Final publishable summary report

Executive summary

Introduction: The plant microbial fuel cell (Plant-MFC) has the potential to become a large-scale electricity generating technology when implemented in wetlands. Such a system can produce in-situ electricity without harvesting the plants, 24 hours per day. In the Plant-MFC living plants provide organic matter to electrochemically active micro-organisms that generate electrons harvested by the fuel cell. It is expected that the Plant-MFC technology can cover 20% of Europe's primary future electricity need. The Plant-MFC produces in-situ renewable electricity. The system can likely operate in a clean, sustainable, and efficient manner.

The objective of this FET project was: "*To explore new areas of science & technology needed to realize the novel, clean, renewable, sustainable, efficient Plant Microbial Fuel Cell as a future bioenergy source in Europe*".

Approach: The PlantPower consortium developed high-tech and sediment Plant-MFC systems. The maximum electricity output improved 16 times up to 1.1 W/m2 projected growth area. This was possible due to research steered on pre-defined prerequisites and standardised set-ups.

***Key outcomes**:* Especially grasses stay healthy and effective in Plant-MFCs. The fuel of the Plant-MFC consists of rhizodeposits like exudates and dead roots. Rhizodeposits are converted within a vigorous anode-rhizosphere typically consisting of cellulosic degrading an electrochemically active bacteria. Still there is significant microbial competition for rhizodeposits. Rhizodeposition depends strongly on plant species, roots-architecture and likely the microbial composition. Especially small carbohydrates released by plant roots can be effective degraded into electrons at Coulombic efficiencies up to 70%. A flat-plate Plant-MFC was designed with a low internal resistance of 0.1 $\Omega.m^2$ which can allow power outputs reaching the theoretical maximum of 3.2 W/m2. Nowadays 0.2 W/m2 long term-output and maximum 1.1 W/m2 is reached. A new effective plant growth medium was developed. Based on model and experimental results we conclude that power output (at lab conditions) is likely limited by fuel availability, proton transport, anode surface area and low (20%) long term coulombic efficiency due to microbial competition and oxygen. Notably, improvement is possible. By integrating novel biocathodes into a flat-plate Plant-MFC, we were able to develop a complete biological sustainable system. A stacked Plant-MFC showed that upscale and energy harvest is possible for e.g. sensor applications. Other interesting added value Plant-MFC applications developed are the constructed wetland Plant-MFC and Green Electricity Roof. These applications look economical feasible. In case the added value is primary electricity production, in case of natural wetlands, still significant costs reduction and/or power output increase is necessary. An early LCA study revealed promising steps to develop a technology which generates electricity from a new source greener than the current electricity supply.

Conclusions: Extensive progress has been made in understanding the Plant-MFC. The Plant-MFC has now a performance that matches the net performance of current crop based electricity systems. The knowledge of this project is disseminated world-wide and an spin-off company is building follow-up projects to exploit our developments. Still, further fundamental research, technological integration, wetlands selection and demonstration in real wetlands is needed to show the full PlantPower electricity potential. After a first full scale implementation a complete environmental and economic performance analyses can be made to prove the potential impact of PlantPower.

More info: www.plantpower.eu.

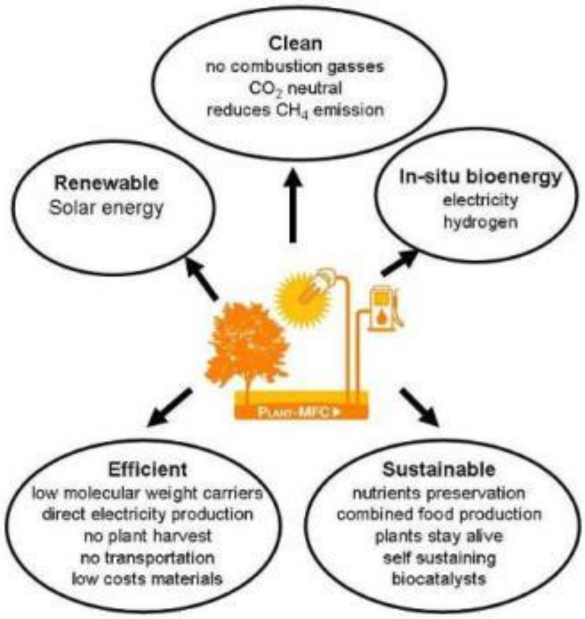
Summary description of project context and objectives

The possibilities of PlantPower

The plant microbial fuel cell (Plant-MFC) has the potential to become a large-scale electricity generating technology. Such a system can produce in-situ electricity without harvesting the plants, 24 hours per day. In the Plant-MFC living plants and micro-organisms form an electrochemical system that can produce sustainable electricity from solar energy, so called *PlantPower*.
It is expected that Plant-MFC technology can cover 20% of European Union's primary future electricity need. This PlantPower project aimed to improve the net power output of the Plant-MFC from 0.0067 W/m2, as achieved in 2007, to 3.2 W/m2 in 2012. Therefore Plant-MFCs' new areas of science & technology were researched. The Plant-MFC concept has several attractive qualities which can transform the current electricity market. The technology can reinforce the competitiveness of Europe on the global energy market because PlantPower's energy source is available everywhere where plants can grow. PlantPower therefore reduces dependency of Europe on external energy resources. Moreover, PlantPower could become a European electricity export product in the future since the Plant-MFC could be implemented world-wide

Benefits

By its nature, the Plant-MFC can become 10 times as efficient as conventional bio-electricity systems (like biogas and bio-ethanol production), because no energy has to be invested to generate electricity. Furthermore, the systems' concept is principally clean, renewable and sustainable. The technology might be implemented in several ways, ranging from local small scale electricity providers to large scale electricity wetlands & islands, high-tech electricity & food supplying greenhouses and novel bio-refineries. This way, affordable electricity can be produced everywhere where plants can grow. This is not limited to Europe. It offers opportunities for developing countries and remote regions as well. Plant-MFCs can be integrated in landscapes invisibly which makes this technology socially highly acceptable.



The PlantPower principle

The principle of the PlantPower project is outlined here. Carbon dioxide is fixed by plant leaves using solar energy. Part of the fixed carbon is transported to the roots and released as small molecular weight components. These so called exudates are partly utilized by electrochemically active micro-organisms yielding carbon dioxide, protons and electrons. Carbon dioxide is returned to the atmosphere. Electrons are transferred by electrochemically active micro-

organisms to the anode for gaining metabolic energy. The anode is coupled to a cathode. Thanks to a potential difference between anode and cathode, the electrons flow from the anode through an electrical circuit with a load to the cathode. To retain electro-neutrality a proton is transported through the membrane from the anode to the cathode. In the cathode oxygen is reduced, with protons and electrons, to water.

The Plant-MFC concept has been enabled by the discovery of electrochemically active micro-organisms in 1911. Since the beginning of this century microbial fuel cells gain renewed attention possibly due to the need for sustainable technologies. The proof-of-principle of the Plant-MFC, which uses plants to provide substrate for the micro-organisms, was independently described in 2008 by two consortium members. This showed for the first time that rhizodeposits from plant-roots can be used as a new electricity source.

Electricity production by a Plant-MFC depends on several processes; photosynthesis, allocation of fixed carbon to the roots, rhizodeposition of carbon-sources to the rhizosphere, electron generation by the microorganisms in the rhizosphere and electricity production by the fuel cell. To achieve a high power density in the Plant-MFC all these elements must be aligned. Using a conservative estimation it was calculated that an average power density of 0.23 W/m2 can be achieved while with enhanced rhizodeposition a maximum up to 3.2 W/m2 can be achieved. Conventional bio-electricity systems under West-European climate conditions achieve 0.3 W/m2 year-round average when producing electricity. It should be noted that in case plant growth is not impaired by the rhizodeposition i.e. exudation, the Plant-MFC power is an additional benefit. Given the potential impact of the technology (a new clean electricity source), the novelty of the concept the following objective has been set for the project:

To explore new areas of science & technology needed to realize the novel, clean, renewable, sustainable, efficient Plant Microbial Fuel Cell as a future bioenergy source in Europe.

To guide our research towards the use of Plant-MFC as a future bioenergy source in Europe we first visualized **two visionary lines for application**. From these visionary lines we defined **6 plant MFC prerequisites**. These prerequisites are the basis for structuring the project and steering the research toward our aim of the Plant-MFC as a future bioenergy source in Europe.

Visionary lines

(1) <u>Development of a high tech Plant-MFC</u>; this is a Plant-MFC which can be located e.g. 1) in an indoor greenhouse which can principally combine food production, heat-cold storage and voltaic solar cells; 2) on top of a buildings; or 3) near highways for street lightning. This closed system will offer a high level of control and a wide range of different upscale possibilities (like designs & cathode processes) compared to the outdoor sediment Plant-MFC.

(2) <u>Development of a sediment Plant-MFC in natural environments</u>; this is a Plant-MFC located outdoors in e.g. sediments, rice fields, flood fields etc. The principle difference with the high tech design is the absence of a membrane and specific measures and conformation to (almost invisible) outdoor application.

Prerequisites

(1) Plants in the MFC must stay healthy in the extreme environment of the MFC with an efficient and continuous production of photosynthates.
(2) Plants must excrete as many exudates & rhizodeposits as possible into the bioanode of the MFC (instead of producing crops).
(3) The rhizosphere must stay healthy; electrochemical active and rhizosphere microorganisms in the bioanode of the MFC must deliver as much as possible energy to the MFC.
(4) The high tech MFC must have a high power output; additionally, the MFC with the plant in the bioanode must be high in coulombic efficiency, low in internal resistances, robust, suited for upscale, good long term operable, cost efficient to material use and especially designed for root integration.
(5) The sediment Plant-MFC in natural environments has additional requirements; it must be insensitive to anode and cathode clogging and fouling, operational under varying water levels, protected from local acidification, composed of non-toxic materials and long term functional under all weather conditions.
(6) The total Plant-MFC concept as described in this proposal must be clean, renewable, sustainable, and efficient for the production of up to 3.2 MW/km^2 bioenergy in Europe.

