. Addition of sulfide in laboratory fuel cells resulted in lower anode potential and increased currents [126]. No sulfide (detection limit: 0.1 mg L⁻¹) was measured in the peat soil. The sulfide/sulfur redox couple acts as a mediator in the marine sediment PMFCs [127]. Sulfide is chemically oxidized to elemental sulfur. Sulfur reducing bacteria are able to anaerobically produce sulfide from sulfur [128].

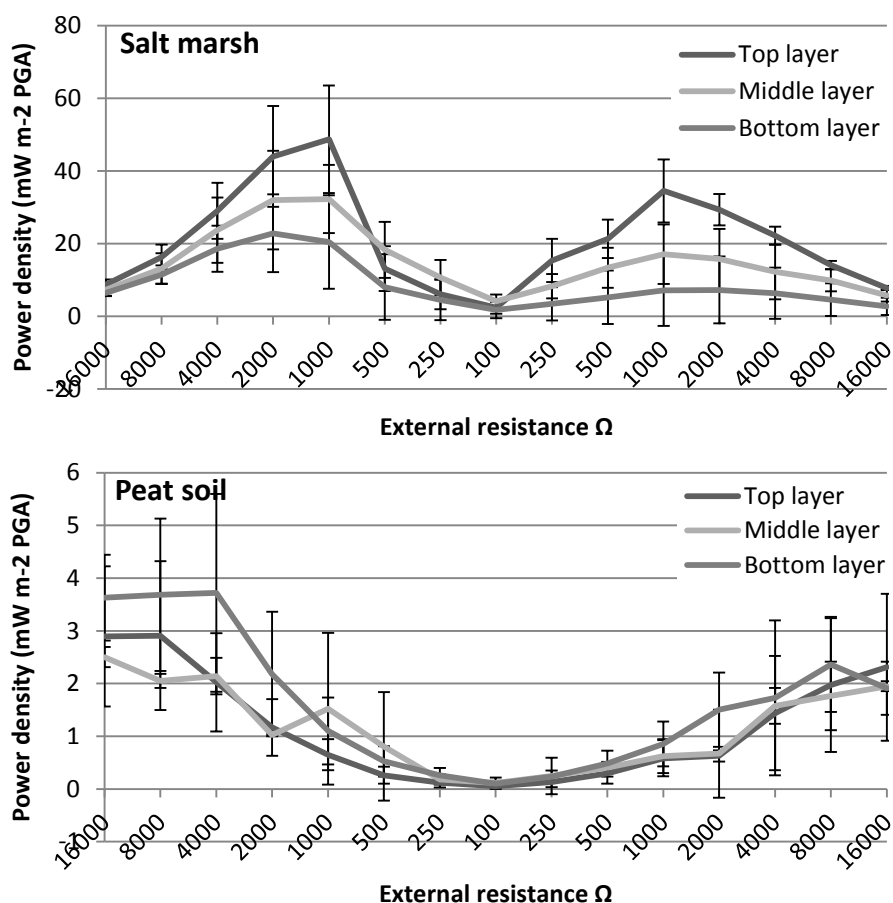


Figure 3: Power density at 10, 30 and 50 cm depth of salt marsh with *S. anglica* and peat soil with *P. australis* with gradually decreasing (16000 to 100 Ω) and increasing (100 to 16000 Ω) external resistance. The error bars indicate 1SD

Table 2: Sulfide concentration (detection limit: 0.1 mg L⁻¹), pH and conductivity of the salt marsh and peat soil

	Salt marsh	Peat soil
S ²⁻ (mg L ⁻¹)	162	0
pH	7.3	5.8
Conductivity (mS cm ⁻¹)	43	0.53

5.3.3 High sulfide concentration in salt marsh results in record volumetric power output

The long term two week average power generation of the salt marsh is higher than the long term power generation of the best performing PMFC per cubic meter anode. The two week average power generation of the salt marsh was 2.9 W m⁻³ anode while the best performing PMFC reached 0.8 W m⁻³ anode [100]. In both experiments the same plants were used. The higher power output is explained by the oxidation of sulfide and not by oxidation of organic material from the plants. The peat soil produced 0.2 W m⁻³ anode. Calculating the power generation in W m⁻³ anode derives more comparable results than calculating them in W m⁻² PGA. The cathode is neglected because two different cathodes are used. In this experiment a ferricyanide cathode was used, the best performing PMFC was operated with an oxygen reducing biocathode. The PMFCs in the wetlands had an anode of only 6 mm in depth, while the best performing PMFC was operated with an anode depth of 15 cm. The power generation in W m⁻³ anode gives new insights towards the applicability of PMFC in wetlands. The experimental results indicate that application of PMFC in salt marsh is promising as even more power is generated than in earlier lab scale experiments. Application of PMFC in a peat soil seems less likely due to the low power output and therefore more material is needed for the same power output. More than 6 times more anode felt is required in peat soil than in salt marsh to reach the same current generation. Our results are comparable to results of earlier research on SMFC in marine sediment which generated 10 W m⁻³ for two days [37]. The higher power density compared to our research (2.9 W m⁻³ anode for two weeks) is likely due to the shorter period of operation.

5.3.4 Top layer of salt marsh reaches highest power output because of tidal advection

The top layer (at 10 cm below the sediment surface) of the salt marsh generates more power than the lower layers (at 30 and 50 cm) (figure 3). The top layer generates a two week average power density of 25 (+/- 7) mW m⁻² PGA while the middle layer generates 18 (+/- 8) mW m⁻² PGA. On average 36 % more power is produced in the top layer than in the middle layer of the salt marsh. The power output in the bottom layer is even lower than in the middle layer, however this is mainly caused by a more depleted ferricyanide in the cathodes in the bottom layer. There are two possible explanations for the higher power generation in the top layer. First, the top layer is the layer with most of the plant roots. In contrast to the peat soil, there are no dead roots or peat in the middle and bottom layer in the salt marsh. Power in the middle and bottom layer is thus mainly generated by oxidizing electron donors in the marine sediment and not from oxidizing dead roots. No roots were visible on the anode felt in the top layer (data not shown), however the presence of the roots likely increased rhizodeposition close to the anode. More rhizodeposition also increased the substrate availability for the PMFC and thus likely more power can be generated. The roots were not yet growing through the anode felt, because the startup period of 45 days for *S. anglica* was not long enough. It is expected that more electricity is generated when the plants are growing through the anode felt because in that case substrate would be generated directly on the anode felt and the plants likely provide more electrons to the PMFC.

The second explanation for the higher power output in the top layer is the influence of the tides. The experimental data clearly shows a tidal related pattern in the power generation of the top layer (Figure 4). The data are generated during operation at an external resistance of 1000 Ω during the period that the external resistance was gradually increased. In the middle and bottom layer power density is nearly constant and tidal pattern is not visible, while the tidal pattern is visible in the top layer. Also the overall power is increasing and likely more time is required for stable operation when the PMFC are influenced by the tides. At low tide, the pressure difference results in advection of sea water in the top layer [129]. This enhances the transfer of electron donors, for example dissolved organic matter and sulfide, towards the anode in the top layer at low tide. The power output in marine SMFCs is limited by mass transfer [36]. Enhancing the mass transfer results in

more electron donors on the anode surface and thus more available substrate for the PMFC. Therefore, more power can be generated in the top layer than in the lower layers. Also in marine SMFC, advection resulted in a higher power output [129].

Next to the plants and tidal effect, also the sediment properties will likely affect power output of PMFC in wetlands. The oxygen concentration, redox potential, pH and trace metal concentration including the electron donor sulfide vary over depth and show seasonal variations [130]. SMFC can also alter the microbial composition and pH of the soil [131]. Further research is required to analyze to what extent these sediment properties affect the power output of the PMFC.

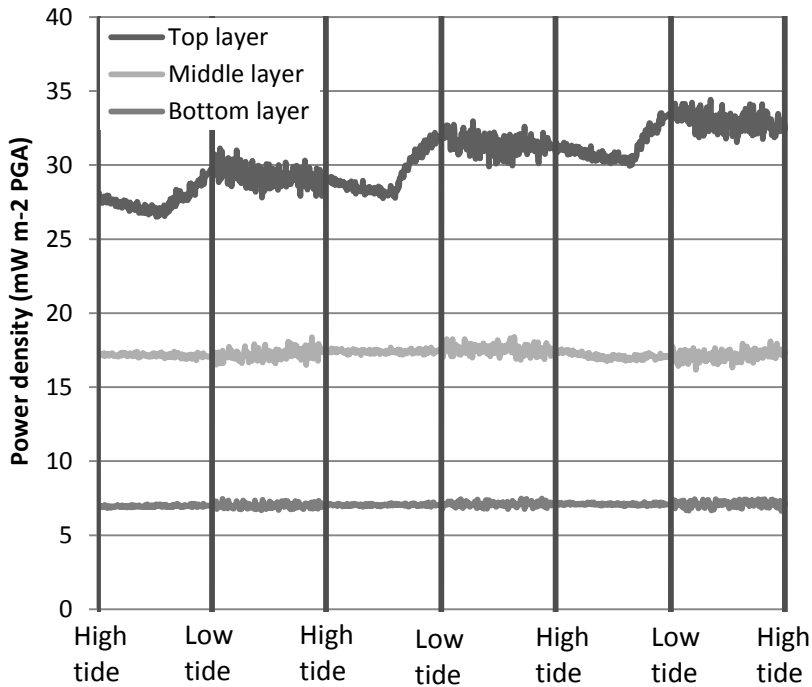


Figure 4: The tidal influence on power generation of the PMFCs in the top layer. Power generation increased towards low tide due to advection. Minor fluctuations are due to tidal pumps. Data is during second time control at 1000 Ω . High and low tide indicates maximum and minimum water level

5.3.5 No significant difference in power generation between different layers in the peat soil

In the peat soil, the bottom layer (at 50 cm) generates $1.6 (+/- 0.7)$ mW m^{-2} PGA which is slightly higher than the top ($1.3 +/- 0.6 \text{ mW m}^{-2}$ PGA at 10 cm) and middle ($1.1 +/- 0.5 \text{ mW m}^{-2}$ PGA at 30 cm)(Figure 3). There is no significant difference in power density between the three layers. Although the presence of roots is different between these three layers. In the top and middle layer, roots were growing through the anode felt, unlike the bottom layer where no roots were growing through the felt (Figure 5). During the installation of the wetlands, clearly roots were abundantly present in all layers of the peat soil. Electricity is generated in all layers of the peat soil, while not in all layers living roots are growing through the felt. This indicates that electricity is likely generated by the hydrolysis of dead roots. These results are in line with previous PMFC research in which a model predicted that power in PMFC was generated by hydrolysis of dead roots and not by the exudates of living roots [105]. Also electron donors could be transported from the top and middle layer to the bottom layer via mediators for example humic acid [132]. Roots were growing through the anode felt and the above ground biomass increased showing the vitality of *P. australis* plants under the laboratorial conditions.

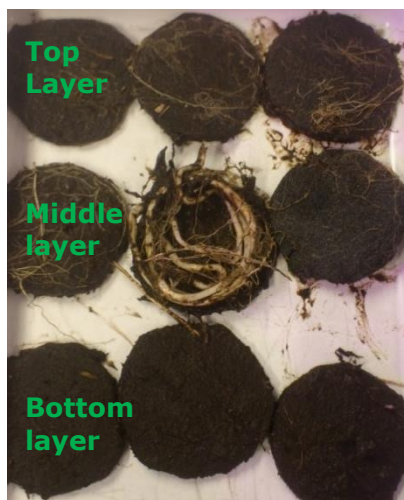


Figure 5: Presence of roots on the anode felt in the peat soil (anodes removed at end of experiment). In the top and middle layer roots are growing through the felt while in the bottom layer no roots are growing through the felt

3.6 Potential current generation likely in the range of 0.21-0.48 A m⁻² PGA for salt marshes and 0.15-0.86 A m⁻² PGA for peat soils

The current generation in PMFC in wetlands is based on the availability of substrate and the actual Coulombic efficiency. The theoretical maximum current generation of the wetlands can be predicted by calculating the availability of substrate, i.e. the electron donors. The electron donors are rhizodeposits (exudates, lysates and secretions [123]) and external carbon flux. Sulfide oxidation is not separately calculated, because the sulfur/sulfide redox couple acts as a mediator with carbon as the energy source [127].

Common rhizodeposition was roughly estimated to be 27 % of the carbon that the plants allocate to the roots [133]. From all rhizodeposits, 61 % (16.5 % of plant carbon allocated to roots) is used for microbial rhizosphere respiration (i.e. carbon consumption by microorganism releasing CO₂) and the rest are mainly soil residues (e.g. fine roots debris) [133]. The carbon used for microbial rhizosphere respiration can be microbially degraded and are therefore also the fuel of the PMFC, while the soil residues likely result in soil accretion. Hemminga et al. provided an overview of the reported below ground production of *S. anglica* and *S. alterniflora* [134]. The reported minimum and maximum below ground dry matter production of *Spartina* is respectively 350 g m⁻² y⁻¹ and 6.5 kg m⁻² y⁻¹ (estimated from figure in Hemminga et al. [134]), which equals a carbon mass of 140-2600 g m⁻² y⁻¹ assuming a carbon mass fraction of 40 % of the dry matter [134]. The dry weight below ground production does not include the common rhizosphere respiration, the respiration both by the microorganisms and by the roots (33% of total carbon allocated to roots of which 50% can be assumed to be used for microbial respiration) [133]. Resulting in more carbon allocated to the roots, ranging from 186-3458 g m⁻² y⁻¹ for *Spartina*. The carbon provided by *Spartina* to the PMFC (16.5 % of the carbon allocation to the roots) is thus in the range of 31-571 g m⁻² y⁻¹. The external carbon flux in the Oosterschelde, the location where the *S. anglica* in this research is collected, is approximately 375 g m⁻² y⁻¹ [135]. In total, a carbon mass of 406-946 g m⁻² is yearly potentially available in salt marshes. This is equivalent to a potential current density of 0.41-0.96 A m⁻² PGA for the PMFC.

The below ground dry matter production of *P. australis* in three locations was estimated by a model [136]. The lowest dry matter production was 1747 g m⁻² and the highest dry matter production was

9874 g m⁻², equaling a carbon mass 751-4246 g m⁻² y⁻¹ assuming a carbon mass fraction of 43 % of the dry matter [137]. The carbon allocated to the roots is 999-5647 g m⁻² y⁻¹ including rhizosphere respiration. Yearly, a carbon mass of 165-932 g m⁻² is used for microbial rhizosphere respiration and thus potentially available in peat soils assuming no external carbon flux. This amount of carbon equals a potential current density of 0.17-0.95 A m⁻² PGA for the PMFC.

The above calculated current densities are theoretical values assuming all carbon for microbial respiration is oxidized by microorganisms in the PMFC. The practical range of current generated by the PMFC is probably lower than the estimated range because these potential ranges do not take into account the alternative electron acceptors [18]. Plants excrete the alternative electron acceptor oxygen to the soil to maintain the necessary aerobic metabolism and to protect the vulnerable parts by oxidizing potential phytotoxins [138]. This process will lower the current generation [105]. Radial oxygen loss of *P. australis* is reported in the range from 1.6 – 12 g m⁻² d⁻¹ [139, 140] which is equivalent to a decrease in current generation ranging from 0.2-1.7 A m⁻² PGA. Subtracting this decrease from the theoretical current generation results in a potential current generation in the range of -1.53 to 0.75 A m⁻² PGA. Detailed long term data of radial oxygen loss of the complete root system is necessary to estimate the practical current generation when applying PMFC in that wetlands.

