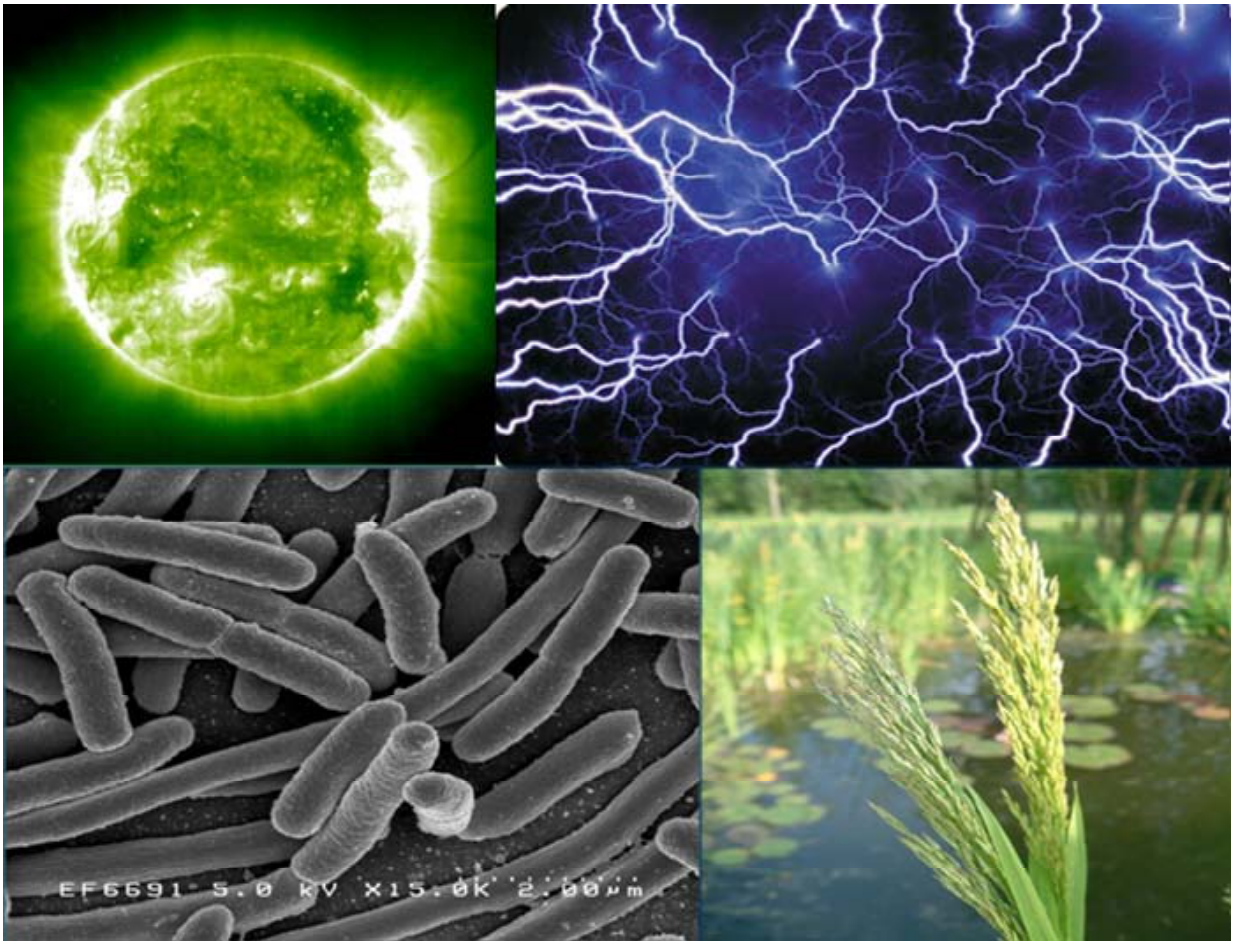
ΠΤΥΧΙΑΚΗ ΕΡΓΑΣΙΑ

ΠΑΡΑΓΩΓΗ ΗΛΕΚΤΡΙΚΟΥ ΡΕΥΜΑΤΟΣ ΜΕ ΤΟ ΣΥΣΤΗΜΑ ΦΥΤΩΝ - MFC

ΕΠΙΔΡΑΣΗ ΑΝΑΕΡΟΒΙΩΝ ΣΥΝΘΗΚΩΝ ΥΠΟΣΤΡΩΜΑΤΟΣ ΚΑΙ ΠΕΡΙΟΡΙΣΜΟΥ ΦΩΣΦΟΡΟΥ ΣΤΙΣ ΡΙΖΙΚΕΣ ΕΚΚΡΙΣΕΙΣ ΦΥΤΩΝ LYCOPERSICON ESCULENTUM ΚΑΙ GLYCERIA MAXIMA



ΚΑΛΑΡΧΑΚΗΣ ΙΩΑΝΝΗΣ

ΗΡΑΚΛΕΙΟ, 2009

Επίδραση αναερόβιων συνθηκών υποστρώματος και περιορισμού φωσφόρου στις ριζικές εκκρίσεις φυτών Lycopersicon esculentum και Glyceria maxima

Περίληψη

Οι κλιματικές αλλαγές του πλανήτη και η ταυτόχρονη ανάγκη του ανθρώπου για ενέργεια έχει φέρει την ανθρωπότητα στο κατώφλι της αναζήτησης ανανεώσιμων, βιώσιμων, αποδοτικών και φιλικών προς το περιβάλλον πηγών ενέργειας όπως είναι η ενέργεια που προέρχεται από τον ήλιο.

Σε αυτή την εργασία παρουσιάζεται ένα σύστημα μετατροπής της ηλιακής ενέργειας που δεσμεύουν τα φυτά με την φωτοσύνθεση, από χημική σε ηλεκτρική. Το MFC (Microbial Fuel Cell) είναι ένα βίο-ηλεκτροχημικό σύστημα όπου υγρά απόβλητα, φυτικά υπολείμματα ή οργανικές ενώσεις αποικοδομούνται από μικροοργανισμούς δίδοντας ενέργεια.. Όταν το σύστημα MFC συνδυάζεται με ζώντα φυτά η ενέργεια που παράγεται προέρχεται από τις εκκρίσεις των φυτών από το ριζικό τους σύστημα στο υπόστρωμα. Τα βακτήρια όταν χρησιμοποιούν τις ουσίες