Scientific & technology approach and key-objectives

From these perquisites we formulated **6 scientific & technologic key objectives** which encompass several main bottlenecks identified by us and potential solutions in these new areas of science & technology. Here we describe the key-objectives and specific objectives per work-package (WP1-6). Besides we had one WP on management (WP7) and dissemination (WP8).

WP1: Solving of specific Plant-MFC bottlenecks for development of high tech Plant-MFCs for application in e.g. greenhouses with an output 3.2 MW/km^2

- Clarification of Plant-MFCs' bioanode coulombic efficiency losses
- Development of a bioanode with a 90% coulombic efficiency
- Clarification of the Plant-MFCs' internal resistances
- Development of a Plant-MFC with a 60% MFC energy recovery
- Development & long term operation with trouble shooting of an up-lab-scaled Plant-MFC with an energy output of 3.2 MW/km^2

WP2: Solving of specific sediment Plant-MFC bottlenecks for development of sediment Plant-MFCs in natural environments with an output 1.6 MW/km^2

- Development of bio-anode for natural plant-MFCs
- Development of cathode for natural plant-MFCs
- Obtain 1.6 MW/km^2 level of energy production
- Development of an upscale natural Plant-MFC
- Determination of long term effects and possible troubleshooting for long term performance

WP3: Exploration of plant functioning and vigour in Plant-MFCs for preservation of healthy plants in the MFC

- Explore plant functioning & vigour of plants under bioanode-MFC conditions
- Explore carbon transfer from the plant to the rhizosphere under MFC conditions.

WP4: Exploration and maximization of rhizodeposition by plants in Plant-MFCs with a 70% rhizodeposition of total carbon fixation

- Knowledge of genetic variation in exudation among plants.
- Understanding the balance between crop/plants growth and exudate production.
- Alternative root architecture for optimal exudate production and collection efficiency.
- Improvement of the rhizodeposition for MFC efficiency.
- Understanding the influence of increased root exudation on bacterial diversity in the rhizosphere

WP5: Exploration and optimising of rhizosphere and anode microbiology in plant-MFCs for the support on 70% rhizodeposition, 60% MFC efficiency and good plant health

- Assessment of the microbial diversity in the rhizosphere and anode under MFC conditions of different plant systems
- Identification of key microbial functions in the rhizosphere and anode to improve the efficiency and sustainability of plant-MFC operation

WP6: Model of Plant-MFC & Early technical, financial and sustainability assessment of Plant-MFC scenarios to reveal the current and future status of the technology as European bioenergy source

- Development and validation of a mechanistic model of the Plant-MFC in a greenhouse & natural environment.
- Apply the model to reveal the Plant-MFC processes, support research and identify major bottle necks
- Early technical, financial and sustainability assessment of Plant-MFC scenarios to reveal the current and future status of the technology as European bioenergy source
- Develop a road map for further development of the Plant-MFC

WP7: Management

- To execute top level coordination, management, financial and administration of the project.
- To monitor, facilitate and ensure the timely fulfillment of project aims and objectives

WP8: Dissemination objectives

- Communicate research findings and outcomes of PlantPower to European research and industrial communities to stimulate applied research and commercial exploitation
- To establish a conference infrastructure on the Plant-MFC as a promising new bioenergy source and clean technology for Europe
- To aid European industry and NGO’s to exploit and deploy Plant-MFC technology effectively
- Management of intellectual property rights

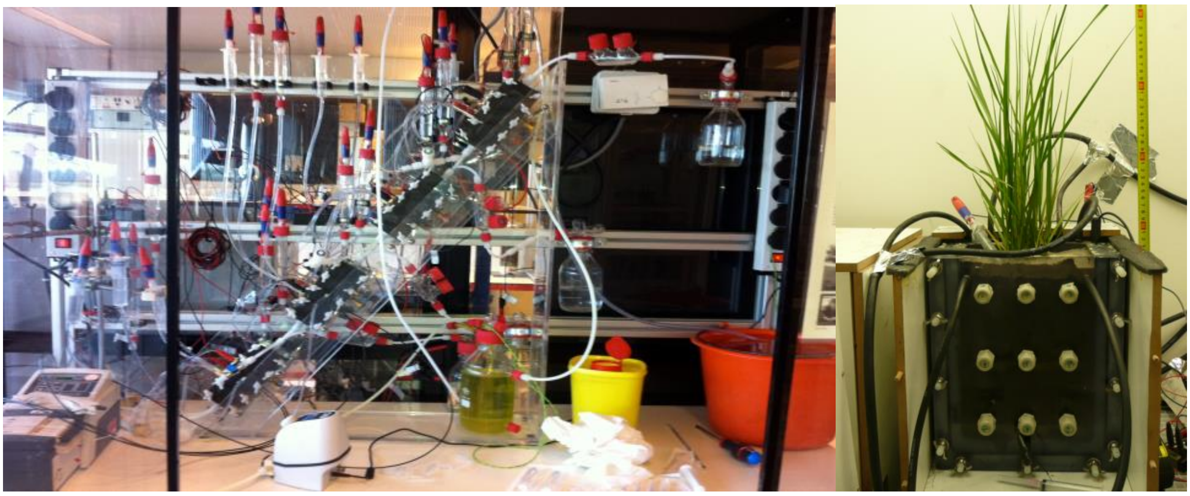
Description of the main S&T results/foregrounds

WP1: High Tech Plant Microbial Fuel Cells

High-tech Plant-MFCs reach long term power density of 0.22 W/m2

The power output of the Plant-MFC depends on the photosynthetic efficiency of the plant, the rhizodeposition, and energy efficiency of the MFC which is a combination of coulombic efficiency and the internal resistances (=voltage efficiency). This WP investigated and developed the high tech Plant-MFC towards the output of 3.2W/m2.

A Plant-MFC with low internal resistance has been realised (0.101 Ω.m2). The plant-growth medium has been adapted such that alternative electron acceptors were removed and both plant-growth and electrochemically active micro-organisms were able to thrive in the Plant-MFC. This has led to an average power density of 0.22 W/m2 and an average current density of 0.47 A/m2.



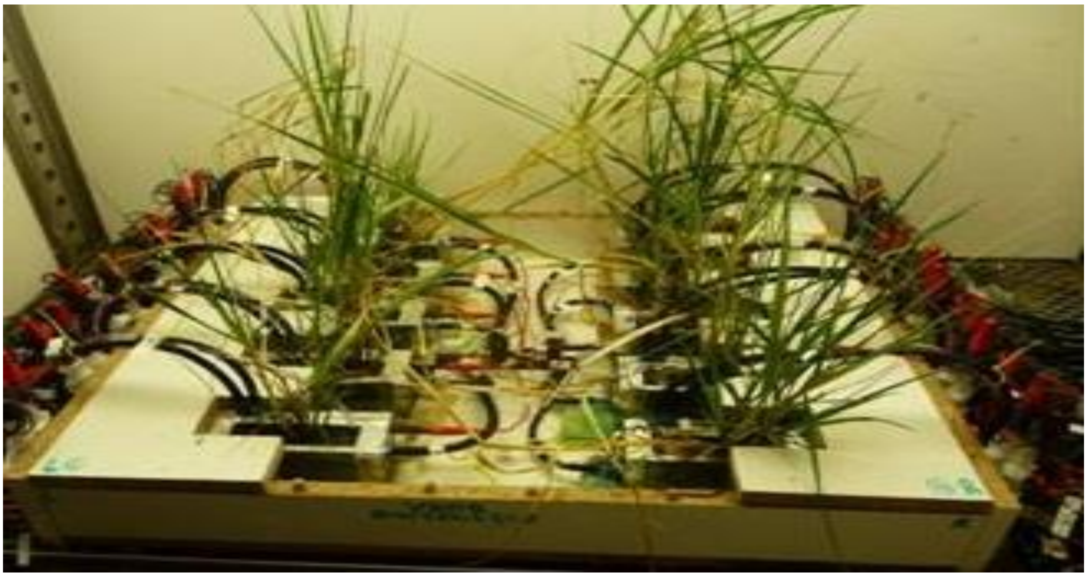
Artificial root-MFCs and flat-plate Plant-Microbial Fuel Cell

Coulombic efficiency depends on rhizodeposits and is estimated at 20%

Coulombic efficiency (CE) is the percentage of electrons present in the rhizodeposits which end up as electrons in the electrical circuit to obtain electricity. CE is affected by alternative electron acceptors (e.g. oxygen), micro-organisms and other parameters in the Plant-MFCs. Lowering the anode potential can lead to a higher power output. Coulombic efficiency of 60-70% was obtained with citrate oxidation. The effect of oxygen on the coulombic efficiency was positive, maybe due to methanogenesis inhibition. However, oxygen can also reduce the Coulombic efficiency since it’s energetically a more effective electron acceptor. Experiments with different rhizopdeposits revealed that the CE in the Plant-MFC is likely 20%.

Design improvements lead to new high performance

Voltage efficiency is percentage of voltage measured compared to the theoretical maximum voltage of the oxidation and reduction reactions. Voltage losses are caused by internal resistances which depends on the systems design, materials and other parameters. We designed a new flat-plate Plant-MFC and a new plant growth medium. Therefore we achieved a voltage efficiency of 40%. The overall resulting energy recovery is estimated at 8%. MFCs running on artificial wastewater can achieve energy recoveries up to 60%. The energy recovery of the Plant-MFC is under lab conditions limited by anode surface area, substrate availability, pH (includes transport resistance) and CE. Improved process control to create advantage for activity of electrochemical microbes (improves CE), selection of plants, selection of wetlands can further improve energy recovery.



Mini flat-plate Plant-MFCs

Roots are an additional source of organic matter but exudates are converted more efficient

Next to exudation, dead roots are an important source of organic matter. Calculation shows that at current exudation-levels organic matter from dead roots would be on a long run the major source of substrate for the micro-organisms. It has been shown that up to 60% coulombic efficiency has been realised with exudates. Although the production of organic acids and sugars is energetically less costly than the production of the complex organic constituents of roots, organic matter is still an attractive substrate for the MFC and the turnover of dead roots should be more thoroughly investigated.