To provide a better estimate of the potential current generation also the methane production in the wetlands can be evaluated as electrochemically active microorganisms are able to outcompete methanogens for electrons. Methane emissions were reduced both in the PMFC and sediment-MFCs when the cells were in operation compared to open cell conditions [39, 141]. By knowing the rate of methane production in wetlands also the current generation can be estimated. Yearly, up to 15 % of the net fixed carbon may be released to the atmosphere as methane in *P. australis* peat soils [142]. Therefore, at least a carbon mass of 150-847 g m⁻² y⁻¹ is available for current generation. According to this theory, peat soils can generate a current density of 0.15-0.86 A m⁻² PGA. This estimation is only suitable for fresh wetlands, because significant lower methane is produced in salt marshes [143] and a large part of the current is generated by oxidizing sulfide. However, also a more realistic potential current generation in the salt marsh can be estimated using the sulfur cycle. Sulfur reducing bacteria reduce sulfur to sulfide while oxidizing carbon. At least 50 % of the

carbon ($203\text{--}473 \text{ g m}^{-2} \text{ y}^{-1}$) is used to produce sulfide in salt marshes [144]. Assuming that all the sulfide is oxidized in the PMFC a current of $0.21\text{--}0.48 \text{ A m}^{-2}$ PGA can be generated in salt marshes.

Determination of the theoretical potential current generation was assessed on two approaches for both the *P. australis* peat soil and the *Spartina* salt marsh. Presently insufficient data is available on e.g. plant growth, rhizodeposition, exudation, radial oxygen loss and accretion, for a more accurate prediction of the theoretical maximum current generation. Only the carbon allocated to the roots is included in the calculation, the effect of the above ground biomass on the rhizodeposition (i.e. burial of plant litter) and thus the current generation is not included. Further insight on the actual processes and data from specific locations is required for a justified detailed estimate. At present, the best method to determine the potential electricity generation for *P. australis* peat soils is the methane emissions approach, because the electrochemically active microorganism are able to significantly outcompete the methanogens [145]. According to this method, the potential current generation is in range of $0.15\text{--}0.86 \text{ A m}^{-2}$ PGA for the peat soil. The best method for *Spartina* salt marshes is the sulfur cycle approach, because the sulfide produced by sulfur reducing bacteria can be directly oxidized at the anode. The potential current generation is ranging from $0.21\text{--}0.48 \text{ A m}^{-2}$ PGA for the salt marsh.

The potential calculated ranges for the *P. australis* peat soil and *Spartina* salt marsh are not in line with the experimental data. The experimental data show a more than 10 times higher power output of the salt marsh compared to the peat soil. The theoretical potential ranges partly overlap and the theoretical maximum is clearly higher for the peat soil. This contrast between the experimental data and potential data is likely caused by the presence of sulfide. Sulfur and sulfate reducing bacteria have produced a high concentration of sulfide in the anaerobic zone of the salt marsh. In absence of an electron acceptor this concentration of sulfide remains high. By providing an electron acceptor, the anode of the PMFC, the sulfide will be oxidized to elemental sulfur. The oxidation of sulfide is fast and therefore when applying PMFC in a salt marsh first the sulfide close the anode will be oxidized and possible higher current density are measured than calculated. Once the sulfide close to the anode is oxidized, then the rate of organic matter oxidation by sulfur reducing bacteria determines the current density.

5.3.7 Outlook: Application of PMFC in wetlands

Spartina salt marshes can potentially generate a power of 0.14–0.34 W m⁻² PGA depending on the plant growth. *P. australis* peat soil potentially generates 0.09–0.52 W m⁻² PGA. This potential power density is based on the calculated potential current density and the best obtained cell voltage. The cell voltage of PMFC in peat soil is assumed to be equal to the cell voltage in the best performing PMFC which operated at a cell potential of 0.6 V [100]. The cell potential of the salt marsh is assumed to be 0.7 V, which is 0.1 V higher than cell potential of the best performing PMFC, due to the lower standard potential of sulfide oxidation (-0.70 V) compared to acetate oxidation (-0.52 V). The lower anode potential results in a higher cell potential. The cell voltage cannot be based on a thermodynamically theoretical value, because also the voltage losses have to be considered. This includes activation energy, microbial energy for maintenance and growth, ohmic losses and mixed potentials due the presence of alternative electron donors in the cathode and alternative electron acceptors in the anode [22].

To make most effective use of the materials per power output, based on current experimental insights, it is possibly most efficient to install the PMFC in the top layer of the salt marsh wetland. Long term experiments can confirm this expectation, however it could be that the sulfide present will deplete more rapidly than it is produced by sulfur reducing bacteria. To reveal the potential power of these investigated PMFC wetlands, further detailed insights are needed on the actual rhizodeposits and other electron donor fluxes and alternative electron sinks within wetlands. Also the outdoor temporal differences, including temperature, wind, seasonality and tidal effect, have to be further analyzed to reveal the actual power output of the PMFC when applied in wetlands [31, 129].

For both wetlands, the potential power generation is clearly lower than the theoretical envisaged 1.6-3.2 W m⁻² PGA. The earlier theoretical value was based on the best known efficiencies of the main processes [32]. Rhizodeposition is the main cause of the lower potential power in this study. The earlier theoretical value was based on 70 % rhizodeposition [32], while common rhizodeposition is estimated to be 11 % of the net fixed carbon [133]. Our study also shows that wetlands specific conditions including plant growth, plant species, carbon allocation, radial oxygen loss and wetland type (e.g. salt marsh or peat soil) will affect the performance of the PMFC wetland systems. Even

though the potential generated power is clearly lower than what was earlier envisaged, still more power can be generated with PMFC than using the same amount of plant growth area for biomass combustion or anaerobic digestion. For example, PMFC in *P. australis* peat soil can generate up to 0.52 W m^{-2} . Every year, 163 GJ can potentially be generated per hectare. Biomass combustion was estimated to generate $27\text{-}91 \text{ GJ ha}^{-1} \text{ yr}^{-1}$ ($0.09\text{-}0.29 \text{ W m}^{-2}$) and anaerobic digestion 2.8 to 70 $\text{GJ ha}^{-1} \text{ yr}^{-1}$ ($0.01\text{-}0.22 \text{ W m}^{-2}$) [32].

In this research, the PMFC operated with the unsustainable ferricyanide cathode. This cathode is not suitable for application of PMFC. Previous PMFC research showed that the power output of an PMFC increased when the ferricyanide cathode was replaced by a sustainable oxygen reducing biocathode due to a higher cell voltage [100]. Also the potential power density is based on this higher cell voltage. Besides a different cathode, also PMFC has to be applied with a different design. The vertical placed PVC tubes are visible in the landscape and can only be used for small cells. To upscale PMFC, a horizontal tubular PMFC with an oxygen reducing biocathode is preferred, which can invisibly be integrated in wetlands [48].

5.4 Conclusions

PMFC in *S. anglica* salt marsh generated more than 10 times more power than the same PMFC in *P. australis* peat soil. The salt marsh reached a two week average power density of 18 mW m^{-2} PGA, while the peat soil reached an average power density of 1.3 mW m^{-2} PGA. The power generation per square meter is clearly lower than the power generation of the best performing lab PMFC (240 mW m^{-2} PGA). However, more power is produced in the salt marsh than in the best PMFC per cubic meter anode, respectively 2.9 W m^{-3} anode and 0.8 W m^{-3} anode. Resulting in a record PMFC power density per cubic meter anode. The *S. anglica* salt marsh generated most power in the top layer likely due to the presence of the plants in this layer and the tidal advection. In the *P. australis* peat soil, there is no significant difference in power density in depth, even though less living roots are present in the bottom layer. The higher power output of the salt marsh is explained by the high concentration of sulfide, which was also oxidized next to the present organic matter. The potential current generation of the *S. anglica* salt marshes is in the range of $0.21\text{-}0.48 \text{ A m}^{-2}$ PGA ($0.14\text{-}0.34 \text{ W m}^{-2}$ PGA). Peat soils potentially generate $0.15\text{-}0.86 \text{ A m}^{-2}$ PGA, which

likely generates a power output of $0.09\text{--}0.52 \text{ W m}^{-2} \text{ PGA}$. More power can potentially be generated with PMFC than using the same amount of plant growth area for biomass combustion or anaerobic digestion.

5.5 Acknowledgement

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Chapter 6

In situ start-up of oxygen reducing gas-diffusion biocathodes in wetland plant microbial fuel cells

Abstract

Application of the plant microbial fuel cell in wetlands should be invisible without excavation of the soil. The preferred design is a tubular design with the anode directly between the plant roots and an oxygen reducing biocathode inside the tube. Oxygen should be passively supplied to the cathode via a gas diffusion layer. The objective of this research is to start-up an oxygen reducing biocathode in situ in a tubular PMFC applied in a *Phragmites australis* peat soil and a *Spartina anglica* salt marsh. PMFCs with a biocathode were successfully started in the peat soil. Oxygen reduction is clearly catalyzed, likely by microorganisms in the cathodes, as the overpotential decreased resulting in an increased current density and cathode potential. The maximum daily average power generation of the best peat soil PMFC was 22 mW m⁻². PMFCs with a biocathode in the salt marsh only started with pure oxygen diffusion reaching a maximum daily average power generation of 82 mW m⁻² PGA. Both wetland PMFCs were successfully started with natural occurring microorganism in the anode and cathode. Calculations show that the power density can be increased by improving the PMFC design limiting crossover of oxygen and substrate.

Keywords

Plant microbial fuel cell, oxygen reducing biocathode, silicone gas diffusion layer, salt marsh, peat soil, *Phragmites australis*, *Spartina anglica*

This chapter has been submitted for publication:

Wetser K., Dieleman K., Buisman C.J.N., Strik D.P.B.T.B. In situ start-up of oxygen reducing gas-diffusion biocathodes in wetland plant microbial fuel cells

6.1 Introduction

The plant microbial fuel cell (PMFC) is an emerging technology which generates sustainable electricity from living plants [9]. Rhizodeposits are oxidized by electrochemical active bacteria in the anode of the PMFC producing electrons. These electrons are transported to the cathode via an external circuit where energy is harvested. Oxygen is reduced to water at the cathode. The PMFC is a sustainable technology as it has no competition for arable land or nature, the substrate (i.e. rhizodeposits) is renewable and the product is plain water [9]. Currently, 0.24 W m^{-2} plant growth area (PGA) is generated continuously in the lab (two week average) [100]. PMFCs were demonstrated in rice paddy fields, green roofs and are commercially applied on 100 m^2 PGA to power public lighting [10, 11, 31, 124].

PMFC is preferably applied in wetlands because of the required anaerobic conditions [9]. Besides the enormous land covered by wetlands ($8\text{-}10$ million km^2 worldwide [28]) also the presence of alternative electron donors is beneficial for PMFC [33]. Additional electron donors likely results in a higher power output in the substrate limited PMFC [100]. Possible alternative electron donors present in wetlands are among others sulfide, organic fertilizer, stored 'fossil' organic matter (e.g. peat matter), biodegradable dissolved organic matter and dead biomass (e.g. phytoplankton, algae, seeds). Electricity is already generated by oxidizing these alternative electron donors in sediment in the so-called sediment microbial fuel cell (SMFC). SMFC have been applied in for example marine and river sediments [36-38]. Application of PMFC in wetlands oxidize both the electron donors from rhizodeposits as the present alternative electron donors.

Oxygen is the preferred as electron acceptor, because of the high redox potential (0.6 V vs Ag/AgCl) and water as product [22]. However, the reduction of oxygen on graphite is slow and must be catalyzed in order to increase the current densities in the PMFC [26, 31]. One of the best and most common catalyst for oxygen reduction is platinum. However, the price of platinum makes it undesirable for application in a low density technology like the PMFC [53]. Oxygen reduction on graphite can also be catalyzed by self-replenishing microorganisms. The first catalysis of oxygen reduction was performed by Bergel et al, who in situ grew a biofilm on a stainless steel cathode in seawater [42]. This breakthrough resulted in plenty of research focusing on oxygen reducing biocathodes in microbial fuel cells [91]. Biocathodes have been among

others applied in the rice rhizosphere [44] and are successfully integrated in PMFC [100]. Oxygen reduction is catalyzed by microorganisms up to a current density of 0.9 A m^{-2} on graphite cathodes [86], which is higher than the maximum long term current density of a PMFC (0.4 A m^{-2} PGA) [100].

Application of PMFC in wetlands should be invisible without excavation of the soil to minimize the disturbance of the wetland. The preferred design is a tubular design with the anode on the outside directly between the plant roots and an oxygen reducing biocathode inside the tube [48] (figure 1). Oxygen should be passively supplied to the cathode, eliminating the need for energy intensive pumping of oxygen. Microbial fuel cells have been applied with passive oxygen diffusion through a polytetrafluoroethylene (PTFE) layer [146]. An alternative for PTFE is silicone, which has a high permeability for oxygen compared to other plastics and elastomers as PTFE [147]. The objective of this research is to start-up an oxygen reducing biocathode in a tubular PMFC applied in wetlands. The biocathode is preferably started in situ using the locally present microorganisms and nutrients without extra inoculation of microorganisms in the cathode. Three tubular PMFC with a biocathode were applied in a *Phragmites australis* dominated peat soil and three in a *Spartina anglica* dominated salt marsh. The differences in biocathode start-up, short and long term biocathode performance, power output and limitations are analyzed and described in this paper. Also possible improvements to the tubular design are suggested.

6.2 Materials and methods

6.2.1 Experimental setup

Three tubular PMFCs were installed at approximately 10 cm below the surface level in a *Phragmites australis* dominated peat soil and three tubular PMFCs in a *Spartina anglica* dominated salt marsh. The wetlands were installed in polypropylene pallet boxes with an area of 1.2 m by 1.0 m and a depth of 1.0 m as explained in earlier research [98]. The tubes consisted from outside to inside of an anode, membrane, cathode and a silicone tube (ISS 6, Lapp Group, Stuttgart, Germany)(figure 1). The anode was made of 3 mm thick graphite felt with an area of 210 by 90 mm (CGT Carbon GmbH, Asbach, Germany) and a graphite stick current collector. The anode is wrapped round the outside of a 300 mm

ultrafiltration membrane with an inner diameter of 24 mm (MEMOS Membranes Modules Systems GmbH, Pfullingen, Germany). 5 extra holes (± 1 mm diameter) were made in the ultrafiltration membrane to enable exchange of microorganisms from the anode to the cathode. The graphite felt cathode had the same dimensions as the anode with a golden wire current collector and is wrapped round a silicone tube and pushed inside the ultrafiltration membrane. The silicone tube has an inner diameter of 6 mm and a wall thickness of 0.9 mm. A silicone tube is selected because silicone is highly permeable for oxygen [147] and as such can provide oxygen in the cathode. PVC tubes are connected at both ends of the ultrafiltration membrane (figure 1). No anolyte or catholyte was added and electrolyte consisted thus purely of the water from the wetlands. Capillaries with 3M KCl were placed in both the anode and cathode of each PMFC and connected to a Ag/AgCl reference electrode. All electrode potentials in this manuscript are therefore in mV vs Ag/AgCl.