αυτές κατά το μεταβολισμό τους σε αναερόβιο περιβάλλον απελευθερώνουν ηλεκτρόνια και πρωτόνια δημιουργώντας φαινόμενα διαφοράς δυναμικού και ταυτόχρονα ροή ηλεκτρονίων που έχει ως συνέπεια την παραγωγή ηλεκτρικής ενέργειας. Το σύστημα φυτών – MFC είναι μια πολλά υποσχόμενη τεχνολογία που μπορεί να χρησιμοποιηθεί στο μέλλον σε υγροβιότοπους, και άγονα εδάφη χωρίς να ανταγωνίζεται την γεωργία ενώ η δυνατότητα παραγωγής ηλεκτρισμού φτάνει τα 21 GJ ha $^{-1}$ year $^{-1}$

Το ενδιαφέρον μας στην εργασία αυτή επικεντρώνεται στην εν δυνάμει αύξηση των εκκρίσεων των φυτών σε σάκχαρα και οργανικά οξέα. Σύμφωνα με προηγούμενες έρευνες ο περιορισμός θρεπτικών στοιχείων και ιδιαίτερα φωσφόρου και σιδήρου ωθεί τους φυτικούς οργανισμούς στην αύξηση του ρυθμού απελευθέρωσης ουσιών από τις ρίζες. Επιπλέον μελετάμε την δυνατότητα των φυτών να αντεπεξέλθουν στις αναερόβιες συνθήκες υποστρώματος που είναι απαραίτητες για τη σωστή λειτουργία ενός συστήματος MFC. Μελετήσαμε φυτά σε διάρκεια 6 εβδομάδων και ενώ δεν μπορέσαμε να εντοπίσουμε εκκρίσεις στα δείγματα μας παρόλα αυτά κατανοήσαμε πολλά γύρω από τον τρόπο που αντιδρούν τα φυτά στις απαραίτητες συνθήκες για τη σωστή λειτουργία ενός αποδοτικού συστήματος συνδυασμού Φυτών - MFC.