Electrode material surface modification effects efficiency of bioanodes and biocathodes

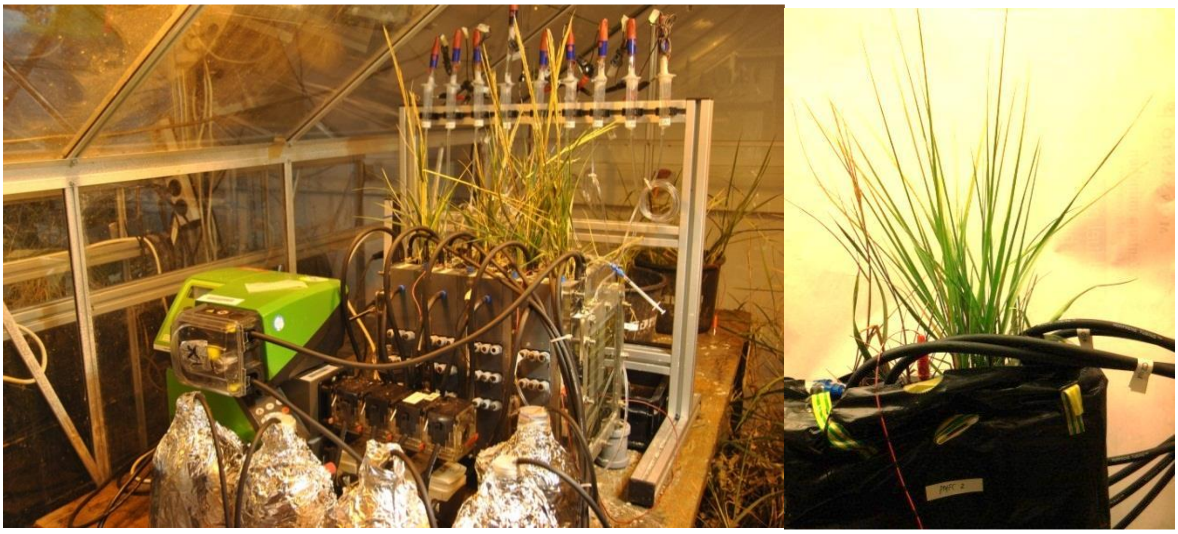
It has been shown that both size and type of carbon granules have an effect on current density of the Plant-MFC. It remains to be seen what determines these differences. Manipulation of the graphite surface with charged groups has shown to improve performance of the electrode compared to non-modified materials. A peer reviewed paper is in preparation.

Attractive airbiocathode was developed using microbes with high catalytic activity

The cathode of the Plant-MFC can limit the power output. A novel airbiocathode was developed that reached compatible current densities for Plant-MFCs. Oxygen-reducing biocatalysing bacteria were enriched on Biocathodes which showed, the highest catalytic activity so far reported. New, unidentified bacteria member of the family of *Sinobacteraceae* were most likely responsible for the effects. The next challenge is to integrate this electrode in a Plant-MFC. A peer reviewed paper is in preparation.

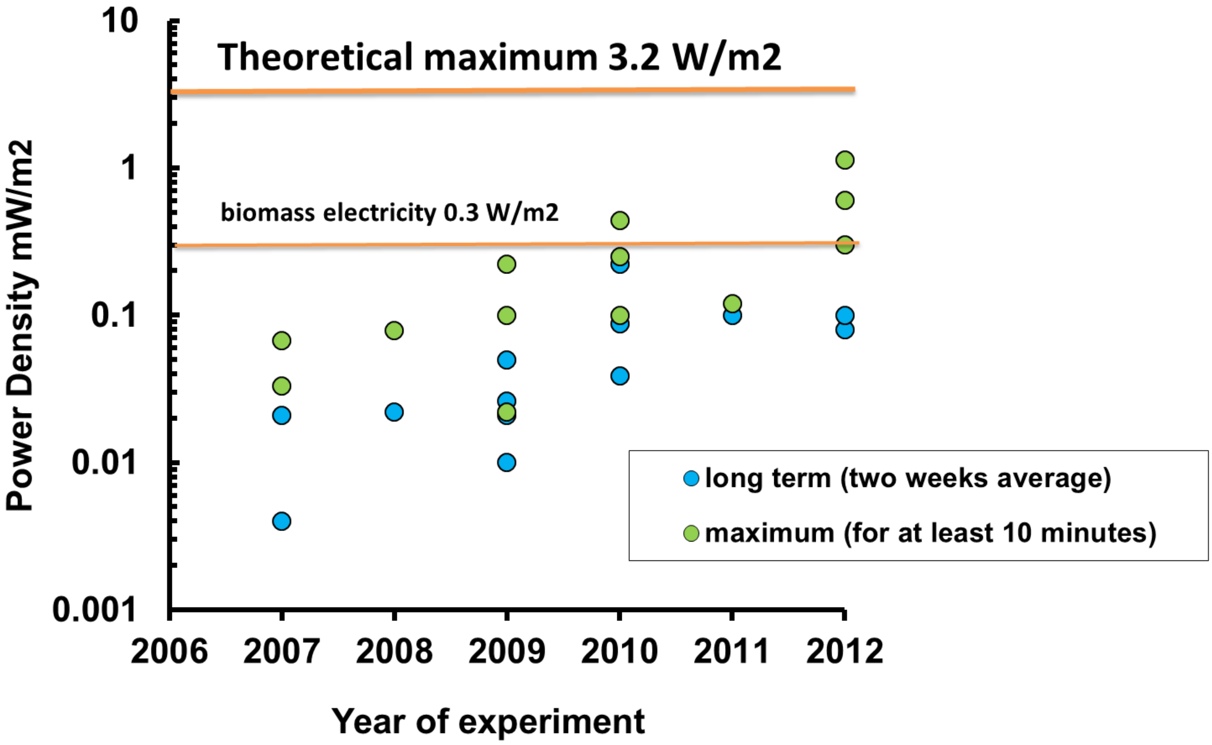
High 1.1 W/m2 power output of a scaled-up Plant-MFC

A stacked Plant-MFC was developed and investigated on performance. The maximum power output reached 1.1 W/m2. The electricity was harvested in capacitors, which can power all kinds of low power requiring applications.



Stacked Plant-MFC in a greenhouse & plant-MFC with biocathode

Based on the developed multidisciplinary knowledge we were able to design and operate Plant-MFC with increasing power output. Below figure shows the PlantPower results on long term power output (average of two weeks) and the maximum power outputs during polarisation curves.



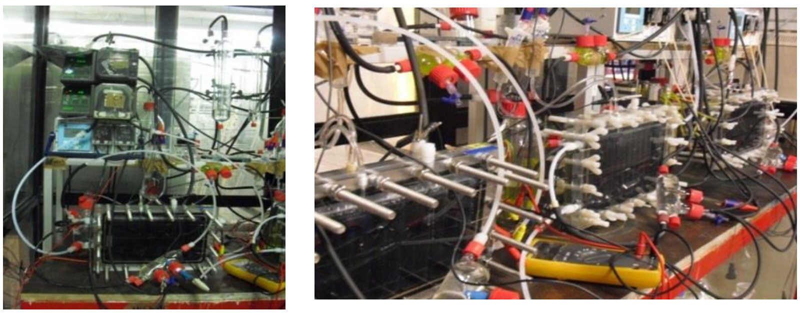
Power output development of FP7 PlantPower research

Plant Microbial Fuel Cell can be operated with sustainable bio cathodes

A flat-plate Plant-MFC was designed and operated with a biocathode. Electricity was generated at for at least 10 minutes at 0.6 W/m2 and the long term performance was 0.22 W/m2.

Biocathodes can also produce high value building blocks for fuel and other chemicals

A new Bio-electrochemical cathode system was developed including mixed cultures. Besides electricity, the Plant-MFC conditions and electrons can be used to produce fuel and other chemicals. Here, new biocathodes were used to produce medium chain fatty acids from low grade biomass, for instance acetate. By combining the Plant-MFC with these cathodes, also fuels and other valuable chemicals may be produced.

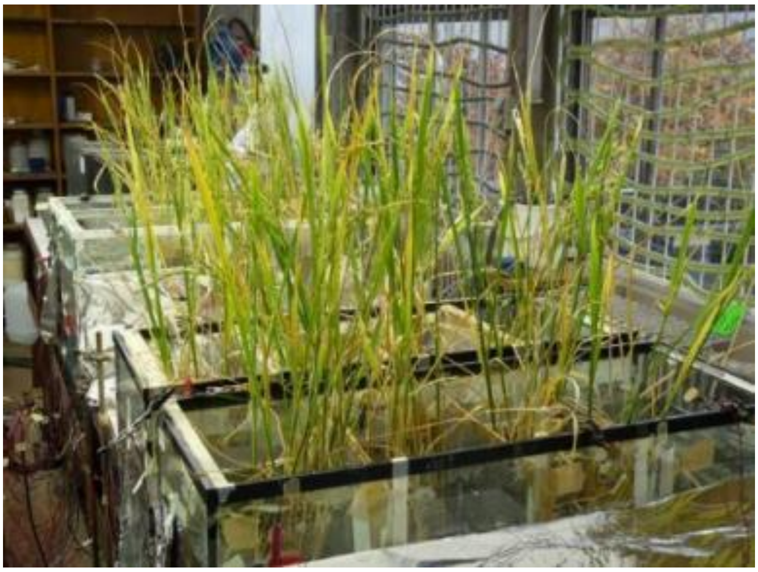


Biocathode producing chemicals

WP2: Sediment Plant Microbial Fuel Cells

Investigation anode materials for sediment Plant-MFCs in natural environments

Sediment plant-MFCs are envisioned to be applied in natural settings. This means that the roots of the plants are not solely in contact with the anode electrode. Sand/soil will also interfere with contact between the roots and the anode. Therefore the amount of anode electrode materials compared to soil volume was investigated. For this work sandy microcosms without plants were used. This was done in order to control the addition of organic matter (electron donor) and ensure the same concentration in each microcosm. Various sizes granules and different types of graphite felt were initially compared. The best performing materials were subject to further study. The further study focused on the amount of materials needed per volume of soil to achieve the same current density as a when no soil is present. It turned out that small granules 0.25-0.5 mm gave a higher current density compared to larger granules (1-5 mm). Rough granules gave a higher current density compared to smooth granules of the same size. This can be related to the different microbial communities that formed on the granules. Felt had an overall superior performance compared to the best performing granular material. Both materials reached current densities of 50-60 mA/m2 but 29 % less felt was needed to obtain this current density. When using granular electrodes, 2/3 of the soil volume should be occupied by the granules to obtain a current density comparable to 100% of the volume being occupied by the granules.