6.2.2 Operation

On day 1, the tubular PMFCs were installed in the wetlands. From the start of the experiment the PMFCs were controlled with a potentiostat at a cathode potential of 150 mV to start the biocathode (MCP, Bank Elektronik - Intelligent Controls GmbH, Pohlheim, Germany)[61, 100]. The three PMFCs in each wetland were controlled with one reference electrode, the reference electrode of PMFC1. The distance of the cathodes of PMFC2 and PMFC3 to the reference electrode was approximately 50 cm. For the peat soil PMFCs the cathode control was from day 1 to day 82. Afterwards the peat soil PMFC2 were controlled by an external resistance between the anode and cathode. The external resistance was set at 4000 Ω on day 84, decreased to 2000 Ω on day 87, further decreased to 1000 Ω on day 114. From day 116 until the end of the experiment (day 160) the external resistance was 500 Ω for the peat soil PMFCs. The salt marsh PMFC were cathode controlled at 150 mV from day 1 to day 82 and also from day 96 to day 136. The salt marsh were controlled by an external resistance from day 84 to day 96 and from day 136 until the end of the experiment (day 160). The external resistance was 1000 Ω from day 84 to day 86, 2000 Ω from day 87 to day 93 and 16000 Ω on day 94, day 95 and from day 136 to day 160. In each wetland, the three cathodes were electrically connected in parallel at both the cathode and anode during controlled cathode

potential operation. However, each PMFC was separately operated when the PMFCs were controlled by an external resistance. Pressurized air was pumped through the silicone tubes. This was changed to pure oxygen from day 41 to day 84 for all PMFCs. For the salt marsh PMFC, pure oxygen was also supplied to the silicone tube from day 89 until the end of the experiment.

The water level in the peat soil was kept at 5 cm below the surface level with a float valve connected to a tap water supply. In the salt marsh, a tidal effect was simulated by increasing and decreasing the water level from 5 cm above to 5 cm below the surface level twice every day by pumping the seawater in and out a rain barrel. The temperature in the lab was approximately 25 °C. Each wetland was illuminated by 2 600 W LED grow lights during 14 hours per day placed 1.5 meter above the surface level.

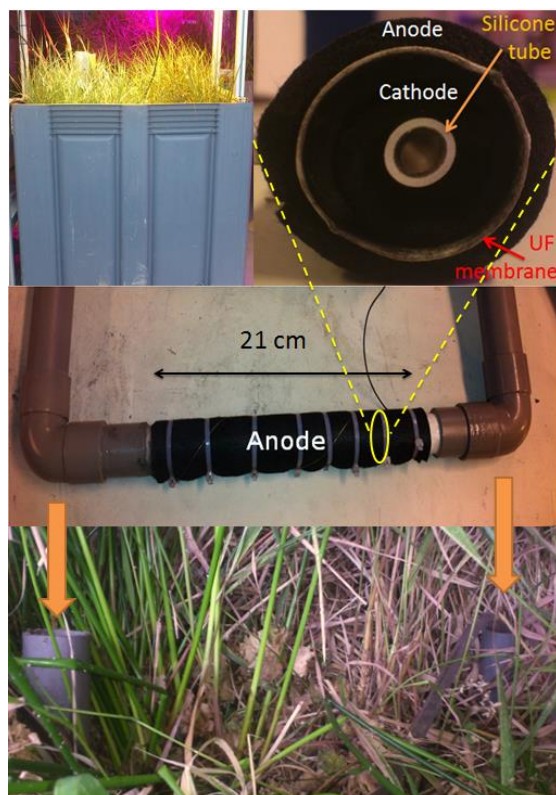


Figure 1: Experimental setup. Top left: Wetland box in lab with *Spartina anglica* salt marsh. Top right: Cross section of the tube. Middle: Completed tubular PMFC before installation in the wetland. Bottom: Tube installed in *Phragmites australis* peat soil

6.2.3 Measurements

The electrode potentials and the cell voltage were measured and logged identically to previous PMFC research [33]. Polarization measurements were done multiple times to analyze the development of the biocathode, bioanode and the short term current and power generation. On day 20 and day 35 (day 38 for peat soil PMFC1), polarizations of all the PMFCs were performed by controlling the cathode potential and measuring the current in a three electrode set up (n-Stat, Ivium Technologies, Eindhoven, The Netherlands). The cathode potential was gradually decreased and afterwards increased starting and finishing at the open cathode potential in steps of 600 seconds. On day 84 and day 88, polarization of all the PMFCs were performed by controlling the cell voltage in a two electrode setup. The cell voltage was gradually decreased from open cell (OCV) to 1 mV and afterwards gradually increased to open cell in steps of 600 mV. A polarization by controlling the cell voltage was also done on day 160 for the peat soil PMFCs. The results of the last second of each step are shown in the results. Deposition on the electrodes was analyzed by an ICP (Vista MPX Simultaneous ICP-OES, Varian Inc., Palo Alto, USA). Samples were first washed with demineralized water, before they were dissolved in aqua regia.

6.2.4 Calculations

All results are reported per m² plant growth area. The PGA in this research is the projected surface area of the part of the tube with the electrodes (210 mm by 31 mm). The internal resistance was calculated as described [98] using the theoretical values of the electrode potentials based on the local pH.

The coulombic efficiency for oxygen reduction was calculated by dividing the current generation by the current equivalent of the oxygen flux through the silicone tube into the cathode (i.e. the current that could be generated by reducing the oxygen) into the cathode of the PMFC. The current equivalent of oxygen in the cathode was calculated according to the following formula based on Fick's law:

$$I_{eq} = AnFD \frac{\partial C}{\partial x}$$

In which, I_{eq} is the current equivalent (in A), A is the area through which the oxygen or sulfide diffuses (in m^2), n is the number of electrons, F is the Faraday constant ($96485 \text{ A s mol}^{-1}$), D is the diffusion coefficient (in $m^2 \text{ s}^{-1}$), ∂C is the difference in concentration (in mol m^{-3}) and ∂x it the distance (in m). Oxygen diffuses from the inside of the silicon tube through the entire cathode. The dissolved oxygen concentration in water (0.258 mol m^{-3} at 25°C and 20.9 % oxygen) is significantly lower than the oxygen concentration in air (8.708 mol m^{-3} at 25°C and 20.9 % oxygen). Therefore, oxygen diffusion through the silicone is neglected in the calculation. Oxygen diffuses over 0.0081 m , which is the thickness of the cathode compartment to the ultrafiltration membrane with an area of 0.0158 m^2 (figure 2). Oxygen reduction reduces four electrons per oxygen (O_2).

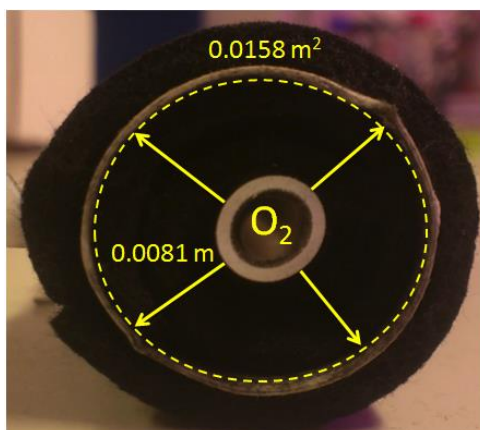


Figure 2: Overview of chosen values for the calculations of the oxygen diffusion in the cathode. Arrows indicate total distance and direction of oxygen diffusion. Dashed circle is used to calculate the area in the equation

6.3 Results and discussion

6.3.1 Biocathode was successfully started in peat soil PMFC

The three tubular PMFCs in the *Phragmites australis* peat soil started to generate electricity 26 days after installation in the wetland (figure 3). The cathodes were controlled at 150 mV, because that resulted in a successful start-up of the biocathode in previous experiments [61, 100]. This method also resulted in a successful start-up of the biocathode in this research as after day 26 a positive current was generated. This

indicates that the onset potential increased from a potential lower than 150 mV to a potential of more than 150 mV. Microorganisms catalyzing the oxygen reduction reaction are known to decrease the overpotential of the cathode and as such increase the onset potential of the cathode [148]. Likely the increase in onset potential in this experiment can also be ascribed to these microorganisms.

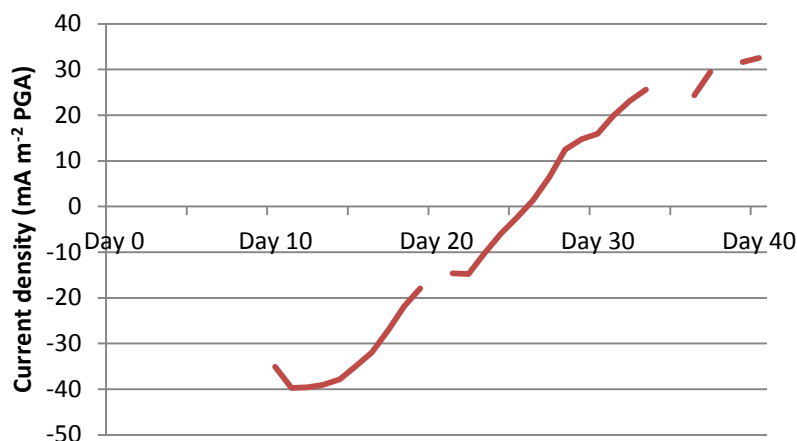


Figure 3: Total current generation of the three PMFCs in parallel connected in the peat soil at a controlled cathode potential of 150 mV. In the first 10 days of the experiment the current generation of the PMFCs were not logged, however the cells were cathode controlled at 150 mV. The other periods without current generation were due to OCV operation

All cathodes were connected electrically in parallel and thus operating as one working electrode. To analyze the start-up of the biocathode of the individual cells, polarization measurements were performed. Also the polarization graphs clearly show an increased onset cathode potential of each PMFC (figure 4). The onset potential increased from 264 mV on day 20 to 521 mV on day 160 for peat soil PMFC1 and from approximately -163/-198 mV (day 20) to 393/449 mV (day 160) for peat soil PMFC2 and PMFC3. The higher onset potential of PMFC1 compared to PMFC2 and PMFC3 on day 20 is likely caused by the differences in start-up of the biocathodes. Already on day 20 the biocathode of PMFC1 is catalyzing the reduction of oxygen. The start-up of the biocathodes clearly varies. The start-up of PMFC 2 is slower than the other two biocathode as can be seen in the polarization on day 88. A possible explanation of the quicker start-up of PMFC1 is the location of

the used reference electrode. The reference electrode of PMFC1 was used to control the cathodes of the three PMFCs. The distance between the cathodes of PMFC2 and PMFC3 to the reference electrode resulted in potential losses which led to a lower and less stable cathode potential for PMFC2 and PMFC3. For example, from day 1 to day 30 the cathodes were controlled at 150 mV. The cathode potential of PMFC1 was on average 150 (± 10) mV. At the same time the cathode potential of PMFC2 and PMFC3 were on average respectively 55 (± 85) mV and 41 (± 73) mV. Earlier oxygen reducing biocathodes were preferably started at a fixed cathode potential of 150 mV [61], which only occurs at PMFC1, possibly explaining the quicker start-up of PMFC1.

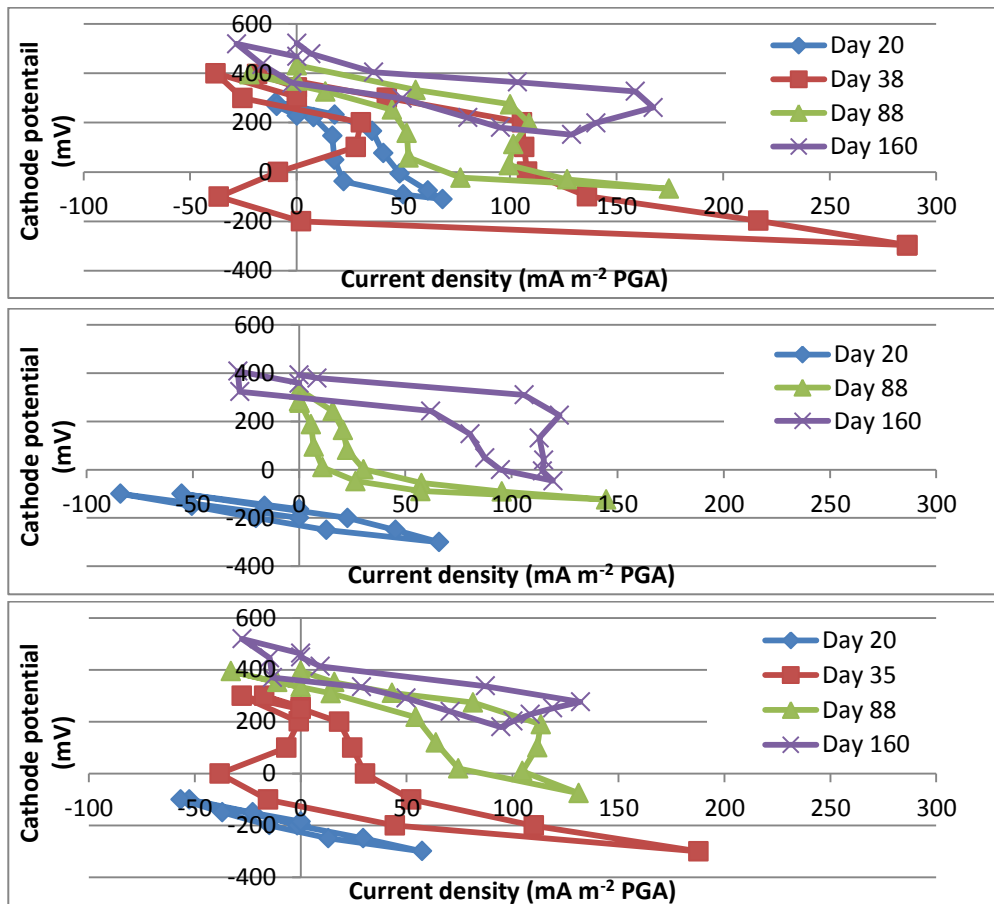


Figure 4: Cathode potential and current density of the three individual PMFC in the peat soil during four polarizations. The polarizations on day 20 and day 35/38 were cathode potential controlled. The polarizations on day 88 and day 160 were cell potential controlled

Even though the start-up of the biocathodes is different, the last polarization on day 160 is similar for all three PMFCs, for both the onset potential as the potential of the maximum current density. The maximum current density is respectively 167 mA m⁻² PGA at 261 mV, 122 mA m⁻² PGA at 225 mV and 132 mA m⁻² PGA at 276 mV for PMFC1, PMFC2 and PMFC3. Further analysis of the maximum current generation is not possible, because the polarization of day 160 is performed by controlling the cell potential and not by controlling the cathode potential. The maximum current density is therefore also determined by the oxidation reactions in the anode. Oxygen reduction is clearly catalyzed likely by microorganisms in the cathodes of all three PMFCs as the overpotential decreased at all current densities. The selected start-up strategy, controlling the cathodes at 150 mV, was thus also successful for starting a biocathode in a *Phragmites australis* peat soil. Therefore, peat soil naturally contains microorganisms which can biologically catalyze the anode in the PMFC and microorganisms which can biologically catalyze oxygen reducing cathodes. As such a bioanode and biocathode were in situ started in the peat soil PMFCs.

6.3.2 Long term power generation with a bioanode and biocathode in the peat soil PMFC

From day 84, the PMFCs were controlled by an external resistance and power was continuously generated. For all three peat soil PMFCs most power was generated at 2000 Ω (figure 5). The maximum long term (two week average) power generation was the highest for peat soil PMFC2, 21 mW m⁻² PGA from day 90 to day 103. Peat soil PMFC1 and PMFC3 generated on average respectively 12 and 8 mW m⁻² PGA. This is clearly higher than the 1.3 mW m⁻² which was earlier generated in the same wetland [98]. The higher power output is likely caused by an improved design and a better cathode performance. Lowering the resistance to 1000 Ω and further to 500 Ω did not result in a further increase in power. After decreasing the resistance the current generation directly increased and afterwards gradually dropped to a current density comparable to the current density at 2000 Ω . The lower resistance resulted in approximately the same current density and thus less power. Current generation in the peat soil PMFC was limited by the anode (figure in supporting information). Lowering the external resistance increased the anode resistance, while the cathode resistance was stable during the entire period that the PMFCs were controlled by an

external resistance (figure in supporting information). Therefore, the anode potential is increasing without donating extra electrons to the anode. Over time, the PMFCs in the peat soil became likely more limited in electron donors, possibly due to diffusion limitation [149], less exudation of the plants [47], slower hydrolysis of dead roots [105] and/or due to the presence of more alternative electron acceptor oxygen via radial oxygen loss [105] and/or via oxygen crossover from the cathode to the anode [24].