Electricity production with the Plant – MFC system

Effects of anaerobic root growth and nutrient limitation to photosynthesis and exudation rate of tomato and reed mannagrass plants.

Kalarchakis Ioannis[1]

Supervised by Jan F.H. Snel[2] and M. Dragassaki[1]

[1]Technological Educational Institute of Crete, Department of Greenhouse horticulture

[2] Wageningen University

I would really like to thank my supervisor F.H. Snel for his support in the Netherlands and my supervisor M. Dragassaki onwards completing my thesis in the TEI of Crete.

I would like to thank Dr. Ioannis Vlachos [1] for his work, offering the great opportunity to students from our department (and me as well) to collaborate with the Wageningen University in the Netherlands.

I would also like to thank for the collaboration: Chris Blok[2], Mary Warmenhoven[2], Rashied Khodabaks[2], Johan Steenhuizen[2], David Strik[2] and Ilse Gerritsen[2]

Contents

Part I

Electricity production with the Plant – MFC system

Introduction

The world is threatened by climate changes in the environment and pollution such as the increase of global warmth and the CO_2 emission from fossil fuel's combustion respectively. Therefore we are in need of efficient and sustainable energy production. We need a source that will protect the environment and will ensure sufficient energy production. Systems used so far such as bio-ethanol and, bio-diesel still carry disadvantages. The competition for the use of fields for food, the additional waste of energy through the need of fertilizations, and the strategies of intensifying the production are not so "ecological" or "sufficient" so that they would be a constant solution in the energy problem of our world.

The sun has been a candidate for a universal power source for centuries. The idea of advancing systems that make solar energy available or the use the energy that is stored in biomass is being constantly studied by the scientific society [Chow 2003]. Biomass has also been produced because of the sun since photosynthesis is the most primary way of absorbing sunlight energy in the plants in the form of carbohydrates. This form of energy is being used by the plants to grow, to survive under stressful conditions, and generally for their daily energy needs until achieving the possibility to reproduce, which constitutes the core of their survival as a species. Perhaps those options could be a solution for the future years to come.

In this study a system is being presented that can convert the chemical energy that is stored by the plants in the form of carbohydrates, into electrical energy. The system is a combination of plants and microbial fuel cells that acts nondestructive for the plants and produces energy in situ.

"Energy provided by sun can be stored through photosynthesis and released from the root system of the plants"

The solar energy and the light

Since as early as the past two centuries, scientists have been researching the concept of photovoltaic cells, in an effort to effectively collect electrical power from the sun's rays. According to studies of the University of Oregon, the entire surface of the earth receives an average of approximately 84 Terawatts of power from the sun in a 24-hour day. Researches that have been done by the Energy Information Association show that the annual worldwide power consumption in the year 2004 was 15 Terawatts. Clearly, the sun provides us with much more energy than we need. Moreover, this energy does not produce waste and is overly available, as shown by the fact that the planet earth intercepts more energy from the sun than is used in an entire year through the burning of fossil fuels [Annual Energy Review 2007]. Η βιβλιογραφία δεν είχε συγγραφέα έτσι είναι σωστή ??