For most studies on MFCs commercially available electrode materials have been used. In order to enhance biofilm formation and anode electrode performance, commercially available electrode materials were modified with various functional groups. The technique of surface modification which has been used is the reduction of diazonium salts which allows the introduction of a wide range of chemical groups on anode surfaces. Numerous modified anodes (graphite plates) have been integrated in conventional microbial fuel cells to create uniform conditions not hampered by the presence of plants and soil. First, different anode surfaces have been modified using an amino group but using different degrees of modification. In this first set of studies, results indicated that modifying the electrode surface by means of controlled electrochemical reduction of the diazonium salt, with a neutral amino group, on the anode surface had an optimal degree of modification. Modifing the electrode surface with a charge up to 28 mC*cm^{-2} resulted in the highest current density. Adding more functional groups to the surface eventually led to a non-conductive surface at 200 mC*cm^{-2}.In a subsequent series of experiments positively charged phosphonium groups and negatively charged acetic acid groups were grafted on anode surfaces and compared to unmodified control anodes. This led to the observation that the

negatively charged group hindered bacterially catalyzed current generation at the anode while adding a positively charged group enhanced charge transfer compared to their respective unmodified controls. Scanning electron microscopic (SEM) observations of the electrode surface indicated that a poor biofilm developed on the negatively modified electrode while a dense biofilm developed on the positively modified electrode. Moreover, Fluorescent In-Situ Hybridisation (FISH) indicated that the dens biofilm contained almost exclusively members of the Geobacter subgroup which contains various current generating microorganisms.
In a second part of the work, anode materials which are used in the project (graphite granules) have been characterized (BET technique, SEM experiments, EDS experiment) and then modified by the chemical reduction of diazonium salts. One kilogram of graphite granules has been modified and sent to one of our European partners (Forschungszentrum Jülich) in order to investigate the effect of the integration of the modified material on the performances of a real Plant-MFC. Results showed that increasing the hydrophilicity of the granule's surface by surface modification (introduction of nitro groups on the surface) did not lead to an increase of the power output of the system.

Integrated applications for sediment Plant-MFCs in natural environments
Sediment Plant-MFCs introduce an electron acceptor in the form of an anode in the waterlogged rhizosphere of a wetland. Usually this rhizosphere is deprived of oxidized electron acceptors such as O_2, NO_3^-, Fe^{3+} and SO_4^{2-} and the main pathway of oxidizing organic matter in the rhizosphere leads to the formation of CH_4. CH_4 is a potent greenhouse gas (25 times the global warming potential of CO_2) and is mainly emitted through the aerenchyma of the plants growing in the wetlands. It is hypothesized that by introducing the anode of a Plant-MFC into the rhizosphere, methanogenic metabolism can be redirected towards current generating metabolism. This gives two advantages at 1) prevention of release of a potent greenhouse gas and 2) concurrent electrical power generation. To study this concept, microcosms with rice as a model plant were set up with a granular anode electrode. Provisions were made to close the aboveground biomass from the surrounding atmosphere and thus determine the methane emission rate at various timepoints during the rice growth season. Results indicated that methanogenesis could be delayed by introducing the electrical circuit. As a sequence in time, first organic matter appeared in the pore liquid, directly followed by current generation. Only after introducing an open circuit period i.e. no electrical current was possible, methane emissions were detected. Closing the electrical circuit as well as lowering the external resistance to allow more current to flow did not result in a decrease in methane emissions. This indicates that for effective mitigation of methane emissions the sediment Plant-MFC needs to be applied with other strategies. Examining the competitive process in more detail indicated that competition between methanogenic organisms and anode reducing bacteria (ARB) was most likely based on competition for acetate as acetoclastic methanogens were only detected of a limited number by means of quantitative Polymerase Chain Reaction (qPCR). ARB cause local acidification during their metabolic activity and acidic conditions are detrimental for methane formation. In this work, the influence of pH on methane emissions was most likely very limited as acidification was not detected in the bulk liquid of the microcosms.
Constructed wetlands (CWs) can be a site where a sediment Plant-MFC can be implemented. Constructed wetlands are currently being used for decentralized wastewater treatment based on sorptive capacities of the reed bed and the microbial transformations that occur at the roots of the plants. From previous studies on

sediment Plant-MFCs it was clear that oxygen reducing (bio)cathode performance was limiting due to overgrowth with algae and 'fouling' with organic matter. Moreover Plant-MFCs are, at times, limited by the amount of biodegradable organic carbon present in the anode/rhizosphere area. Therefore a combination between a constructed wetland and a MFC was envisioned. Here the CW is used for a fast filtration step collecting soluble organic carbon from plant roots and wastewater thereby retaining the solids from the wastewater in the reedbed. The liquid containing organic matter is sent to the anode of a reactor-type MFC where an electrical current is produced. At the cathode oxygen reduction can take place resulting in electrical power. However, at the cathode one can also opt to produce hydrogen peroxide, a potent disinfectant. Thus by combined the wetland and the (P)MFC a new concept for decentralized wastewater treatment is proposed.

The use of surface modification to optimize the O_2 reduction at the cathode side of a Plant-MFC

One drawback of using dioxygen as an oxidant at the cathodic side of a (Plant)-MFC is that an important over potential is observed compared with the thermodynamical potential (by using a carbon electrode). Then, one way to tackle this bottleneck is to use catalysts. In this work we considered the use of chemical and microbial catalysts.

First, chemical catalysts (metaloporphyrins) have been immobilized on cathode surfaces by the electrochemical reduction of diazonium salts. Then, the modified cathodes have been implemented in MFCs, and performances and stability of the catalyst have been studied. We observed that it was possible to increase *ca.* two times the power output of the set up by modifying the cathode surface. However, a poor stability was also revealed (< one day) which was not suitable for a Plant-MFC application.

Microbial catalysts have also been considered. Indeed, it has been shown that micro-organisms are able to catalyze the dioxygen reduction at the cathodic side of a MFC. In this study we wanted to study the effect of pre-modifying cathode surfaces on the performances of biocathode. Different cathodes have been modified by the electrochemical reduction of diazonium salts. Especially, we tuned the hydrophilicity of the cathode surfaces by using a hydrophobic group (decyl-anyline) and a hydrophilic group (nitro group) as modifiers. The modified cathodes have been integrated in different MFC set ups simultaneously with an unmodified control and the current produced by the cathodes has been followed. It appeared that the unmodified control exhibited the highest current density followed the surface modified with the hydrophobic group and the surface modified with the hydrophilic group. One way to explain these observations was that the degree of modification was too important and then it decreased the electron transfer rate between the modified surface and the biofilm. Then, a perspective of this work should be to modify cathodes with lower degrees of modification.

Even if modifying the surface did not lead to an increase of the performances of the set up, a high stability (~2 months) was observed in all cases (cathodes pre-modified or not). In another hand, we also studied the effect of changing the pH (from pH 5 to 9) and the temperature (from 14 to 31°C) on the performances of oxygen reducing microbial biocathodes. It was observed that the oxygen reducing microbial biocathode exhibited the highest performances at acidic pH and at high temperature.

WP3: Plant functioning and vigour in Plant-MFCs

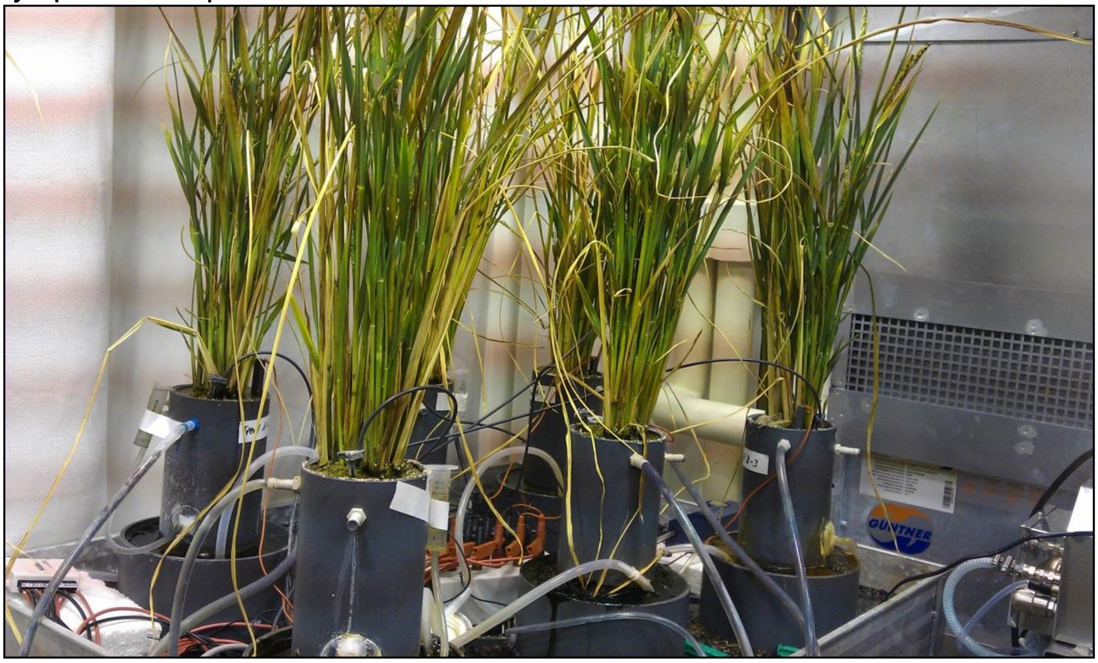
The environment in the anode of a Plant-MFC is a harsh environment for plants as compared to regular growing substrates (i.e. carrier material) of plants. The permanent waterlogging of the system, but also interactions of the artificial substrate (usually graphite granules) with the plants can cause inhibiting effects on plant-growth and thus prevent a healthy development of the plants. In order to improve plant growth - and especially root exudation (i.e. the release of organic carbon from the roots to the soil), it is necessary to understand the functioning and vigour of plants in Plant-MFCs.