PMFC2 produces most power (figure 5), while the cathode potential of PMFC 2 is lower than of the other two PMFCs (figure 4). The difference in power output between the PMFC is explained by the lower anode potential and resistance of PMFC2 (table 1). The lower cathode potential of PMFC2 compared to PMFC1 and PMFC3 is not caused by a worse cathode performance, but by the higher current of PMFC2 (figure 5). This is confirmed by the cathode resistance of PMFC2, which is only slightly higher than the cathode resistance of PMFC1, while the cathode resistance of PMFC3 is clearly higher. The anode resistance of PMFC2 is clearly lower than the anode resistance of PMFC1 and PMFC3. This shows that the difference in power output is explained by the differences in anode performance.

Table 1: Power, potentials and resistances of the peat soil PMFCs during the two week maximum power generation

	PMFC1	PMFC2	PMFC3
Power (mW m^{-2} PGA)	12	21	8
Anode potential (mV)	-41	-255	23
Cathode potential (mV)	375	265	336
Membrane potential (mV)	21	3	-10
Anode resistance ($\Omega \text{ m}^2$ PGA)	12.8	4.4	18.2
Cathode resistance ($\Omega \text{ m}^2$ PGA)	9.7	10.2	13.5
Membrane resistance ($\Omega \text{ m}^2$ PGA)	0.7	0.1	-0.4

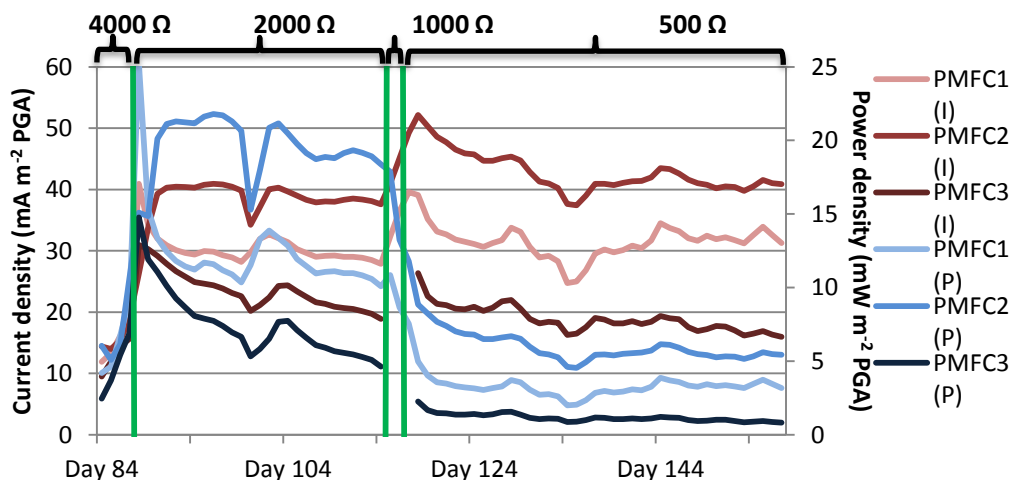


Figure 5: Long term current (I) and power (P) generation of the three peat soil PMFCs at different external resistances to study the individual performance of the cells. The green lines indicate the moment that the external resistance was lowered

The differences in anode performance can be explained by local conditions. A better anode performance (i.e. lower anode resistance) can be explained by higher local availability of electron donors, less electron acceptors, the microbial population and/or other beneficial local conditions. The electron donors depend on exudation, hydrolysis of dead roots and other organic matter and likely vary due to local variations in root growth. The main electron acceptor in the anode is oxygen, which can be present in the anode due to radial oxygen loss from the plants [105] or via diffusion from the cathode through the ultrafiltration membrane. Roots were clearly growing through the anode felt in the peat soil PMFCs (figure 6) which likely results in local variations in exudation and radial oxygen loss and thus in the availability of electron donors and acceptor. The other factors which can vary are local pH and redox. A locally lower pH results in an higher anode potential and thus an higher anode resistance. Local conditions, as pH, redox and oxygen, will also influence the microbial population and density [150]. Preferably the required electrochemically active microorganisms are enhanced and not outcompeted by other microorganisms [18]. All these factors will influence the anode performance. Differences in the cathode performance are caused by the same factors as for the anode: local variation in electron donors, acceptors, microbial community and local conditions. Also root growth likely affected the cathode performance as

small roots penetrated through the ultrafiltration membrane to the cathode. Such roots may be beneficial while they can provide oxygen [44]; though it is expected that on the long term the roots provide more rhizodeposits which allow electron donor supply and oxidation in the cathode. As such, internal currents in the cathode can occur which may diminish the intended cathode function of the PMFCs.



Figure 6: Roots are growing through the anode felt of peat soil PMFC1. Picture from the inside of the anode felt of the dismantled peat soil PMFC1 at end of experiment

6.3.3 Improving the design of the peat soil PMFC to avoid oxygen diffusion in anode

The current and power generation decreased when more oxygen was supplied to the cathode by pure oxygen diffusion instead of by air diffusion due to oxygen crossover from the cathode to the anode. Showing that oxygen supply is an important design criteria since it can be too high and too low. The polarization with pure oxygen diffusion on day 84 and air diffusion on day 88 illustrates this. The maximum power density of the PMFCs during the polarization on day 84 was on average $6.1 (+/- 1.8) \text{ mW m}^{-2} \text{ PGA}$ and on day 88 on average $32 (+/- 19) \text{ mW m}^{-2} \text{ PGA}$. This difference can be mainly attributed to the anode as the anode resistance decreased on average from $11 (+/- 6) \Omega \text{ m}^2 \text{ PGA}$ to $2.6 (+/- 0.7) \Omega \text{ m}^2 \text{ PGA}$. Clearly, the performance of the anode in the peat soil decreases when supplying more oxygen to the cathode. Thus it

is likely that oxygen in the cathode diffused through the ultrafiltration membrane, resulting in internal currents, lowering the voltage coulombic efficiency and thus decreasing the power output [24].

Further optimization of the oxygen supply is necessary, because also oxygen crossover from the cathode to the anode occurred during air diffusion as the anode is also during that period the main resistance (figure 6). This expectation based on the resistances is confirmed by the modelled coulombic efficiency of oxygen reduction. 55 % of the oxygen available in the cathode is related to current generation. The rest is transported to the anode and/or reduced in side reactions. The coulombic efficiency was predicted using Fick's law, assuming an ideal wetland PMFC in which all the oxygen is consumed in the cathode and no electron donors diffuse from the anode to the cathode. The oxygen diffuses from the silicone tube in to the cathode compartment. The dissolved oxygen concentration is 0.258 mol m^{-3} (20.9 %) directly next to the silicone tube and diffuses through the complete cathode compartment (0.0081 m) where all oxygen is consumed. The diffusion coefficient for oxygen through water is $1.97 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$. This results in an oxygen diffusion flux of $9.92 \cdot 10^{-10} \text{ mol O}_2 \text{ s}^{-1}$ (table 2) which is equivalent to a current of 0.38 mA. The peat soil PMFCs produced on average a current of 0.21 mA/PMFC during the two weeks in which the maximum long term power was generated. The coulombic efficiency is calculated by dividing the measured current by the theoretical value. Only 55 % of the available oxygen is thus used for oxygen reduction.

To increase the power output of the PMFCs in the peat soil, the design should be improved to make sure that all the oxygen is used in the cathode and no oxygen diffuses to the anode. This can be done by increasing the current density of the peat soil PMFCs to reduce more oxygen or by decreasing the oxygen diffusion in the cathode. The current density can be increased by using a larger anode. Based on Fick's law, possible improvements to the design for less oxygen diffusion to the cathode are the use of an air diffusion tube with a lower diffusion coefficient, a smaller size or a larger thickness. Also increasing the thickness of the cathode compartment or a lower oxygen concentration in the silicone tube will result in less oxygen diffusion. The search for the optimal design in which no oxygen diffuses to the anode is an important factor in development of the PMFC as oxygen diffusion to the anode results in internal currents and power losses.

Table 2: Passive oxygen diffusion in the cathode compartment and the oxygen current equivalent in the peat soil PMFCs during air supply

	Air diffusion	
Distance oxygen transport	0.0081	m
Diffusion coefficient	$1.95 \cdot 10^{-9}$	$\text{m}^2 \text{s}^{-1}$
Oxygen concentration difference	0.258	mol m^{-3}
Area ultrafiltration membrane	0.0158	m^2
Oxygen diffusion flux	$9.92 \cdot 10^{-10}$	$\text{mol O}_2 \text{s}^{-1}$
Oxygen current equivalent	0.38	mA

6.3.4 Biocathode in salt marsh PMFC only started with pure oxygen diffusion

The biocathode of the salt marsh PMFCs was started using the same method as the peat soil PMFCs, i.e. by controlling the cathode potential at 150 mV and by pumping pressurized air through the inner-side of the silicone tube. The oxygen diffuses passively from the inner-side of the silicone tube to the cathode. This method was not successful for the salt marsh PMFC, as there was no increase in current density in the first 40 days (figure 7). The start-up problems were likely caused by the limited availability of oxygen in the cathode. The open cathode potential of the salt marsh PMFCs was -377 (+/- 4) mV before the polarization on day 38, which is lower than the minimum open cathode potential recorded for the peat soil PMFCs (-200 mV). The lower open cathode potential of the salt marsh PMFC is likely caused by a lower oxygen concentration as can be calculated from the Nernst equation of the oxygen reduction reaction [22]. Possibly, the oxygen which diffused through the silicone tube was directly reduced in the cathode while oxidizing the sulfide present in marine sediment [36]. Due to these internal currents, likely not enough oxygen was present for oxygen reduction in the PMFCs. Therefore, the oxygen concentration in the cathode compartment was increased by pumping pure oxygen instead of pressurized air through the silicone tube resulting in more diffusion of oxygen from inside the silicone tube to the cathode. More oxygen diffusion to the cathode, likely resulted in a higher concentration of oxygen in the cathode and did result in the start-up of the biocathode as the current density increased at the same cathode potential (figure 7). The overpotential thus decreased and the oxygen reduction reaction was catalyzed, likely by the microorganisms in the cathode [148].

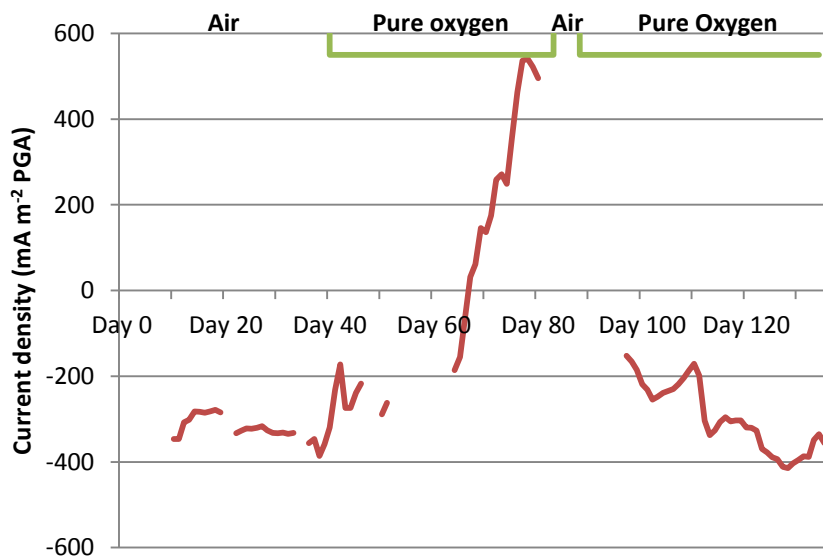


Figure 7: Total current density of the three salt marsh PMFCs during 150 mV cathode control. Line on top indicates period of pure oxygen diffusion instead of air diffusion. In the first 10 days of the experiment the current generation of the PMFCs were not logged, however the cells were cathode controlled at 150 mV. From day 84 to day 96, the cells were controlled by an external resistance. The other periods without current generation were due to OCV operation

The maximum daily average current density was achieved on day 78 and 541 mA m⁻² PGA (i.e. 3.52 mA/PMFC) was generated. The coulombic efficiency for oxygen reduction was 192 % that day calculated by dividing the generated current by the potentially available current (i.e. 1.83 mA/PMFC based on Fick's law). More oxygen was reduced in the cathode, than what was supplied through the ultrafiltration membrane. Pure oxygen was already supplied for 32 days likely resulting in more oxygen in the cathode than reduced by the PMFC. The extra oxygen was reduced once the biocathode started to catalyze oxygen reduction. The higher concentration of oxygen resulted in a higher current by the PMFC than what was supplied through the silicone tube and thus a coulombic efficiency of more than 100 %. The current generation decreased on day 79 and 80, likely due to the decrease in oxygen concentration in the cathode compartment. Probably, a maximum current density of 1.83 mA could be maintained in a long term.

The salt marsh PMFCs did not generate power for a long term. Therefore, the maximum two week average power generation cannot be compared with the data from the peat soil PMFCs. However, for a shorter period both wetlands can be compared. The maximum daily average power density of the salt marsh PMFCs was 82 mW m^{-2} PGA on day 72. The current density increased after day 72 (figure 7), but no more power was generated due to a lower cell voltage. The maximum daily average power generation of the best peat soil PMFC was 22 mW m^{-2} PGA. Almost 4 times more power was generated by the salt marsh PMFCs than by the peat soil PMFCs. The higher power density of the salt marsh PMFCs is likely caused by the alternative electron donor sulfide, which is oxidized at the anode of the PMFC to elemental sulfur. The sulfide-sulfur couple is a mediator in the PMFC with organic carbon as the source [98].

On day 84, again pressurized air was pumped through the silicone tube to analyze the current generation without pure oxygen. The individual PMFCs were connected with an external resistance of 1000Ω . Directly after the change to pressurized air the current density and cathode potential dropped (figure 8). Clearly, again not enough oxygen diffused through the silicone tube to the cathode. The drop in current density and cathode potential of PMFC3 was smaller than of the other two PMFCs (figure 8), likely due to a broken connection of the silicone tube, resulting in air bubbles in the cathode compartment and thus more oxygen. The drop would likely be comparable to the drop of the other two PMFCs if the connection was not broken. Also the polarization on day 84 with pure oxygen diffusion and day 87 with air diffusion shows a clear drop in cathode potential, current and power density. The maximum power density during the polarization decreased from 86 mW m^{-2} PGA to 0.5 mW m^{-2} PGA for salt marsh PMFC1, from 109 mW m^{-2} PGA to 5.2 mW m^{-2} PGA for salt marsh PMFC2 and from 156 mW m^{-2} PGA to 39 mW m^{-2} PGA for PMFC3. Pressurized air diffusion through a silicone tube with these dimensions results in insufficient amount of oxygen for the oxygen reduction reaction at the cathode. Therefore, on day 87, pure oxygen was again pumped through the silicone tube. Unfortunately, the biocathode did not directly restart at the same current densities as before the air diffusion. Possibly sulfide was able to diffuse from the anode to the cathode as a result of a lower oxygen concentration and current density when switching from oxygen to air diffusion. This resulted in less oxidation of sulfide at the anode of the PMFC or due to less internal currents and thus likely higher

concentrations of sulfide in the cathode. The toxic sulfide may have killed the microorganisms [151]. Also the formation of elemental sulfur on the cathode may substantially decrease the oxygen reduction reaction [152]. The biocathode were controlled at 150 mV with pure oxygen diffusion until day 136 which did not result in the restart of the biocathode.