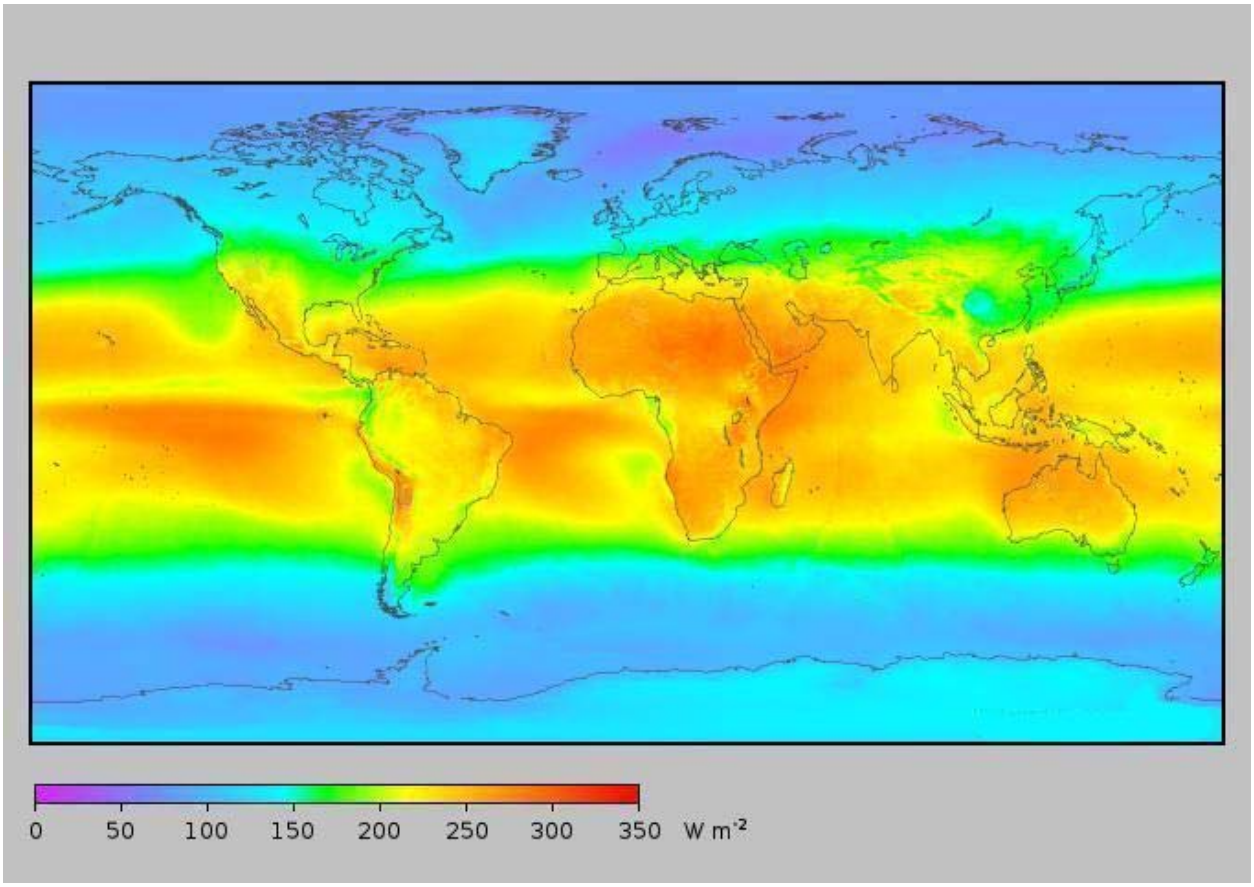


Figure 1. Map showing the average insolation on the earth's surface as measured in 1991[Bishop and Rossow 1991]

The light in a broad sense is the total spectrum of the electromagnetic radiation given off by the sun which includes the infrared, the visible and the ultra-violet light. It is known that the visible light, the light that we see, is just a small part of the light that sun sends to earth. Light can be thought of in two different forms. The one is the form of discrete "packages" that are called photons or quanta. In the other form light is a wave with a given velocity and wavelength.

Sunlight is mixture of (sun) rays with different wavelengths, which for the visible light, that matters in photosynthesis, is between 390 to 760 nm. In Figure 2 we can see the different wavelengths of light.