Plant species specific tolerance to Plant-MFCs

This work package investigated the performance of plant growth in Plant-MFCs by use of different experimental set-ups and methods. Some of the used methods were invasive or destructive and others were non-invasive methods. We investigated a variety of plant species that are known to be waterlogging tolerant, because the adaptation to waterlogging is of course a prerequisite for surviving MFC conditions, since a Plant-MFC is kept waterlogged permanently. But other parameters seem to control the survival of plants, because we identified two species that do not survive MFC conditions, although they are well adapted to waterlogging conditions: *Viminaria juncea* ("Golden Spray", native to Australia) and *Lythrum salicaria* ("Purple Loosestrife", native to Central Europe). On the other hand, the following three species appeared to grow without any severe loss of vigour under MFC-conditions: *Phalaris arundinacea* ("Reed Canarygrass", native to Central Europe), *Glyceria maxima* ("Reed Mannagrass", native to Central Europe) and *Oryza sativa* (Asian Rice, cultivated across the world). Plant-Microbial fuel cells planted with these species generated comparatively high amounts of power and produced even high amounts of biomass that could be harvested frequently. Other well performing plant species are *Spartina anglica* ("Common Cordgrass, native to Central Europe), *Arundinella anomala* ("Small Cane", native to China) and *Hemathria altissima* ("Limpo Grass", distributed across the world).

Photo from a well growing Phalaris arundinacea individual (left hand side) and a dying Lythrum salicaria individual (right hand side) in operating Plant-MFCs.

Obviously, there is only one major difference that separates the two non-tolerant species from the MFC-tolerant plant species: all tolerant species are grasses. In the end this could be one key feature, because it is known that grasses display strong differences in their physiology compared to other species and they are able to cope with a broad range of environmental stresses. For example it is well known, that grasses are able to produce so called phytosiderophores (e.g. mugineic acid) in their roots, if they are subjected to low iron stress in soils. These phytosiderophores are large molecules exuded by the roots and they improve the bioavailability of iron in such deficient soils. Hence, maybe grasses can survive the MFC-conditions due to a family specific adaptation.



The figure above shows well growing Rice plants in tubular Plant-MFCs. Some of these plants started flowering and formed seeds as well.

Nutrient availability and plants nutrient mobilization capability are key for plant survival

This is in line with another key finding of our research: The results of our experiments strongly indicate that the availability of macro- and micronutrients in combination with the ability of the plants to modify the pH of the anode solution is key for plant survival and plant health. In detail: the surface of the graphite material is obviously capable to adsorb large quantities of plant nutrients like Calcium (Ca), Potassium (K), Magnesium (Mg), Iron (Fe), Manganese (Mn) or Phosphorous (P). Depending on the procedure of watering the Plant-MFCs, this results in a quick loss of bioavailability of especially Fe, Mn or P (even below the limit of detection). A deficiency of one or more of these nutrients immediately affects plant growth and health. For example Fe deficiency strongly affects the chlorophyll content in the leaves (chlorosis) and as a consequence the photosynthetic efficiency of the plants. Iron is essential for synthesis of proteins and ribosomes in leaves. The roots of iron deficient plants also show strong losses of vigour, i.e. an inhibition of root elongation. Hence, a long-term Fe deficiency will result in a severe loss of plant vigour.

MFC-tolerant plant species are able to overcome this constraint by modifying the pH of the anode solution below pH 6.5 within less than one week of growth in graphite (initial pH = 8). This will cause a release of the previously adsorbed nutrients from the surface of the graphite material due to ion exchange processes and by this alleviate the nutrient deficiency. On the other hand MFC-non-tolerant plant species are not able to modify the pH of the anode solution and therefore have to cope with the severe deficiency of nutrients. As long as these plants have no other adaptations towards nutrient deficiency they will die in a Plant-MFC.

We therefore recommend replenishing of the anode solution frequently with fresh nutrient solution. A continuous circulating of the anode solution might result in a severe deficiency of key nutrients. Another option or additional procedure could be the manipulation of the anode solution and by this mobilize the adsorbed nutrients. A regular flushing of the anode with demineralized water, to ensure that no surplus of the macronutrients will occur, could accompany these procedures. Another alternative could be the modification of the surface of the anode material in a way that all binding sites for nutrients are occupied. This could hamper a further adsorption of the nutrients as well.

Quick carbon allocation into the rhizosphere

Another aim of our work package was to provide information on the plant internal carbon dynamics. By this we wanted to improve our understanding how quickly the photoassimilates (e.g. sugars) that are formed during photosynthesis in the leaves are transported to the roots and even out of the roots into the environment of the roots (rhizosphere). This information will help to estimate the impact of dynamic environmental conditions on the carbon supply of the rhizosphere bacteria, especially the electrogenic bacteria.

This carbon transport under MFC conditions was investigated by labelling plant leaves with 11-CO2. The short-lived radioisotope 11C ($t_{1/2}$ = 20.4 min) is used as a tracer for non-invasive analysis of carbon transport and for 3D and live imaging or carbon transport by Positron Emission Tomography (PET/ PlanTIS). The applied 11C is incorporated in photoassimilates during photosynthesis and are transported into the roots via a plant internal translocation pathway (the phloem system). Using external detectors, the carbon distribution between shoot and root systems was non-invasively measured. In addition a plant nutrition system was established allowing sampling of the nutrient/ soil solution so that 11C- radiolabelled exudates were detected in the outflow.

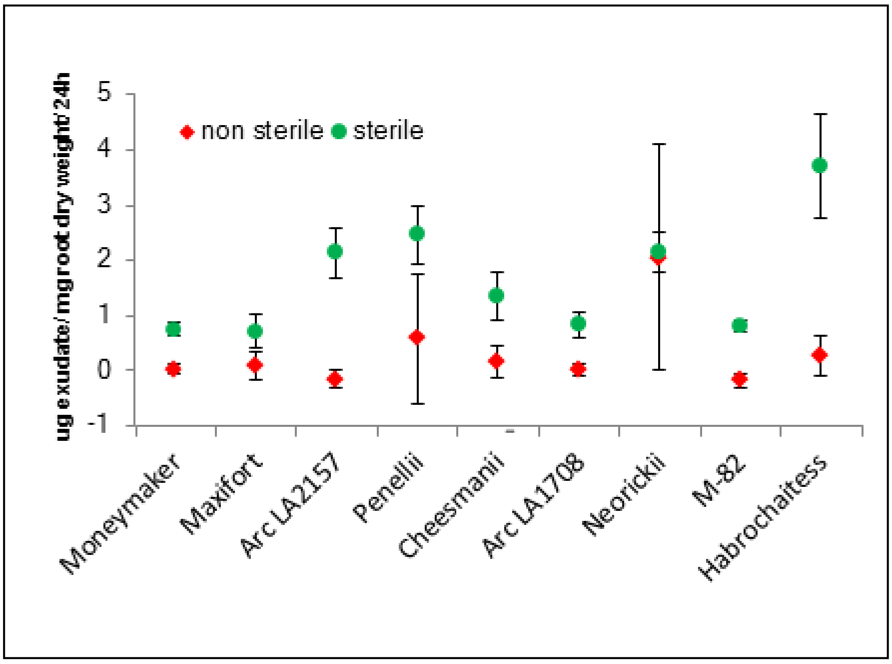
In our experiments we could demonstrate that the labeled carbon, derived from the uptake of 11-CO2 in the leaves of *Phalaris arundinacea* growing in waterlogged sand (serving as our standard reference substrate) was transported into the root systems after less than 40 minutes. Furthermore, after about 90 minutes the first release of the labeled carbon to the rhizosphere could be detected. This released carbon was a mix of respired CO_2 (by roots and microorganisms on the root surface) and root exudates. This quick transport of freshly assimilated carbon compounds from the leave through the entire plant into the soil also demonstrates the potential sensitivity of the underlying transport processes. Because any change in the photosynthetic activity of the leaves will almost immediately affect the supply of this carbon transport. One factor that controls the photosynthetic activity is for example the stomatal conductance (gs) of the leaves. This parameter is known to be very sensitive towards environmental parameters like humidity, light and temperature, as well as towards plant stress.

Grasses are most promising plant species for Plant-MFCs

Taking into account the information from other experiments in the frame of the PlantPower project it becomes very clear that only a healthy plant with minimal or moderate stress can supply the electrogenic bacteria in the anode compartment sustainably with rhizodeposits (root exudates and decaying roots). The most promising plant species are grasses, since they overcome certain limitations of the Plant-MFC systems and are able to produce high shoot biomass during the operation of the Plant-MFC systems. This biomass might even be used for other purposes like biogas production or animal feed.

WP4: Exploration and maximization of rhizodeposition

Genetic variation in exudation among plants.
Root exudation can be the limiting factor in electricity production by high-tech Plant-MFC's. The potential of exploiting genetic variation in exudation has, therefore, been a major topic of this work package. The research was carried out using tomato, a horticultural model species. The first important result was the development of a test system with which genetic variation in root exudation could be studied quantitatively. It was shown that micro-organisms in the rhizosphere break down externally added exudate species rapidly and therefore it was concluded that a sterile root environment is a prerequisite for studying root exudation. In a second study root exudation of a number of wild and commercial tomato varieties was compared under identical conditions using a sterile root environment. It was shown that there is indeed genetic variation in root exudation within the species tomato.