Deposition on the cathode felt directly at the silicone tube was clearly visible when dismantling the PMFC at the end of the experiment (figure in supporting information). Crushing the black formation resulted in a white powder. ICP analysis showed a clear increase in calcium concentration on the deposition compared to a part of the felt without deposition. Addition of HCl formed gas bubbles, likely carbon dioxide. Therefore, the deposition is probably calcium carbonate which may have been formed due to the pH increase caused by the reduction of oxygen. The black color could be metal sulfide deposition. The addition of aqua regia may have caused the evaporation of hydrogen sulfide and as a result the sulfide was not measured by the IPC. The deposition may have blocked the diffusion of oxygen to the cathode. The dismantling of the PMFC also showed that no roots were present in both the anode and cathode felts. The plants were vital at the start of the experiment and as such providing rhizodeposits. During the experiments, the plant vitality dropped until the plants died resulting in no roots growing through the felt. The plants likely died due to the not ideal growth conditions. Even at this time, the cells can still be considered PMFC as also dead plants generate electricity [105].

The current design is not suitable for PMFCs in this salt marsh with air diffusion. To also start-up the biocathode with air diffusion more oxygen should diffuse into the cathode and/or less sulfide should diffuse into the cathode. More oxygen will be present in the cathode when an air diffusion tube with a larger diameter, smaller thickness and/or a gas diffusion material with a larger diffusion coefficient for oxygen is used. Also a smaller cathode likely results in a start-up of the biocathode PMFC in the salt marsh. The diffusion of sulfide to the cathode can be blocked with the use of an ion selective membrane instead of the used ultrafiltration membrane. However, this will increase the costs of the PMFCs [153].

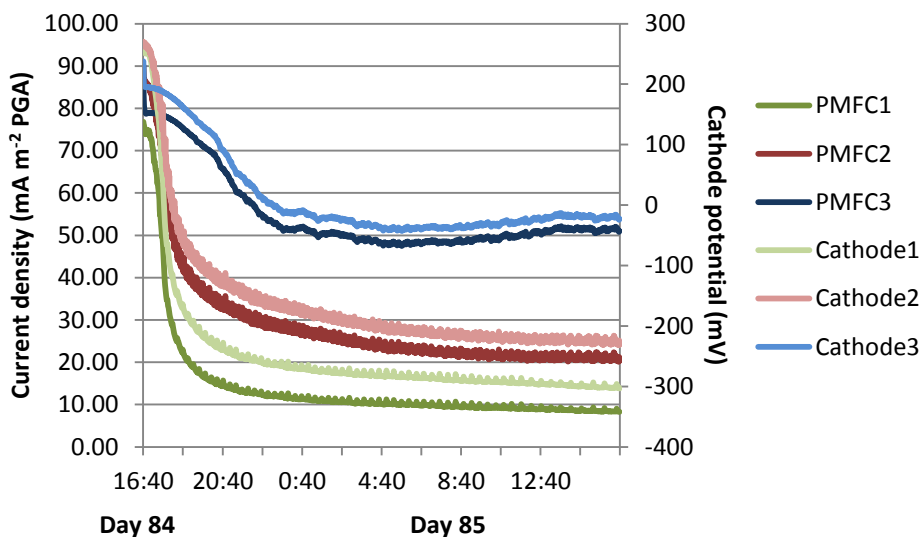


Figure 8: Cathode potential and current density of the three salt marsh PMFCs during 24 hours after the switch from pure oxygen diffusion to air diffusion at an external resistance of 1000Ω

6.4 Conclusions

PMFCs with a gas-diffusion oxygen reducing biocathode were successfully started in *Phragmites australis* peat soil. Oxygen reduction is clearly catalyzed, likely by microorganisms in the cathodes as the overpotential decreased resulting in an increased current density and cathode potential. The long term two week average power generation of the best peat soil PMFC was $21 \text{ mW m}^{-2} \text{ PGA}$ with a maximum daily average of $22 \text{ mW m}^{-2} \text{ PGA}$. The anode of the peat soil PMFC limits the current generation, likely due to oxygen crossover from the cathode to the anode. PMFCs with a biocathode in *Spartina anglica* salt marsh only started with pure oxygen diffusion reaching a maximum daily average power generation of $82 \text{ mW m}^{-2} \text{ PGA}$. Startup of the biocathode with pressurized air was not successful, likely due to crossover of sulfide from the anode to the cathode, resulting in insufficient amounts of oxygen and possible toxic conditions for the microorganisms in the cathode. To further increase the power, the design of the PMFCs in both wetlands has to be improved to limit the crossover of oxygen from the cathode to the anode for the peat soil PMFCs and to limit the crossover of sulfide from the anode to the cathode for the salt marsh PMFCs.

6.5 Acknowledgements

This project was carried out within the research programme of BioSolar Cells, co-financed by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. Furthermore, this research is financially supported by Alliander and Plant-e. We would like to thank It Fryske Gea and the province of Zeeland for their support with the collection of the wetlands.

Chapter 7

General discussion and outlook

7.1 Successful installation of the plant microbial fuel cell (PMFC) in laboratory wetlands

In this thesis, we installed PMFCs in a *Phragmites australis* dominated fen peat soil and a *Spartina anglica* dominated salt marsh. The anode of the tube was located directly between the plant roots, allowing the roots to grow through the anode felt. The cathode was located at the inside of the tube and oxygen was passively supplied into the cathode through a silicone tube with a high permeability for oxygen diffusion. Both the anode and the cathode were biologically catalyzed in this new design. We showed that PMFC can be applied in wetlands without the need for energy intensive pumping thus increasing the net power output and applicability of the PMFC.

Although the tubular PMFC has only been applied in a fen peat soil and a salt marsh, the PMFC is expected to generate electricity in all wetlands with plants. According to the wetland classification presented in the introduction, the PMFC can thus be applied in bogs, fens, swamps, temporary lakes, marshes and permanent lakes. Worldwide, 8-10 million km² of wetland could be used for electricity generation by the PMFC [28].

In this chapter, the application of the PMFC in wetlands is discussed. First, the worldwide potential electricity generation of the PMFC is estimated based on the insights from chapter 5. Second, the tubular design used in chapter 6 is assessed. The maximum costs of the tubular PMFC to be economically feasible are presented and possibilities to reduce the costs are discussed. In the third section, the potential electricity generation of the *Phragmites australis* peat soil and the *Spartina anglica* salt marsh used in this thesis are further evaluated based on the theoretical electricity generation calculated in chapter 6. The used plants are collected respectively in national park Alde Feanen and in the Oosterschelde. For each location, a separate case is presented including the potential electricity generation and possible local uses for the generated electricity. In the last section, potential applications and extra profits besides electricity generation in wetlands are discussed in order to increase the value of the PMFC.

7.2 Potential power generation of PMFC

7.2.1 Site specific prediction required for realistic potential power output

In chapter 5, a site specific power output for the PMFC was predicted for the *Phragmites australis* peat soil and the *Spartina anglica* salt marsh. The potential power generated of the wetlands is respectively 0.09 to 0.52 W m⁻² plant growth area (PGA) for the *Phragmites australis* peat soil and 0.14 to 0.34 W m⁻² PGA for *Spartina anglica* salt marshes. The maximum power generation of the PMFC in Western European conditions was earlier envisioned to be 1.6-3.2 W m⁻² PGA based on the best known efficiencies of the main processes [32]. These calculations included a solar radiation of 150 W m⁻², photosynthetic efficiency of 5 %, 70 % rhizodeposition and 60 % energy recovery by the PMFC [32]. There are a few major concerns regarding the earlier envisioned power generation explaining the higher theoretical power output. First, rhizodeposition of 70 % is not a common value rhizodeposition. Common rhizodeposition is estimated to be 11-17 % [14, 133]. Second, the energy recovered by the PMFC in the earlier envisioned power output did only include the Coulombic efficiency and not the voltage efficiency. Third, also the photosynthetic efficiency used to calculate the maximum power generation is more than twice as much as usually achieved by plants [154]. The theoretical maximum power generation of the PMFC lowers significantly when including the voltage efficiency and a common value for rhizodeposition and photosynthetic efficiency.

The new predicted power output was calculated using a different approach. The power generation of the PMFC is wetland specific and varies from wetland to wetland. Each wetland is different in for example plant species (e.g. primary production), climate (e.g. solar radiation and temperature) and external carbon inflow. In the new predictions, these local conditions are included. The solar radiation and photosynthetic efficiency are replaced by the primary production of the plants, in our case *Phragmites australis* and *Spartina anglica*. The primary production includes solar radiation and photosynthetic efficiency and is site specific. In the new calculations, a common value for rhizodeposition was used, local external inflow of carbon was added and both the Coulombic efficiency and the voltage efficiency were included. However, the Coulombic efficiency of the PMFC is not based on the Coulombic efficiencies reported in literature, but on the percentage of the carbon

used by microorganism in the soil. The Coulombic efficiency was assumed to be 15 % for the *Phragmites australis* peat soil, which is based on the carbon use by methanogens (15 % of the net fixed carbon) [142]. For the *Spartina anglica* salt marsh, the Coulombic efficiency was assumed to be 8.25 %. This is based on a microbial respiration of 16.5 %, from which 50 % is oxidized by sulfur reducing bacteria to produce sulfide. The sulfide is subsequently oxidized by the PMFC to generate electricity. The voltage efficiency is based on the voltage efficiency achieved in the best performing PMFC in chapter 2 of this thesis.

7.2.2 Wetland methane emissions estimate worldwide potential power generation of the PMFC

The new predicted power generation of the PMFC is wetland and location specific. Therefore, specific data are required for every wetland to estimate the worldwide potential power generation of the PMFC. In chapter 5, we showed that the power generation of the PMFC applied in a fresh *Phragmites australis* peat soil can be estimated based on methane emissions of the wetland. This estimation is based on the ability of the electrochemically active bacteria to outcompete the methanogens for electron donors [39]. As a result, the methane is not emitted and the carbon electron donors are instead used for electricity generation in the PMFC.

Modelled methane emission of wetlands varies from 141 to 264 Tg yr⁻¹ [155]. However, not all the methane which is produced in the wetlands is emitted into the air. 76 % of the methane produced by methanogens in the wetland is reoxidized to carbon dioxide by methane oxidizing bacteria in the rhizosphere of the wetlands [142]. The total methane production by methanogens in wetlands is therefore in the range of 588 to 1100 Tg yr⁻¹. This equals a total potential power generation of fresh water wetlands of 0.53 to 1.01 TW. The power generation is calculated as shown in box 1.

Box 1 – Potential power generation of the PMFC in fresh water wetlands

The potential power generation of PMFC installed in fresh water wetlands is calculated from the total methane emission of these wetlands (eq 1).

$$P = \frac{JnFU}{Mt} \quad (\text{eq 1})$$

In which, P is the power (in TW), J is methane production (in Tg yr⁻¹), n is the number of electrons (8), F is the Faraday constant (96485 A s mol⁻¹), U is the voltage of the PMFC (assumed 0.6 V, same as in the site specific prediction), M is the molar mass of methane (16 g mol⁻¹) and t is the number of seconds in one year (in s yr⁻¹).

7.2.3 Up to 60 % of the global electricity consumption could be produced by PMFC in wetlands

The average worldwide power consumption in 2012 was 2.25 TW [156]. PMFC could generate 0.53 to 1.01 TW when applied in all fresh wetlands over the world. Therefore, 24 to 45 % of the total energy consumption could be produced by the PMFC. Worldwide, more power can be generated by the PMFC when also the one million km² of salt and brackish wetlands are included in the calculations [28]. However, the methane emission model is not suitable for salt and brackish wetlands. Methanogens are the least competitive group of microorganism and are outcompeted in case of the presence of other major electron acceptors [143]. Sulfate is the major electron acceptor in salt and brackish wetlands and therefore the methanogens are outcompeted by the sulfate reducing bacteria.

A different method is required for a detailed estimation of the potential power generation of PMFC applied in salt and brackish water. A first rough estimate of the power generation is possible assuming that all salt and brackish wetlands produce the same amount of power as the *Spartina* salt marsh described in chapter 5 (i.e. 0.14 to 0.34 W m⁻² PGA). In that case, the one million km² of salt and brackish wetlands produce between 0.14 to 0.34 TW and could cover 6 to 15 % of the electricity consumption. In total, PMFC applied in wetlands are roughly estimated to be able to produce 30 to 60 % of global electricity consumption.

7.2.4 Wetland PMFC generates potentially most power in the tropics

The potential power generation of PMFC in wetlands is shown in the world map in figure 1. This map is based on a methane emission map [155, 157] and shows the potential power generation of PMFC by adjusting the legends from methane emission to power generation according to eq 1. Melton et al. compared eight methane emission models from which this model was selected for the PMFC calculations because of the high spatial resolution, the area of the wetlands (i.e. 9 million km², thus within the earlier mentioned range of 8-10 million km²) and the methane emission close to the average methane emission of all models [155].

The world map clearly shows that most power can be generated in the tropics, mainly in the Amazon in South America, Sub-Saharan Africa and South Asia. In this model, 70 % of the potential power of the PMFC is generated in the tropics from 30° N to 30° S. Application of PMFC is therefore preferably in the tropics as the expected power generation per m² PGA is higher than in other regions. The electricity should, if possible, be used at the same location as the electricity is generated because of the electricity losses associated with the transmission of electricity as can be calculated with Ohm's law (box 2). Therefore, also uses for the electricity has to be available close to the location PMFC is applied. Sub-Saharan Africa and South Asia have a higher population density than the Amazon [158]. As a results, they also have a higher electricity need and are more suitable for application of the PMFC. After the tropics, most electricity can be generated at the high northern latitudes (figure 1). These locations are influenced by permafrost [159] and are therefore less suitable for electricity generation by the PMFC as the PMFC does not generate electricity when frozen [31]. Also the population density in the northern latitudes is low and the electricity can thus not be used in this location [158]. Application of PMFC in salt and brackish wetlands is also suitable, because these wetlands are located close to the shore where in general also many people live [158]. The electricity can therefore likely be used directly.

Box 2 – Voltage and power losses due to electricity transmission

The voltage and power losses due to electricity transmission are calculated with Ohm's law.

$$U_{loss} = IR \quad (\text{eq 2})$$

$$P_{loss} = I^2 R \quad (\text{eq 3})$$

In which, U_{loss} is the voltage loss (in V), P_{loss} is the power loss (in W), I is the current (in A) and R is the resistance of the transmission wires (in Ω).

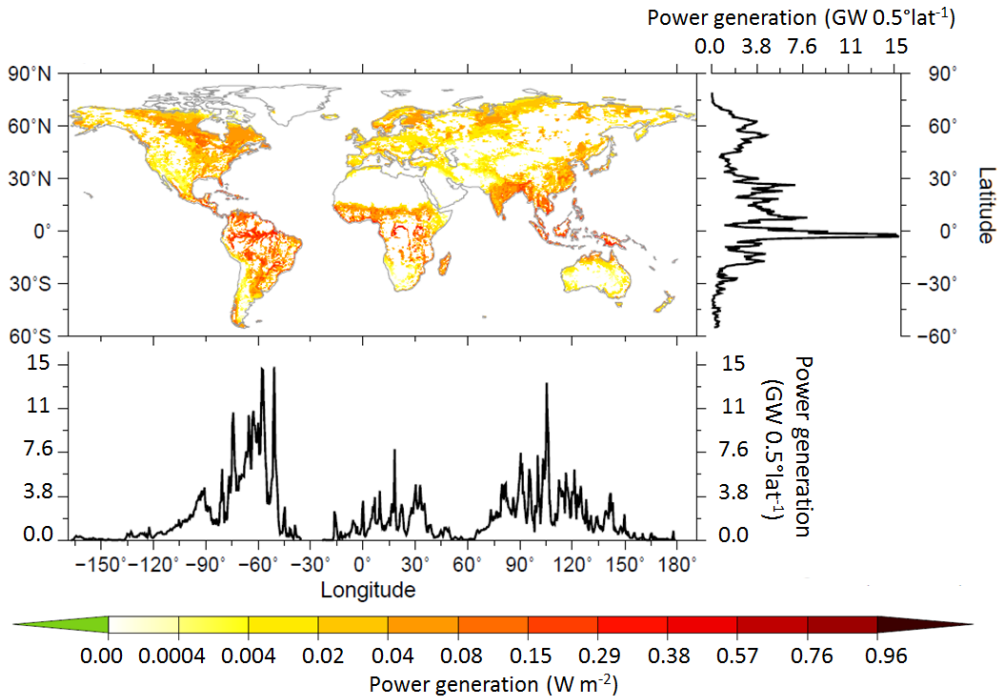


Figure 1: Potential power generation of the PMFC when applied in fresh wetlands. Power generation based on methane emissions. Legend of map adjusted from [155]. Original research by [157]

7.3 Technological assessment of the tubular PMFC in wetlands

7.3.1 Designing the tubular wetland PMFC

In chapter 6, we showed that the design of the tubular PMFC is crucial for the power generation of the PMFC. Both, calculations and experiments with oxygen supply through the silicone tube showed that the oxygen supply should be balanced with the electron donors to

harvest most power. Supplying too much oxygen results in oxygen diffusion to the anode and the oxygen reacts with the electron donors in the wetland. When not enough oxygen is supplied, electron donors diffuse to the cathode and react with the oxygen in the cathode. In both cases, internal currents occur which lower the current and power output of the PMFC. Ideally, all the oxygen is reduced in the cathode and all the electron donors are oxidized in the anode. As a result no oxygen diffuses to the anode and no electron donors diffuse through the cathode and the maximum current is harvested. As the electron donors vary per wetland (as shown in chapter 5), also the oxygen supply is wetland dependent and should be adjusted to the local situation. Next to the oxygen supply into the cathode also the oxygen supply from the outside air into the silicone tube, the length of the tubular PMFC, the possible use of an air pump or ventilator for oxygen supply and the potential costs of the PMFC are important aspects for scaling up the tubular PMFC in an economically feasible and energy efficient way. These aspects are discussed in this section.

7.3.2 Maximum length of current tubular PMFC with passive oxygen supply is 0.31 m in the salt marsh and 0.77 m in the peat soil

Oxygen can be supplied from the outside air into the silicone tube passively via diffusion or actively for example by an air pump or ventilator. For passive diffusion, the length of the tube is an important factor in the design of the tubular PMFC as oxygen diffusion is limited. A maximum distance to where oxygen diffuses can be calculated. This shows the maximum length of the tubular PMFC over which oxygen can be supplied via diffusion and depends on the current density and the diameter of the silicone tube. The maximum length of the tube can be calculated according to the equations in box 3.

The best peat soil tubular PMFC in chapter 6 produced 1.3 mA m^{-1} and could thus be maximally be 0.77 m long assuming a desired end of tube oxygen concentration of 55 %. The 55 % oxygen concentration at the end of tube is selected because the tubular PMFC in the peat soil had a 55 % cathodic Coulombic efficiency. 55 % oxygen in the tube is minimally required to reach the current of 1.3 mA m^{-1} . Less oxygen results in a drop of the current in the tubular PMFC and is therefore not desired. The maximum length of the tube decreases in case the current

Box 3 – Maximum length of tubular PMFC with passive air diffusion

The maximum length of the tubular PMFC with passive oxygen diffusion is calculated with eq 4. This calculations is derived from the calculation of the theoretical oxygen diffusion (J_{th} in mol s^{-1}) and the oxygen consumption (J_c in mol s^{-1}). The theoretical oxygen diffusion is calculated by multiplying the diffusion flux from Fick's Law (J_d in $\text{mol m}^{-2} \text{s}^{-1}$) by the inside area of the silicone tube (A in m^2) as shown in eq 5. The oxygen consumption can be calculated from the current of the tubular PMFC (eq 6). In a steady state PMFC, the oxygen flux equals the oxygen consumption (eq 7) and thus x can be calculated according to eq 4.

$$x = \sqrt{\frac{D\partial C\pi r^2 nF}{I_x}} \quad (\text{eq 4})$$

$$J_{th} = J_d A = D \frac{\partial C}{x} \pi r^2 \quad (\text{eq 5})$$

$$J_c = \frac{I}{nF} = \frac{I_x x}{nF} \quad (\text{eq 6})$$

$$J_{th} = J_c = D \frac{\partial C}{x} \pi r^2 = \frac{I_x x}{nF} \quad (\text{eq 7})$$

In which, x is the length of the tube (in m), D is the diffusion coefficient of oxygen in air ($1.76 \cdot 10^{-5} \text{ m}^2 \text{ s}^{-1}$), ∂C is the difference between the outside air oxygen concentration and the desired oxygen concentration at the end of the silicone tube (i.e. the part of the tube farthest away from the inlet) (in mol m^{-3}), r is the radius of the inside of the silicone tube (in m), n is the number of electrons involved in oxygen reduction (4), F is the Faraday constant ($96485 \text{ A s mol}^{-1}$), I is the current of the tubular PMFC (in A) and I_x is the current density of the PMFC (in A m^{-1}).

density of the tubular PMFC increases due to a better design or application in a wetland with more available electron donors. For example, the PMFC applied in the salt marsh generated a daily average current of 8.0 mA m^{-1} during the day that the maximum power was generated. The maximum length of the tubular PMFC decreases to 0.31 m assuming air diffusion and a cathodic Coulombic efficiency of 55% . The maximum length of the tubes should be increased preferably by increasing the diameter of the silicone tube to allow more oxygen diffusion into the silicone tube. However, this results in an increased diameter of the tubular PMFC, while not increasing the power output. Therefore, increasing the diameter of the silicone tube results in a lower power density both per m^2 PGA and per m^3 tubular PMFC.

7.3.3 *Actively supplying oxygen results in 18 to 31 % net power losses*

Active supply of oxygen by an air pump or ventilator is only interesting for application when the power consumption of the pump is easily supplied by the tubular PMFC. The silicone tube used in the tubular design has an inner diameter of 6 mm. Small ventilators have a diameter of 10 mm and a flow rate of 1100 mL min^{-1} at 0.165 W (Sepa Micro-Fans, Sepa Europe GmbH, Eschbach, Germany). The ventilator is larger than the current silicone tube and therefore not suitable for the current design. An air pump (CurieJet, Taiwan, gas MicroPump GS51C) with a flow rate of 20 mL min^{-1} at 0.050 W could be used. The percentage of produced power used by the air pump is calculated as shown in box 4.

A tubular PMFC with a CurieJet gas MicroPump would ideally have a length of 397 m in the peat soil and 63 m in the salt marsh. The distance is calculated based on a maximum daily average current density and power densities of the tubular PMFC in the peat soil and salt marsh (chapter 6) and a 55 % oxygen concentration at the outlet of the tube to reach a cathodic Coulombic efficiency of 55 %. The peat soil produced a maximum daily average current of 1.3 mA m^{-1} and power of 0.68 mW m^{-1} . The salt marsh produced 8.0 mA m^{-1} and 2.5 mW m^{-1} . A tubular PMFC of 397 m in the peat soil would generate in total 0.27 W and thus 18 % of the produced power is used by the air pump. The salt marsh tube would produce in total 0.16 W from which 31 % is used for the air pump.

The calculations exclude the power required to overcome the pressure drop inside the silicone tube. This can be calculated by the Darcy–Weisbach equation (box 5). The pressure drop in the 397 meter peat soil tubular PMFC would be 73 Pa which equals 75 J m^{-3} . The power required to overcome the pressure drop is 0.025 mW at a flowrate of 20 mL min^{-1} . This is less than 0.1 % of the power of the pump, although this does not include the efficiency of the pump. The pumping pressure of the CurieJet gas MicroPump is up to 5000 Pa. Therefore, the pressure drop can be neglected in the calculations of the peat soil tubular PMFC and thus also in the shorter salt marsh tubular PMFC.

Box 4 – Percentage of power used by the air pump and length of tubular PMFC with air pump

The percentage of produced power used by the air pump can be calculated by dividing the power consumption of the pump by the power production of the PMFC using eq 8. The calculations require the length of the tube which is calculated by dividing the current equivalent of oxygen supply (by the air pump) by the current density of the PMFC (eq 9).

$$\eta = \frac{P_{\text{pump}}}{x P_{\text{PMFC},x}} \cdot 100 \quad (\text{eq 8})$$

$$x = \frac{\frac{\partial C Q n F}{I_{\text{PMFC},x}}}{I_{\text{PMFC},x}} \quad (\text{eq 9})$$

In which, η is the percentage of power produced by the tubular PMFC is used for the pump (in %), P_{pump} is the power consumption of the air pump (in W), x is the length of the tubular PMFC (in m), $P_{\text{pump},x}$ is the power density of the tubular PMFC (in W m^{-1}), ∂C is the difference between the outside air oxygen concentration (i.e. inlet tube) and the desired oxygen concentration at the end (i.e. outlet tube) of the silicone tube (in mol m^{-3}), Q is the flowrate of the air pump (in $\text{m}^3 \text{s}^{-1}$), n is the number of electrons involved in oxygen reduction (4), F is the Faraday constant ($96485 \text{ A s mol}^{-1}$) and $I_{\text{PMFC},x}$ is the current density of the tubular PMFC (in A m^{-1}).

Shorter tubular PMFCs with an air pump are possible by intermitted air supply, because less oxygen is supplied that way. However, this would require more air pumps for the same total length of tubes and thus higher capital costs. Intermitted air supply does not affect the percentage of power used for the air pump. Decreasing the current density and/or increasing the cell voltage would decrease the relative power consumption of the air pump because relatively less electrons are reduced for the same power and thus less oxygen is required. The benefit of using an air pump is the possibility to adjust the air flow into the silicone tube for example by placing a redox sensor in the anode of the PMFC. In case the redox sensor detects oxygen in the anode due to oxygen crossover from the cathode, the air flow in the silicone tube can be lowered. Adjusting the flow of the air pump could be part of the adapted control strategy for the maximum power output of the PMFC proposed in chapter 2. The PMFC is a dynamic system with a changing current and power output, as such also the oxygen supply should preferably be adjusted. The maximum power point tracker (chapter 2) and redox sensor could in this case be linked to the air pump.

Box 5 – Darcy–Weisbach equation

The pressure drop inside the silicone tube is calculated with the Darcy–Weisbach equation (eq 10). The friction factor required for the calculations is calculated from the Reynolds number (eq 11 and 12).

$$\Delta p = f \cdot \frac{x}{2r} \cdot \frac{\rho Q^2}{2A^2} \quad (\text{eq 10})$$

$$f = \frac{64}{Re} \quad (\text{eq 11})$$

$$Re = \frac{2r\rho Q}{\mu A} \quad (\text{eq 12})$$

In which, Δp is the pressure drop (in Pa; $1 \text{ Pa} = 1 \text{ kg m}^{-1} \text{ s}^{-2} = 1 \text{ N m}^{-2} = 1 \text{ Nm m}^{-3} = 1 \text{ J m}^{-3}$), f is the Darcy friction factor (-), x is the length of the tubular PMFC (in m), r is the radius of the inside of the silicone tube (in m), ρ is the density of air (1.225 kg m^{-3}), Q is the flowrate of the air pump (in $\text{m}^3 \text{ s}^{-1}$), A is the inside area of the silicone tube (in m^2), Re is the Reynold's number (-) and μ is the dynamic viscosity of air ($1.797 \text{ kg m}^{-1} \text{ s}^{-1}$).

7.3.4 Natural ventilation through the silicone tube could replace the air pumps

The calculations in the previous section show that a significant amount of power is used by the air pump. Replacing the air pump by a ventilator could reduce the relative power used to supply oxygen. However, still power is required for the ventilator. Natural ventilation could replace the air pump and as such circumvent the power required by the air pump and the capital costs of the air pump. Wind and temperature driven ventilation could be used as natural ventilation in the tubular PMFC [160]. Temperature driven ventilation can likely be created by installing the tubular PMFC with a slight slope instead of completely horizontal. This results in a difference in temperature between the inlet and outlet and likely natural ventilation. Possibly, also the pressure difference inside the silicone tube due to a decreasing oxygen concentration could result in natural ventilation. Further investigation is required to explore the feasibilities of replacing the air pump by natural ventilation.

7.3.5 Predicted maximum current output can likely not be reached in the peat soil

The site specific theoretical current output of the PMFC was predicted in chapter 5. Peat soil and salt marsh PMFCs could produce respectively 0.15 to $0.86 \text{ A m}^{-2} \text{ PGA}$ and 0.21 to $0.48 \text{ A m}^{-2} \text{ PGA}$. The tubular PMFC in chapter 6 produced a volumetric power output of $1.68 \text{ A m}^{-3} \text{ PMFC}$ for

the peat soil PMFC and 10.6 A m^{-3} for the salt marsh PMFC. The maximum peat soil current of 0.86 A m^{-2} PGA can be reached by installing 0.51 m^3 tubular PMFC per m^2 PGA. This requires 34 % of the rhizosphere to be filled with PMFC assuming a maximum rooting depth of 150 cm for *Phragmites australis* [161]. In the salt marsh, the maximum can be reached by installing 0.045 m^3 tubular PMFC per m^2 PGA which requires 7.5 % of the rhizosphere to be filled with tubular PMFCs assuming a *Spartina anglica* rooting depth of 60 cm [63]. In the peat soil, it is likely not possible to easily install PMFC in 34 % of the 150 cm deep rhizosphere. This could only be possible when installing multiple PMFCs on top of each other, by increasing the diameter of the tube significantly assuming no drop in current density (per m^3 PMFC) or by increasing the current output of the PMFC (per m^3 PMFC). However, increasing the current output seems not likely as the anode was already the main limitation in the tubular PMFCs in the peat soil. Therefore, it is likely not possible to reach the maximum predicted current of 0.86 A m^{-2} PGA and less current will be generated. The maximum current that can be generated depends on the percentage of the rhizosphere filled with PMFCs (figure 2).

Further research is required to determine the maximum percentage of rhizosphere to be filled with PMFC and the resulting maximum current that can be reached. Both possible environmental and technical issues should be analyzed. The PMFC should not negatively affect the ecosystem (e.g. plants, root development and animals). Therefore, the effect of the PMFC on the ecosystem should be further evaluated, especially when installing many PMFCs in the rhizosphere. Possible technical issues may be the decrease in power density when using tubes with a larger diameter or multiple tubes close to each other. Also the root density should be taken into account when determining the maximum current (per m^2 PGA) of tubular PMFC in the wetlands. The root density is not evenly distributed over the depth. Tubular PMFC close to the highest root density produce most current as shown in chapter 4 and 5. Therefore, the tubes should preferably not be distributed evenly over the entire depth.