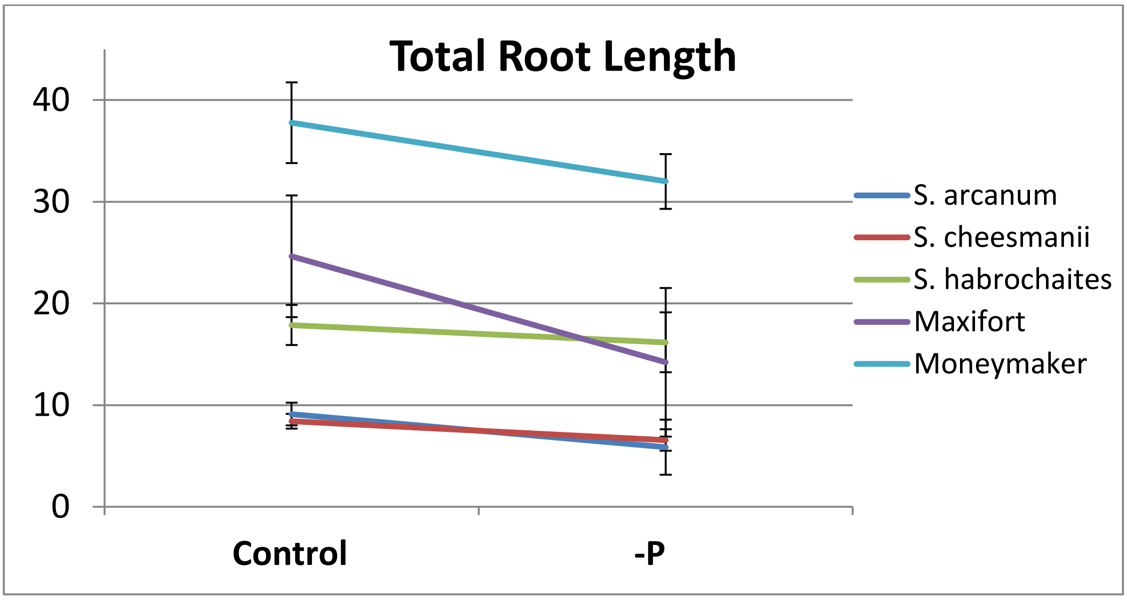


Total organic acid exudation (malate, citrate, oxalate, acetate) of nine tomato cultivars and species grown in sterile and non-sterile root environments (µg exudate (mg root dry mass)$^{-1}$ 24h^{-1})

The variation depends on the type of organic acid that is exuded. Under the observed conditions there was a factor 3-4x difference in root exudation of organic acids (malate, citrate, oxalate, acetate) between the highest and the lowest exuding tomato cultivar. Oxalate is, quantitatively, the most important exudate species in tomato. The popular commercial root stock cultivar Maxifort showed the lowest exudation, while the wild tomato species *Solanum habrochaites* exuded the most.
In general, the cultivated tomato genotypes show a lower exudation than the genotypes derived from the wild. This knowledge on exudation can be used to increase crop yields in high-tech greenhouses by lowering exudation even more. On the other hand, more exudation might contribute to increased yield stability by increasing the tolerance of crops against nutrient limitation in poor soils. More exudation can also be used to achieve better phytoremediation. This is the cleaning of contaminated soils with pollutant accumulating plants.

Understanding the balance between crop/plants growth and exudate production.
The above study on genetic variation within tomato species was carried out with intact plants. As the various species have quite different shoot characteristics and biomass it was not known whether the genetic differences in exudation in tomato were determined by root- or shoot traits. Therefore a new experiment was designed. Root systems of different tomato varieties (genotypes) were grafted onto a single shoot genotype and exudation was measured. Strong clues were obtained that it is indeed the root genotype that causes the variation in root exudation. To verify this finding, shoots of various tomato genotypes need to be grafted onto a single rootstock genotype.

Alternative root architecture for optimal exudate production and collection efficiency.
Root systems architecture (RSA) is important for the Plant-MFC for two reasons. First of all it can contribute to increased exudation. Secondly, it can increase collection efficiency by bringing the exudates closer to the electrochemically active bacteria. RSA is influenced by genotype and environment. Before the effect of optimization of RSA on exudation and on the presence of electrochemically active bacteria can be estimated, it should be clear what kind of genetic variation in RSA exists.
Five tomato varieties were studied to see if there is genetic variation in RSA. The results showed that such a genetic variation indeed exists. For several varieties total root length (figure below), total lateral root length, primary root length, lateral root number and average lateral root length were different. Under phosphorus limitation, which is known to increase root exudation in plants, these differences in RSA were still observed. No clear correlation between RSA and previously found exudation could be observed.
But can these differences improve collection efficiency? Electrochemically active bacteria are likely mostly located at the graphite granules in the anoxic zones. In the Plant-MFC this means in the lower part of the bioanode. Longer primary roots would mean the exuding root tips are deeper in the bioanode where the oxygen concentration is lower and collection/conversion efficiency is higher. Longer root length is also associated with lower oxygen loss from the roots into the bioanode. This also helps to increase coulombic efficiency.
Breeding for RSA traits is possible since there are differences in total root length. However, more research needs to be done to find genes responsible for these root traits.



Genetic variation of Root system architecture

Improvement of rhizodeposition for MFC efficiency.
To improve rhizodeposition for Plant-MFC efficiency the quantity can be increased or the composition can be changed. For the Plant-MFC concept to be successful, both quantity and composition require attention.

Phosphorus limitation is one of the most promising environmental factors that can increase root exudation. The effect of phosphorus (P) limitation on exudation by rice was studied under Plant-MFC conditions. A rice cultivar tolerant to P deficiency (Milyang 23) and intolerant to P deficiency (IR 5440-1-1-3) reacted on P stress with an increase in exudation. The cultivar tolerant to low P showed an eight times higher exudation under P limitation, compared to control conditions. The P deficiency sensitive cultivar (Milyang 23) showed higher exudation rates (0.98 ± 0.59 [mg $g^{-1}d^{-1}$]) and a ~40% increase in biomass compared to the P deficiency intolerant cultivar IR 5440-1-1-3. Extra biomass formation can be beneficial for Plant-MFC functioning through the supply of extra organic material like senescing roots. Both increased biomass formation and increased exudation can improve power output of the Plant-MFC. P starvation is a tool to influence both.
Besides increasing exudation quantity, the exudation composition –or quality- can be improved. It is known that the secondary metabolites in root exudates can have a large effect on the microbial population at the root surface. In the ideal Plant-MFC with a plant growing in the anode compartment and the roots surrounded by conductive graphite granules a large community of electrochemically active bacteria like *Geobacter* will dominate the granule surfaces in the rhizosphere promoted by the root exudates they can metabolize. However, root exudates may also inhibit bacterial growth.. Recently it is becoming clear that plant roots produce and exude antimicrobial metabolites, notably phytoalexins and phytoanticipins. The first step in achieving this is to know if there is variation between and within plant species in exudation of these metabolites. We took this first step by showing that large differences exist between the secondary metabolite profile of exudates of two tomato varieties. At least 8 metabolites were present in Moneymaker and not in *S. arcanum and* 7 were present in *S. arcanum* and not in Moneymaker. The next steps include identification of these metabolites, quantification of the genetic variation and assessing the effect on the microbial community.

Understanding the influence of increased root exudation on bacterial diversity in the rhizosphere.

As mentioned above, one can use the secondary metabolites in the root exudate as a starting point when investigating the effect of root exudation on bacterial diversity. However, one can also use the bacterial community in the bioanode as a starting point and see how biological and environmental factors affect the community.

The assessment of the bacterial diversity in the bioanode at different rhizodeposition scenarios was done by comparing Plant-MFC's that received additional dead roots to support root degrading bacteria with control Plant-MFC's without extra organic carbon input. Although the results were heterogeneous, the assessment revealed that the addition of extra dead root material had no significant effect on total bacterial abundance. There was, however, a slight positive effect of the added dead root material on the abundance of *Clostridium sporosphaeroides* and the electrochemically active *Geobacter* sulfurreducens. This indicates that not only the root degrading bacteria benefit from this altered rhizodeposition scenario, but also the current generating bacteria. This highlights a change of bacterial community structure and supports the idea of the necessity of a complex bacterial network for optimal Plant-MFC functioning.

In order to see if a quick reduction of plant photosynthesis had an effect on the occurrence of *Geobacter* in the bioanode, all above ground parts of the rice plants were removed. After some days, when the internal pool of carbohydrates was used up, the abundance of *Geobacter* was assessed and compared with the abundance before the treatment. FISH analysis showed that there was a tendency towards a higher *Geobacter* density after the clipping of the rice shoots. Especially one sample showed a very strong increase of *Geobacter* after the clipping. But there was also a strong heterogeneity of bacterial colonization along the roots and granules. This outcome is surprising at first sight, because we expected that the decline of root exudates would hamper the growth of *Geobacter*. However, it might be that *Geobacter* profited again from the breakdown products of other bacteria, especially the root degrading bacteria colonizing the roots that die as a result of the shoot clipping.

These results show that rhizodeposition conditions in the rhizosphere affect the occurrence of *Geobacter*. It also shows that the microbial ecology in the rhizosphere shows complex interactions between bacteria, plant and the environment. With the current knowledge it is not possible to define optimal rhizodeposition conditions.