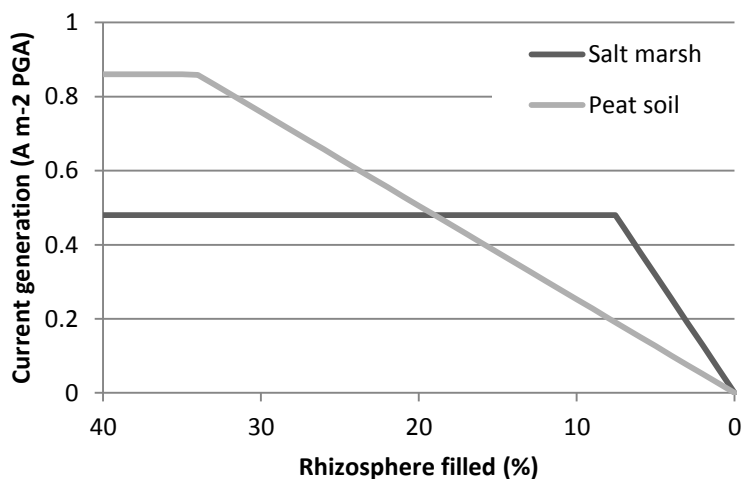


Figure 2: Current generation of the PMFC in relation to percentage of rhizosphere used for PMFC. Figure based on the results of the tubular PMFC in chapter 6. Current generation cannot be higher than the predicted maximum current density calculated in chapter 5. The rhizosphere is assumed to have a depth of 150 cm for the peat soil and 60 cm for the salt marsh

7.3.6 Vertical installation of tubular PMFC to limit excavation of the soil

The long tubular PMFC with an air pump could be installed in the wetlands using horizontal drilling (figure 3). However, to reach the maximum output per m² PGA the power losses related to the use of the air pump have to be avoided and a large total length of tubular PMFCs has to be installed (figure 2). Therefore, short tubular PMFCs without air pumps are preferably used when the maximum power output has to be reached. Horizontal drilling is not possible for multiple short tubular PMFCs. The tubular PMFCs have to be installed on top of each other which requires excavation of the soil. The tubular PMFC can also be installed vertically with less excavation of the soil than horizontal installation while generating the same power output (figure 3). The vertical PMFCs are easily installed in the soil by placing them in a hole made with a soil drill or by a pole driver. Vertical installation of the tubes is especially interesting in the salt marsh. In chapter 5, we showed that most power can be generated in top layer (around 10 cm below the surface level) of the salt marsh so most electron donors are present at this top layer. As a result also most oxygen is required in the top layer. With the tubular design, most oxygen will be present in the top part

because the diffusion of oxygen into the silicone tube causes a gradient of oxygen through the silicone tube. At the inlet the oxygen concentration equals the oxygen concentration of the outside air, which decreases into the silicone tube. Both the oxygen concentration and the electron donor concentration follow a similar trend and as a result internal currents could be limited.

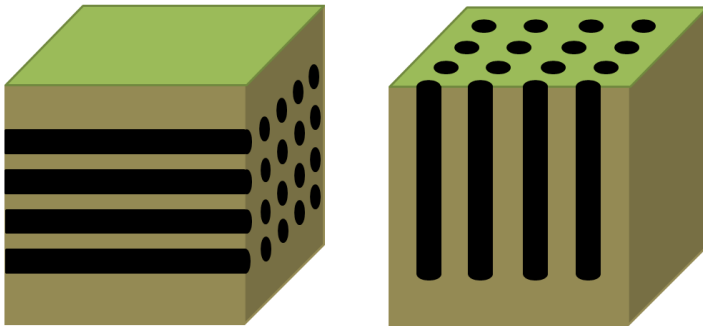


Figure 3: Impression of installed tubular PMFCs. Left: Horizontal, preferred for the long tubular PMFCs with active air supply. Installation by horizontal drilling. Right: Vertical, short tubular PMFCs with passive air supply preferred to reach maximum power out per m^2 PGA. Installation with soil drill or pole driver

7.3.7 Cost reduction required for economically feasible tubular PMFC

In practice, more power can be generated in the salt marsh than in the peat soil as was shown in chapter 5 and chapter 6. Application of the PMFC in the salt marsh seems therefore to be more favorable than application in peat soil. Although, more oxygen should be supplied into the cathode to be able to start the oxygen reducing biocathode with air diffusion (chapter 6). This is possible by using a silicone tube with a larger diameter or a higher diffusion coefficient.

The chapter 6 tubular PMFC in the salt marsh generated 0.53 mW (= 2.5 mW m^{-1} PGA). The tubular lab-scale PMFC had a total cost of 7.75 euro excluding the current collector. The total costs include the costs of the graphite felt (2.34 euro, 62 euro m^{-2} electrode, PGF grade, CGT Carbon GmbH, Asbach, Germany), ultrafiltration membrane (5.04 euro, 24 euro m^{-1} , 24 mm, MEMOS Membranes Modules Systems GmbH, Pfullingen, Germany) and silicone tube (0.36 euro, 1.82 euro m^{-1} , ISS 6, Lapp Group, Stuttgart, Germany). The used golden wire current

collector was excluded as it can be easily replaced by a graphite stick current collector. The payback time of only the material is 40410 year in the salt marsh based on the Dutch electricity generation price (0.041 euro kWh⁻¹ in 2014 [162]). The same tubular PMFC in peat soil has a payback time of 150618 year. The payback time for the tubular PMFC decreases to respectively 7530 year in the salt marsh and 28070 year in the peat soil when compared to the Dutch household electricity price of 0.22 euro kWh⁻¹ [163]. The PMFC electricity generation costs (in euro kWh⁻¹) have to be lowered to decrease the payback time. Figure 4 shows the required reduction in costs to be economically feasible in the salt marsh at different payback times. The costs of the PMFC should decrease to 0.5 % of the current costs to be able to compete with the Dutch electricity generation prices when the payback time of the PMFC is 200 years. 0.08 % of the current PMFC costs are required when the desired payback time is 30 years [12]. For household application, the costs should be reduced to 0.4 % of the current costs to be economically feasible at a payback time of 30 years.

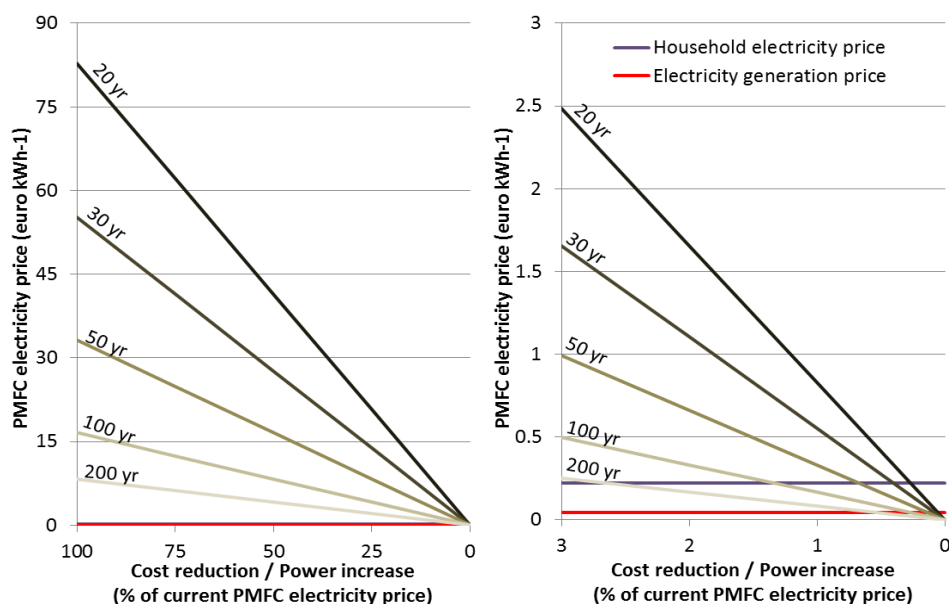


Figure 4: Required reduction of PMFC electricity generation costs of the tubular PMFC to reach economic feasibility in the salt marsh at different payback times (20 to 200 year) for application in the Netherlands taking into account only the material costs of the tubular PMFC. Figure is based on a power output of 0.53 mW per tubular PMFC in the salt marsh. Each tubular PMFC has currently a material cost of 7.75 euro

The costs of the tubular PMFC have to be decreased. The graphite felt anode and cathode should be replaced by a cheaper electrode material, for example graphite granules or granular activated carbon [164]. Also the application of PMFC with the even cheaper biochar as electrode material is promising [165]. Biochar prizes are approximately 0.0005 euro m⁻². Replacing the graphite felt by biochar reduces the costs of the PMFC to 70 % of the current costs. Next to the electrode material, also the costs of the membrane (e.g. cation exchange, ultra- or microfiltration) are high and should be lowered [153]. The main function of the membrane is to physically separate the anode and cathode avoiding short circuit with a minimal distance between the electrodes. The membrane could thus also be replaced by a cheap spacer. This spacer should preferably be small (to minimize the ionic and transport losses [166]), cheap, not electric conductive, ion conductive and should block roots avoiding roots and rhizodeposition in the cathode and the associated internal losses. A possible replacement of the membrane is water permeable landscape fabric as it meets the set requirements. The costs of this landscape fabric are lower than 0.03 euro per tubular PMFC [167]. Also the silicone tube can be replaced by a cheaper silicone tube which would costs approximately 0.04 euro per tubular PMFC [168]. The material costs of the tubular PMFC could be less than 0.10 euro per PMFC (of 21 cm). Thus less than 1 % of the current PMFC costs and the PMFC could thus be economically feasible in approximately 50 years when only taking into account the material costs. Certainly, the use and long term stability of cheaper materials must be further investigated in PMFCs. For application at real scale, also the costs of current collectors, production, installation and electricity transmission should be included.

7.3.8 Current collectors could significantly increase costs of tubular PMFC with air pump

Current collectors are required for the transmission of the electrical current from the anode to the cathode. The current collectors have to cover the entire length of the anode and cathode as the resistance of the graphite felt is large (0.14 Ω cm, PGF grade, CGT Carbon GmbH, Asbach, Germany) and results in major voltage losses when transported over the length of the tubular PMFC with an air pump. Relative cheap electric conductors like aluminum and copper cannot be used in the PMFC as both are easily oxidized at the potential of the PMFC. Gold and titanium are stable conductors at the potential of the PMFC and are

therefore suitable as current collector [164]. Titanium is significantly cheaper than gold and is therefore the preferred current collector for the PMFC. The total volume and costs of the current collector can be calculated (box 6).

The length of the tubular PMFC with an air pump would be 397 m in the peat soil and 63 m in the salt marsh as calculated in section 7.3.3. The costs of a titanium current collector for these tubular PMFC would respectively be 12202 euro and 309 euro. This is calculated assuming a voltage loss of 0.1 V, a current density of 1.3 mA m^{-1} for the peat soil and 8.0 mA m^{-1} for the salt marsh, a resistivity of titanium of $43 \cdot 10^{-8} \Omega \text{ m}^{-1}$, a density of 4506 kg m^{-3} and a titanium price of 3.97 euro kg^{-1} [169]. The costs of titanium per meter tubular PMFC would be 31 euro for the peat soil and 4.9 euro for the salt marsh, thus significantly increasing the costs of the tubular PMFC.

In the calculations, the length of the current collector is to the power two. Increasing the length of the tube results in relatively higher costs for the current collector. Thus, especially at large length the current collector significantly increases the costs of the PMFC. The costs of the current collector could be decreased by using cheaper conductors like aluminum and copper. However, these current collectors should be coated with gold, titanium or even graphite [170] to avoid the oxidation of the conductor. Replacing the titanium current collector by an aluminum current collector reduces the total costs of the 397 m current collector in the peat soil to 153 euro (0.39 euro m^{-1}) and the 63 meter current collector in the salt marsh to 3.87 euro (0.06 euro m^{-1}) based on a resistivity of aluminum of $2.65 \cdot 10^{-8} \Omega \text{ m}^{-1}$, a density of 2700 kg m^{-3} and an aluminum price of 1.35 euro kg^{-1} [171]. This significant lower costs of the current collector shows the importance of graphite coated aluminum current collectors for the application of the PMFC. However, experiments have to show the stability and applicability of these current collectors prior to application in the PMFC.

Figure 5 shows the costs of a titanium and aluminum current collector in the salt marsh in relation to the total material costs of the PMFC at different lengths. The potential costs of the tube are approximately 0.50 euro per meter as calculated in section 7.3.7. Currently, the costs of the air pump are approximately 20 euro. However, economy of scale would reduce the costs of the air pump. In the calculations, 2 euro per air pump is assumed. The costs of the current collector are calculated according to box 6. The lowest costs of the tubular PMFC with a titanium current collector are 0.82 euro m^{-1}

Box 6 – Volume and costs of the current collectors

The total volume and costs of the current collectors are calculated with eq 13 and 14. The calculations of the volume of the current collectors are based on Ohm's law (eq 2) and Pouillet's law (eq 15).

$$V = \bar{A}2x = \frac{\rho \bar{I}(2x)^2}{U} \quad (\text{eq 13})$$

$$\epsilon = V\rho\epsilon_m \quad (\text{eq 14})$$

$$R = \frac{\rho 2x}{\bar{A}} \quad (\text{eq 15})$$

In which, V is the volume of the current collectors, \bar{A} is the average cross sectional area of the current collectors (in m^2), x is the length of the tubular PMFC (in m), ρ is the resistivity of the current collectors (in $\Omega \text{ m}$), \bar{I} is the average current density in the current collectors of the tubular PMFC (i.e. half the maximum current as the current is linearly increasing) (in A), U is the voltage loss in the current collectors (in V), ϵ is the total costs of the current collectors (in euro), ρ is the density of the current collectors (in kg m^{-3}), ϵ_m is the price per kilogram current collector (in euro kg^{-1}) and R is the resistance of the current collectors (in Ω). The length is multiplied by 2 in eq 13 and eq 15 as both the anode and the cathode have a current collector.

at a total length of 9 meter. The costs per meter increase significantly for longer tubular PMFCs. Replacing the titanium current collectors by aluminum current collectors reduces the minimum cost to 0.57 euro m^{-1} at a length of 40 meter. Increasing the length of the tubular PMFC towards the maximum of 63 meter does only increase the costs of the tubular PMFC by a few cents per meter. The figure clearly shows that titanium current collectors are not suitable for the installation of long (i.e. $> 20 \text{ m}$) tubular PMFC in the salt marsh.

7.3.9 Installation and electricity transmission further increase costs of PMFC

The production and installation costs of the tubular PMFC are difficult to evaluate as it depends on the time it takes to install a tubular PMFC and on the tools used for installation. The installation costs could be reduced by the use of tubes with a larger diameter, because in that case less tubes have to be installed to reach the same power output per m^3 PMFC assuming the same power density per m^3 . In chapter 4, we showed that current is generated at the same location as the plant roots are present. A larger (i.e. thicker) anode allows more roots to grow in the anode compartment and thus a higher current per meter tubular PMFC. Field experiments are required to show if also the same current per m^3 anode

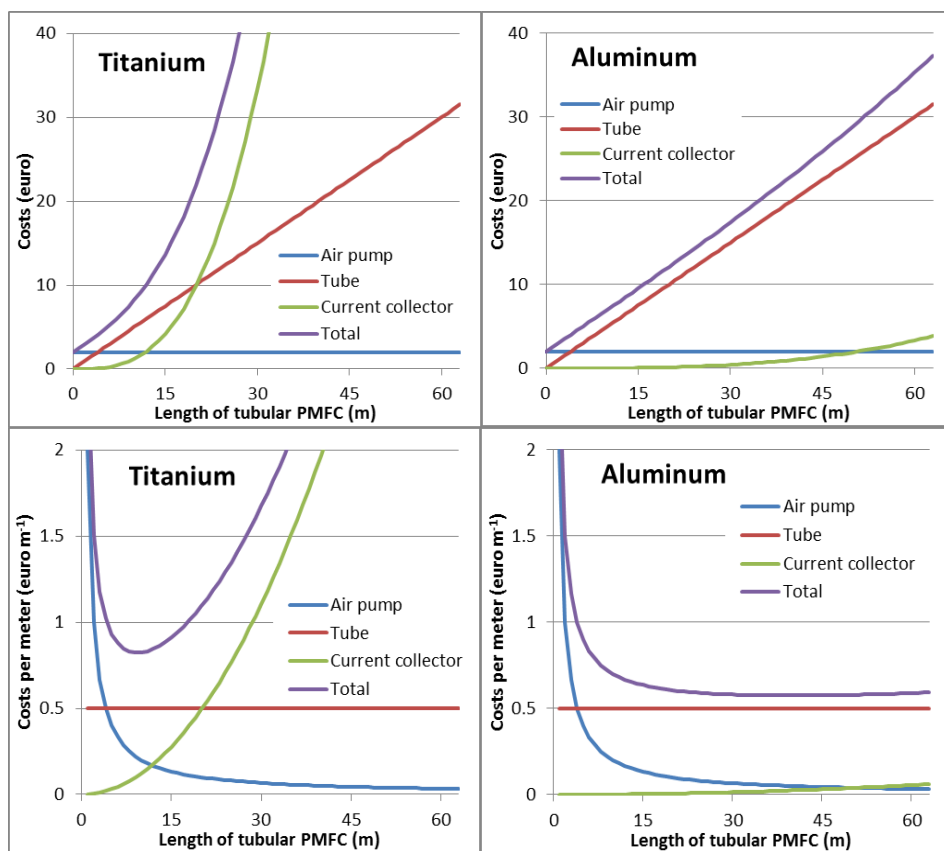


Figure 5: Total material costs (top) and costs per meter (bottom) of the tubular PMFC with a titanium (left) and aluminum (right) current collector applied in the salt marsh. The figures include the costs of the air pump (assumed 2 euro), the costs of the tube (0.50 euro m⁻¹) and the costs of the current collector (as calculated in box 6)

can be generated. The installation costs could also be decreased by installing PMFC in an area which is already under construction, by combining PMFC with an existing application like drainage systems in soils or new wetland formation for example in the Markermeer [172]. Further assessment of the installation costs is required for a detailed effect of these costs on the payback time of the PMFC.

Electricity transmission could increase the costs of the PMFC significantly. The costs of electricity transmission can also be calculated with the equations from box 6. For example, one hectare of *Spartina anglica* salt marsh could generate 4800 A. Transmission of the electricity over 250 meter with a maximum allowed voltage drop of 0.1 V would cost more than 1 million euro for the aluminum based on a resistivity of

aluminum of $2.65 \cdot 10^{-8} \Omega \text{ m}^{-1}$, a density of 2700 kg m^{-3} and an aluminum price of $1.35 \text{ euro kg}^{-1}$ [171]. The costs per meter tube are 1.93 euro assuming 600,000 meter of tubular PMFC in one hectare. The 600,000 meter of tube are calculated from a current generation of 8 mA m^{-1} and a total current of 4800 A. The high costs of transmission wires shows that the produced power by the tubular PMFC should preferably directly be used without transmission. In the case that electricity transmission is required the voltage should be increased with a voltage booster to reduce the costs for the transmission wires [173]. For example, when the voltage is increased from 0.7 V to 5 V, the costs for aluminum transmission wires decreases to 22,719 euro (0.04 euro per meter tubular PMFC). The significant lower costs are explained by the decrease in current and the absolute increase in maximum voltage loss when the relative voltage loss is assumed to be the same percentage as without power booster. However even with a voltage booster of 5 V, transmission of the power produced should be limited. The voltage should be further increased when electricity transmission over longer distances is required.

7.3.10 Increasing the economic feasibility of the tubular PMFC

The economic feasibility of the tubular PMFC in the Netherlands can be increased by increasing the electricity price for PMFC electricity. However, consumers of PMFC should in that case be willing to pay more for their electricity. In general, people are willing to pay more for green electricity, but the willingness to pay varies per source [174]. The willingness to pay more for PMFC electricity is expected to be more than for other renewable electricity sources as PMFC uses plants which do not affect the aesthetics of the surrounding like other sources do. A survey regarding the willingness to pay more for PMFC should be conducted to confirm this expectation.

PMFC application in locations outside the Netherlands could also increase the economic feasibility of the tubular PMFC. Interesting locations are locations without electricity, higher electricity prices or higher potential power generation by the PMFC. Worldwide, 1.1 billion people had insufficient access to electricity in 2012. This is 15 % of the world population, from which 88 % lived in Sub-Saharan Africa and South Asia [175]. In these locations electricity is more valuable than in the Netherlands. Interestingly, these locations also have a higher potential power production than what can be achieved in the

Netherlands (figure 1). Thus more power can be produced in Sub-Saharan Africa and South Asia which also has more value. Likely, PMFC is thus more economically feasible in Sub-Saharan Africa and South Asia and can as such be part of the realization of the objective of universal access to electricity by 2030 which was set to decrease the amount of premature death due to air pollution [175]. Further case specific analysis needs to be done at smaller scales to evaluate the case specific economic feasibility.

7.4 Outlook of PMFC application in the Netherlands: two cases

7.4.1 Salt marsh in the Oosterschelde could produce the power required for a village

The Oosterschelde is a tidal basin in the southwest of the Netherlands. The *Spartina anglica* salt marsh used in this thesis was collected at this tidal basin. In 2014, the total area of *Spartina anglica* salt marsh in the Oosterschelde was 390.9 ha [176]. In theory, the salt marsh could generate 0.34 W m^{-2} PGA (chapter 5). Therefore, application of PMFC in the Oosterschelde could generate 1.3 MW (i.e. 11.6 GWh yr^{-1}). An average person uses 1393 kWh yr^{-1} , based on an average household use of 3500 kWh yr^{-1} [177] and average household size of 2.19 persons [178]. The Oosterschelde could thus cover the electricity consumption of 8360 persons. As such, PMFC applied in the Oosterschelde could generate all the household electricity consumption of an average village like Yerseke (6670 persons [179]) located directly at the Oosterschelde. The produced electricity could thus be used directly at the Oosterschelde.

More electricity could be generated in the Oosterschelde by oxidizing the electron donors in the sediment. The external carbon flux in the Oosterschelde is approximately $375 \text{ g m}^{-2} \text{ yr}^{-1}$ [135] generating 0.13 W m^{-2} when oxidized (calculated in chapter 5). The total surface of the Oosterschelde, 336 km^2 [180], could generate 44 MW and thus potentially generating the electricity of 279,835 persons. The Oosterschelde could generate the electricity consumption of 73 % of the inhabitants of the province of Zeeland. However, the electricity generation is clearly lower at locations without plants decreasing the economic feasibility of the application.

7.4.2 Peat soil in the Alde Feanen could be used to stimulate eco-tourism

Alde Feanen is a national park in the north of the Netherlands. The area is mainly peat fen from which 223 ha is covered by *Phragmites australis* [181]. PMFC applied in the *Phragmites australis* peat soil in the national park could theoretically generate 10 GWh yr⁻¹ based on the predicted power generation of 0.52 W m⁻² PGA (chapter 5). This power generation will likely not be reached in the peat soil by the PMFC as mentioned in section 7.3.5. Tubular PMFCs in the peat soil could possibly generate 0.25 A m⁻² PGA assuming installation of the PMFC in 10 % of the rhizosphere (figure 2). This results in a power generation of 0.13 W m⁻² PGA using the tubular PMFCs of chapter 6. The Alde Feanen could thus produce 2.5 GWh yr⁻¹. The generated electricity is preferably directly locally used in the national park for example for tourism purposes. Large part of the recreation in the Alde Feanen is by boat. The local government stimulates the use of electric boats and even plans to have 'electric only' boat routes in the Alde Feanen [182]. PMFC could provide the electricity at public charging points and as such support the local government in their goal to use more renewable electricity. Also PMFC electricity could be locally used for example at the charging points for electric bikes and in the holiday park. Possibly, the price of PMFC electricity will be more than of other sources of electricity and the tourists of the national park should in that case be willing to pay more for their electricity. The willingness of tourists to pay extra for electricity depends on sufficient information and their environmental consciousness [183]. Nature and serenity are the main reasons for tourists to visit the Alde Feanen National Park [184]. Therefore, these tourists are expected to be environment conscious, especially as most tourist would like more calm recreation (e.g. electric boats)[184]. Likely, the tourist in the Alde Feanen are thus willing to pay more for the electricity.

Electricity could also be generated by oxidizing the peat at areas without plants. When the peat is not renewed it is a fossil source. Electricity generation from oxidizing the peat is as such thus not renewable, however clearly more environmental friendly than burning the peat, as no fine particles are emitted. Burning peat for electricity production is still common in countries like Ireland [185] and could thus be replaced by the environmental friendly PMFC.

7.5 Potential additional values and applications of PMFC

The worldwide area of wetlands decreases because wetlands are converted for agricultural and aqua-cultural purposes [186]. Wetlands have several natural biological functions as food chain productivity and habitat, coastline protection, groundwater recharge, flood and storm water storage, water quality improvement and also cultural values [29]. The conversion of wetlands for agricultural and aqua-cultural purposes results in unacceptable losses in biodiversity and eco-hydrological functions [186]. Furthermore, up to 60 % of the local population in low income countries depends on the natural resources and functions of wetlands and are affected by the conversion [186]. The importance of wetlands for these people is often ignored [186]. Electricity generation by the PMFC can be an additional value to wetlands increasing its importance. This may result in less conversion of wetlands and thus maintaining the values and functions of wetlands.

The application of PMFC in wetlands has more potential values than just electricity generation (figure 6). The electrochemical active microorganisms outcompete the methanogens and the methane emission from wetlands is therefore decreased when applying the PMFC [39]. Methane has a significant contribution to the greenhouse effect [187] and 32 % of the worldwide methane emission is emitted by wetlands [188]. Therefore, PMFC could decrease the greenhouse effect not only by replacing fossil fuels by renewable fuels but also by lowering the methane emission of wetlands.

PMFC could also be beneficial for plant growth. A high concentration of sulfide is toxic for plants. The presence of sulfide significantly reduces root biomass and may result in dieback of the plants [189, 190]. The PMFC applied in salt marshes oxidize sulfide, thus reducing the sulfide concentration in the soil. The lower sulfide concentration may enhance plant growth and avoid dieback of the plants. This is for example valuable for seagrass which currently declines significantly [191]. Seagrass provides key ecological services (e.g. carbon production, nutrient cycling and sediment stabilization) and thus conservation and regrowth of seagrass is required to maintain these services [191]. Application of PMFC in salt marshes may enhance plant growth and as such aid in the regrowth of plants like seagrass and improve the coastal protection [192]. Experiments are needed to prove this theory.

In case large scale application of the PMFC is not economically feasible, PMFC could still be applied on smaller scale at remote locations. In this case PMFC could be applied independent from the grid avoiding the costs for electricity transmission lines to remote locations. A possible remote application for PMFC is remote sensors in wetlands powered by electricity directly from the wetland [37].

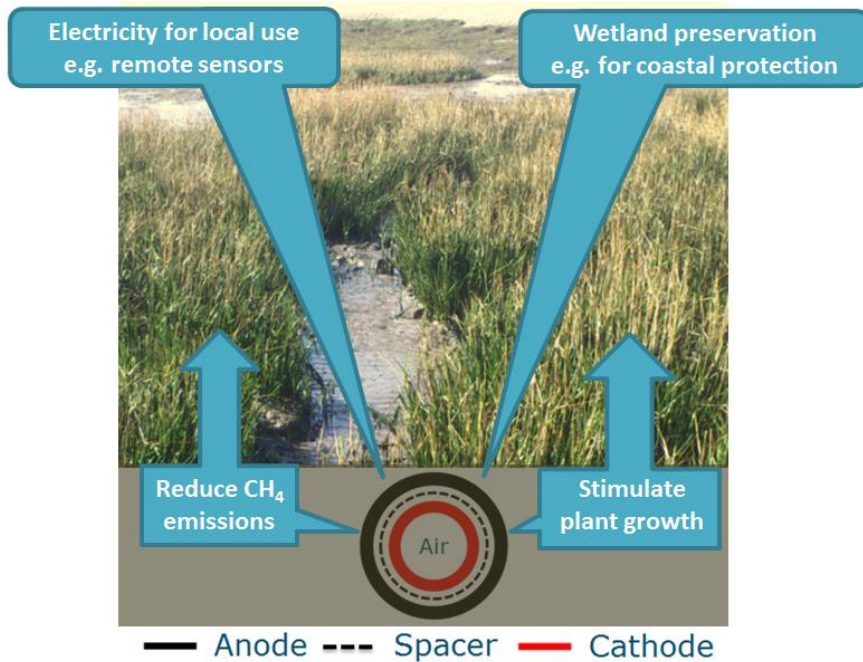


Figure 6: Visualization of potential additional values of the PMFC besides electricity generation

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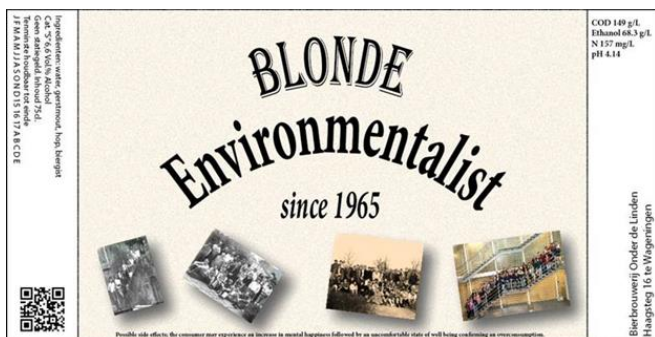
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About the author

I was born on the 14th of April 1986 in the very beautiful and atmospheric 's-Hertogenbosch in the south of the Netherlands. After finishing my high school, I travelled in New Zealand for a couple of months. Here I found out that I wanted to study something related to the environment and therefore decided to study Physical Geography at Utrecht University. During my bachelor, I realized that I prefer a more applied education with a higher societal relevance. Therefore, I moved to Wageningen University to study Urban Environmental Management. This master was not technical enough for me and so after finishing this master, I started a PhD in Environmental Technology at the Wageningen University. The PhD was about the application of the plant microbial fuel cell in wetlands. This PhD was related to the application of an environmental and societal relevant technology, thus combining the aspects of my interest.





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- o MSc Biological Processes for Resource Recovery, Wageningen University, The Netherlands (2013)
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Management and Didactic Skills Training

- o Supervising six MSc students with their MSc thesis, Wageningen University, The Netherlands (2012-2016)
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- o *Electricity generation within wetlands*. Environmental Technology for Impact Conference (ETEI2015), 29-30 April 2015, Wageningen, The Netherlands
- o *Continuous electricity generation in a plant microbial fuel cell by oxidizing rhizodeposits*. Rhizosphere 4 Conference: "Stretching the Interface of Life", 21-25 May 2015, Maastricht, The Netherlands

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Cover photos taken in National Park the Alde Feanen. The peat soil used in this thesis was collected in this National Park.