Concluding remarks

Increasing root exudation

It has been shown that most of the root exudates are used to support microbial metabolism in the root biofilm and not for current generation. Genetic variation in exudation of carbonic acids in tomato was about 4x in terms of quantity. In grass species, a response to low phosphorus supply led to a difference in exudation of around 8x under Plant-MFC conditions. All the described experiments were performed under a light intensity of 150-400 $\mu mol.m^{-2}.s^{-1}$. Under natural conditions this can easily rise up to 600 $\mu mol.m^{-2}.s^{-1}$, allowing more photosynthesis and more exudation. In the most optimistic scenario, these results could be combined and exudation would increase by a factor 4x8x2=64x. This is with respect to exudation in tomato (cv Moneymaker) and assuming that P limitation affects tomato in the same way as rice.

But even a much smaller increase in exudation can already have significant effects on Plant-MFC efficiency. Timmers *et al.* [3] argued that in a *Glyceria maxima* bioanode oxygen loss from the roots was sufficient to support aerobic degradation of the exudates. The rate of oxygen loss, however, surpassed the rate of exudation by only a factor 2 implying that a 5-fold increase in root exudation - without increase in oxygen loss – would make more than half of the exudates available for the electrochemically active bacteria. Using energetically cheap primary metabolites is to be preferred above complex compounds as in dead root material. Making exudates available for electrochemically active bacteria would be an enormous step forward in achieving the desired Plant-MFC efficiency.

Future research directions

The existing genetic variation in root exudation is already valuable, but combining the genes responsible for increased root exudation with genes for enhanced biomass production could increase exudation even more. With genetic studies identifying the genes involved and marker assisted breeding this can be accomplished within 10 to 15 years. The relation between root exudation and oxygen loss from the roots into the bioanode is another important issue. With plant (eco)physiological studies the relationship between root exudation and oxygen loss under MFC conditions can be better understood. With this knowledge possibilities for improving the exudate/oxygen ratio in the Plant-MFC can be identified.

Root exudation research is relevant to more applications than the Plant-MFC only. Root exudation is also used to remove heavy metals from polluted soils and to increase crop yields in (organic) agriculture with less input of chemical fertilizers. In fact, combining research on exudation in these application areas might be very beneficial. Breeding research has increased crop and livestock yields considerably in the last 50 years. There is a wealth of knowledge about them and often the complete DNA sequence is known. To achieve maximum exudation of primary metabolites as substrate and selected secondary metabolites to facilitate a stable microbial community in a fast and effective way, investment in breeding tools is the most cost-efficient way. When the genes and corresponding DNA sequences responsible for better root and exudation traits are available in tomato, the same genes in different crops and wild relatives can be found easier. In this way, investment in root exudation research in crops species, e.g. tomato, benefits exudation research for all crops and wild plant species.

WP5: Assessment microbiology anode-rhizosphere and biocathodes

Analysis of microbial community structures in the anode compartment

In a plant microbial fuel cell (Plant-MFC), the plant roots are located at the anode of the MFC, where rhizodeposition provides electrochemically active bacteria (EAB) with electron donors. The MFC anode and the rhizosphere make up a complex system, where it is likely that different micro-organisms compete for electron donors. Competition for electron donors among the different micro-organisms results in a decrease of the electron donors available for EAB and thus lower current generation. Analysis and localisation of the microbial community is needed to gain insight into the competition for electron donors in a Plant-MFC.

Therefore, we firstly determined the microbial communities present in a Plant-MFC rhizosphere and localised the active EAB on the electrode of the Plant-MFC. Additionally, we investigated the electron donors and electron pathways in the Plant-MFC by using next generation sequencing technology (454) and latest bioinformatic tools to identify and roughly quantify relevant Bacteria and Archaea To verify the relevance of the identified dominant bacterial groups and to localize them, the bacteria of interest were specifically labelled with fluorescent markers using the fluorescent in situ hybridisation (FISH) technique in combination with confocal laser scanning microscopy (CLSM), which enabled us to observe, three dimensionally without cutting, fluorescently labelled bacteria colonizing roots. This first study was performed comparatively on samples of high-current-generating and low-current-generating *Glyceria maxima* (Reed Mannagrass) Plant-MFCs.

With the applied techniques two species could be identified to be connected to high current generation (0.164 A/m2) in a Plant-MFC: *Clostridium sporosphaeroides* and *Geobacter sulfurreducens*. *C. sporosphaeroides* was located as the dominant bacterial species on and even within roots, while *G. sulfurreducens* was the most numerous colonizer on the electrically conductive graphite granules surrounding the roots. *C. sporosphaeroides* has been described as a biomass degrading (cellulolytic) organism in biodegradation of plants and as it was so closely associated with older, probably dead root parts, it is very likely that it fulfils the same function in a Plant-MFC. *G. sulfurreducens* is known for its ability to reduce solid electron acceptors by respiration with low molecular mass carbon sources and has frequently been described to be associated with anodes in various MFC systems. This led us to the conclusion that by breaking down plant biomass to small carbon sources *C. sporosphaeroides* provides small molecular organic carbon which is then metabolized by *G. sulfurreducens.* During this process *G. sulfurreducens* can easily get rid of the produced electrons via the graphite granules connected to the electrical circuit of the Plant-MFC, thus generating a current. This type of metabolism provides it with a competitive advantage in this system compared to other bacteria metabolizing the same substrate.

These results could be verified by analysing three further Plant-MFC systems with different set-ups (flat plate, tubular) and plants (*Glyceria maxima, Spartina anglica, Alnus glutinosa, Salix caprea).* Although sometimes there were other biomass degrading Clostridia detected as well as other electrochemically active *Geobacter* species, the principal functional combination of plant matter degradation and EAB was constantly found in all studied systems. Additional analysis of the same *S. anglica* Plant-MFC at a later time point confirmed that the microbial community was rather stable and the abundance of the biomass degraders even increased over time.

So it can be stated that in general there is a functional as well as a bacterial species consistency when observing the same set-up over time. Analyzing different set-ups revealed some variation in bacterial species composition but the function provided by these species remained constant.

The abundance and role of Archaea was also studied in different Plant-MFC systems with different plants including *Glyceria maxima* and rice (*Oryza sativa*) by means of 454 sequencing and quantitative polymerase chain reaction (qPCR). In general the presence of acetate utilizing methanogens, like members of the family *Methanosarcinaceae*, were associated with low performing Plant-MFCs (0.067 A/m2), while *Methanobacteriaceae*, forming methane from H_2 and CO_2, were more numerous in Plant-MFCs producing a higher current (0.164 A/m2). This means that acetate metabolizing Archaea are direct substrate competitors for EAB and, similar to members of the *Comamonadaceae* and *Rhodocyclaceae* within the Bacteria, will reduce power output by limiting the available substrate for EAB.

Application of a bacterial inoculum at the anode compartment

One way to ensure the establishment of a high current generating Plant-MFC anode microbial community is to selectively inoculate certain bacteria which were found to be associated with high current production. As an inoculum a combination of *C. sporosphaeroides* and *G. sulfurreducens* was selected as these bacteria have been identified to be functionally associated with the development of a high current Plant-MFC in the first experiments. The inoculum was tested on *Spartina anglica* and rice Plant-MFCs, the first being inoculated three weeks before the end of the experiment the latter being inoculated at the starting point and compared to an undefined mixed culture inoculum from the anode of a MFC without a plant. In the rice Plant-MFCs the inoculum successfully established in the inoculated Plant-MFCs and usually led to a considerably higher number of inoculated species (up to a factor of 10) compared to the undefined inoculum. However, regarding current generation, no clear beneficial effect could be observed, which might have been due to other factors, like e.g. the oxygen supply in the cathode, that obscured the effect of the inoculation. In the *Spartina* Plant-MFC neither an increase in numbers of the inoculated species nor a higher current generation could be demonstrated.

Photo of a tubular shaped Plant-MFC (anode in the central cylinder and cathode in the bottom cylinder) planted with rice and inoculated with the bacteria C. sporosphaeroides and G. sulfurreducens

Considering these results it seems to be important to perform inoculation at an early stage of the experiment, preferably before beginning to operate the system. By this the beneficial bacteria get a head start, while the development of detrimental groups of organisms is suppressed. In later stages the established microbial community of a well operating Plant-MFC might be to a large extent resistant to an inoculation, as the added species do not find any colonization space. Regarding a higher current generation, an inoculation can only unfold its full potential if all other Plant-MFC factors are kept in a well operating range.

Assessment of the microbial community in the cathode compartment

Besides the bacteria in the anode compartment also the cathode compartment plays a crucial role for a stable high current producing Plant-MFC. Therefore we aimed to identify dominant bacterial species in a biological cathode compartment of a MFC with a chemical anode. DNA from the cathode liquid (catholyte) and the biofilm that formed on the cathode electrode was prepared from six MFCs with differing performance (from 0 A/m2 to – 0.85 A/m2). Additionally, graphite samples were collected for fluorescent labeling of the target bacteria with fluorescent in situ hybridization (FISH). After a preanalysis with a DNA fingerprinting technique (DGGE) revealed striking differences between the microbiology of samples from high and low current generating MFCs a next generation sequencing approach (454) was used to identify key bacterial players in the different systems. The obtained results clearly showed members of the bacterial family of *Sinobacteraceae* to be dominant in cathode biofilms of the four well performing MFCs (– 0.5 to – 0.85 A/m2), while they were clearly lowered in numbers or even absent in the two MFCs with low current output (0 to – 0.2 A/m2) and in the catholyte of all analyzed Plant-MFCs. The family of *Sinobacteraceae* has not been described so far in relation to this specialized habitat. Not even isolates are available for the closest relatives of the detected species based on sequence comparison. Although this made the finding very interesting, it hampered a further analysis as there were just too little data and no isolates available. Therefore also an inoculation approach like in the anode compartment could be performed with pure cultures but must be realized with the transfer of the mixed biofilm of a running (P)MFC cathode. Nevertheless we could prove for the first time that members of this bacterial family were dominating the cathode biofilm of high power producing Plant-MFC.

Regarding these results from a microbiological point of view it seems to be advisable for an optimization of Plant-MFC performance to inoculate both the anode and the cathode compartment at the beginning of the Plant-MFC operation with scraped of biofilms or biofilm coated particles (root pieces, graphite granules) from a well operating (P)MFC. This could be preferable even if pure culture inoculates are available, because an established biofilm is much more resistant towards changes in environmental conditions than liquid cultures are.

WP6: Model of Plant-MFC & Early technical, financial and sustainability assessment

Sustainability in this project was assessed, based on the broadest definition, as economic feasibility, environmental performance and social acceptability. This was combined with technological feasibility derived from the project to assess the applicability of the Plant-MFC in society.

Economic feasibility

The economic feasibility of the Plant-MFC is dependent on several factors that differ per specific application:

- Power output
- Material use and price
- (Local) Electricity costprice
- Lifespan of the Plant-MFC

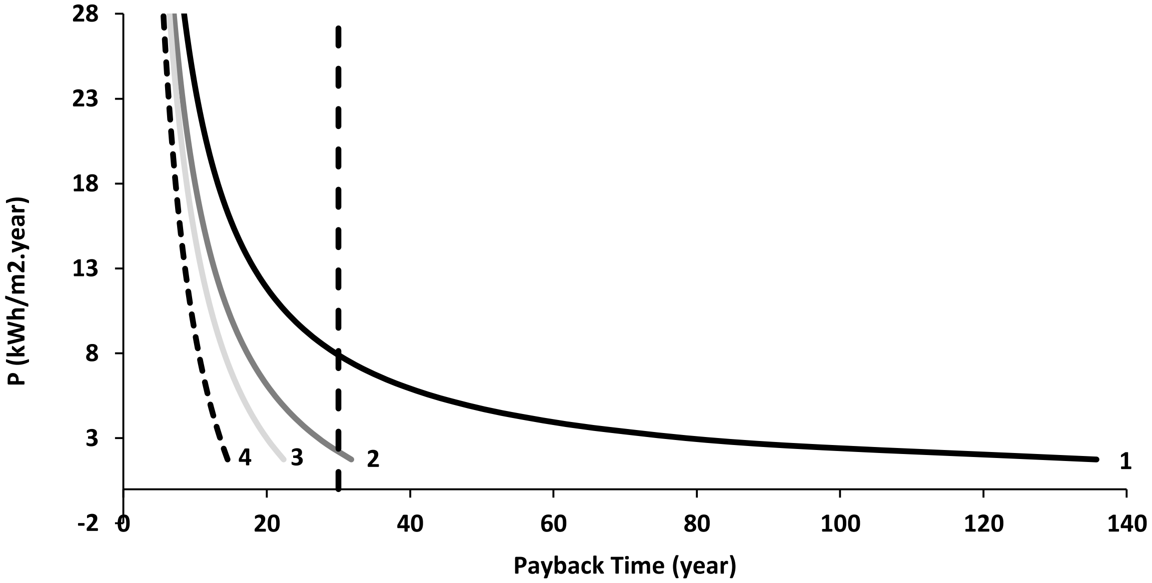
For economic feasibility we assumed a payback time of 30 years for the Plant-MFC. This would be technologically feasible as well. The power output of the Plant-MFC was forecasted based on the achieved results from the PlantPower project (0.2 W/m2) ranging towards the theoretical maximum of 3.2 W/m2. At a power output of 0.2 W/m2 the Plant-MFC would not be economically feasible in any application at any site except when applying the system on very large scale and an economy of scale could be assumed. This application would not yet be technologically feasible though, so it is assumed that power output needs to be increased or material use needs to go down in order to produce an economically feasible Plant-MFC.

Specific application of the Plant-MFC comes with specific (local) electricity prices and in some cases added advantages for the system that increases its profitability. Different scenarios for applying the Plant-MFC were worked out in business cases in order to assess the most promising applications for the system. In general two main markets can be distinguished: developed countries and developing countries. Within these two markets, roughly the following products can be envisioned that would be technologically feasible:

1. gadgets;
2. education;
3. decentralised electricity production;
4. centralised, large scale electricity production.

Of these specific products, the gadget-type, has already been launched into the market in the Netherlands by spin-off company Plant-e (www.plant-e.com/webshop). At the end of 2012 over 100 gadgets had been sold already. For educational purposes different specific projects are being set-up.

Decentralised electricity in developed countries can be envisioned in the form of a green electricity roof. It was calculated that the green electricity roof is not yet economically feasible based on electricity production only. When including insulation of the building, which is one of the added advantages of a green roof, in the calculation it shows that even at the already achieved power output of 0.2 W/m2 the green electricity roof would be an economically feasible application for the Plant-MFC.

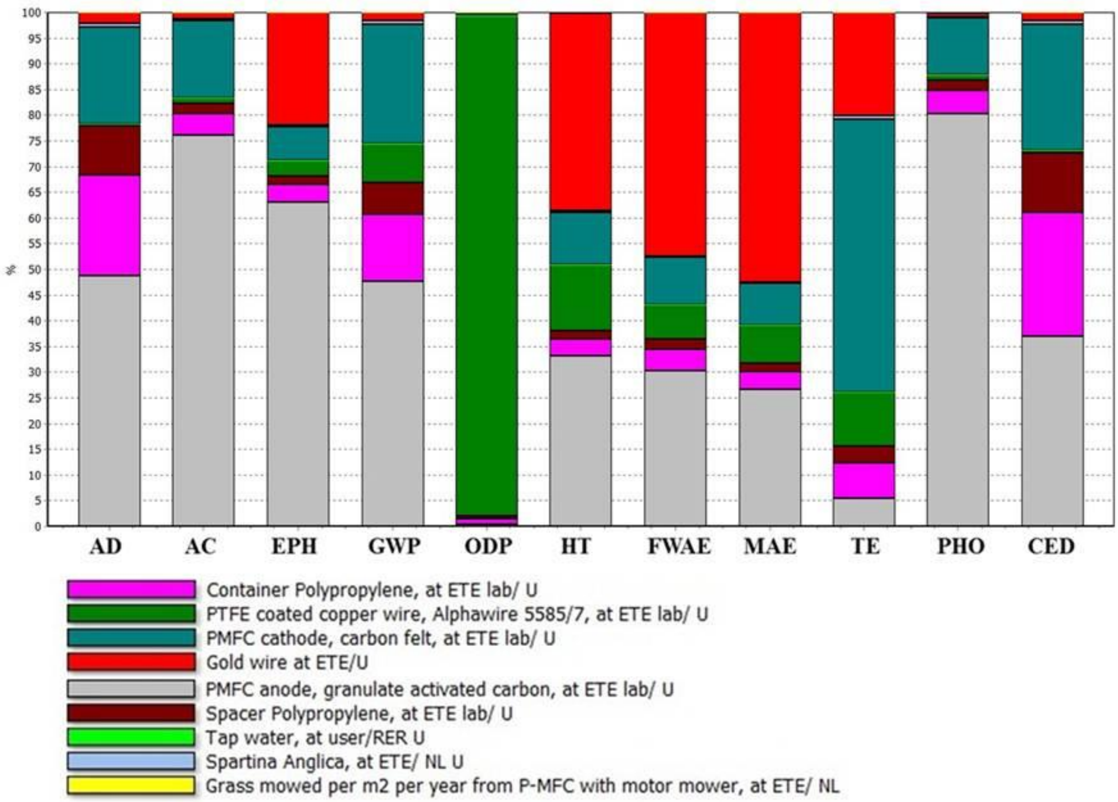


Payback time - when energy input in system equals energy output - of the Plant-MFC applied on a green roof based on different power output of the Plant-MFC ranging from 1.75 kWh /m2 year^{-1} to 28 kWh /m2 year^{-1} (0.2W /m2 - 3.2 W /m2) for four scenarios: 1) without co-products, 2) with heat insulation as a co-product, 3) with biogas production as a co-product, and 4) with both heat insulation and biogas production as co-products.

Centralised, large scale electricity production would be very interesting both for developed and developing countries. Technological feasibility of this type of product needs yet to be determined, though, and more research into this specific application is needed.

Environmental performance

The environmental performance of the Plant-MFC was assessed based on one of the specific business cases: the green electricity roof. A pilot-scale green electricity roof was developed during the research project, so calculations could accurately be performed. It has shown that the Plant-MFC is not yet environmentally friendly. This is mainly caused by the relatively low power output, as well as the use of some specific materials. Materials that need to be avoided in order to increase the environmental performance are: Teflon coated copper wires, goldwires and the amount of activated carbon for electrodes needs to be reduced.



The contributions of the different parts of the Plant-MFC construction and grass mowing to the selected impact categories (calculated for 1 kWh electricity supply in the reference scenario), in which AD=Abiotic Depletion, AC=Acidification, EPH=EutroPHication, GWP=Global Warming Potential, ODP=Ozone DePletion, HT= Human Toxicity, FWAE=Fresh Water Aquatic Ecotoxicity, MAE=Marine Aquatic Ecotoxicity, TE=Terrestrial Ecotoxicity, PHO=PHothochemical Oxidation.

It can reasonably expected that at a higher power output of the Plant-MFC environmental performance of the system will increase and the Plant-MFC can be considered an environmentally friendly system.

Social acceptability

Since the Plant-MFC can be integrated with the normal landscape, social acceptability of the system is expected to be high. Introduction of the gadget-type product by Plant-e as well as media interest in the research project have shown that public interest in the technology and its application are high. The Plant-MFC offers an opportunity for introducing a completely green and sustainable electricity technology into the market. Aesthetic value is high and the system is appealing. Social acceptability of the technology at specific sites and applications can only be assessed when specific applications are being developed. Some aspects to take into account in this respects are the following:

- Maintenance needed
- Robustness of the system
- Use of valuable components (in relation to vandalism)

When looking at a decentralized application for developing countries social acceptability of the Plant-MFC will probably be high. Farmers are amongst the people with the lowest income around the world. Producing electricity with a product that they are familiar with, plants, will offer them an opportunity to increase profit. Moreover, applying the Plant-MFC as a decentralized system for electricity production will offer (part of) 1.2 billion people around the world that don’t have access to electricity to develop economically and socially.