When light comes in contact with an object a part of it is been absorbed and part of it is been reflected. The wavelength of light that is reflected back is which gives the impression of the colour to that object. Therefore, as an example to have a better understanding of the subject, those things that reflect the green sunrays appear as green to us. If they absorb all sunrays, they appear black because black or if they reflect all light they appear as white and if they do not reflect or absorb then they appear as transparent (e.g. water).

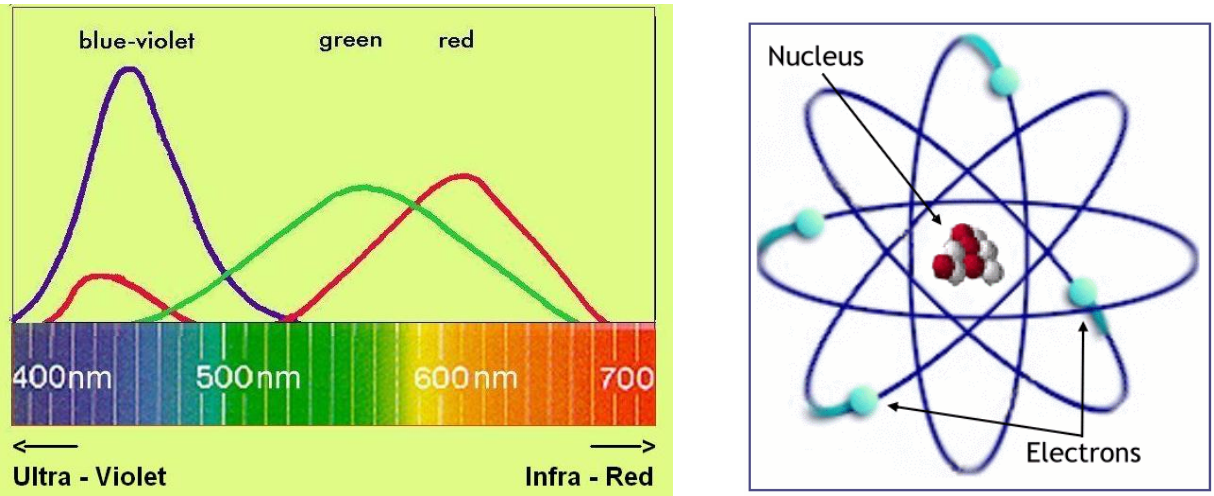


Figure 2. To the left we see an illustration of the wavelengths of light and to the right a picture of the the Atom.

The ability of an object to absorb light depends on the structure of its molecules and atoms. When a photon comes in contact with an atom (see fig 2.) of an object then an electron of that atom is been charged by the energy taken from the photon and reaches a higher energy stage. Afterwards, this electron returns to its normal stage. From that moment it tends to release the energy taken from the photon. This happens either in the form of i) heat or in the form of ii) light of longer wavelength (fluorescence) or with iii) a photochemical reaction as we will see. In the photochemical reaction of photosynthesis of plants the pigment chlorophyll is responsible for absorption of the light and below we are going to see how eventually the energy of the light provided by sun is being trapped by the plants with photosynthesis.

The mechanism of photosynthesis

From the start of life's existence in the planet, the plants have reached the solution of

their energy needs with photosynthesis providing either directly or indirectly the survival of all species in earth. This mechanism is the natural production of carbohydrates from inorganic CO_2 with the contribution of chlorophyll and sunlight. It can be done by every living organism that contain chlorophyll and belong to the autotrophic organisms meaning those that produce their own food in contrast to the heterotrophic organisms that actually live on the carbohydrates produced by the autotrophic organisms.

In the process of photosynthesis the energy that is converted from the sunlight energy into chemical energy. Its substrates are carbon dioxide and water, the energy source is light (electromagnetic radiation) and are used for the formation of carbohydrates. In Figure 3 below we can see an example of how suntlight is trap from the leave step by step .

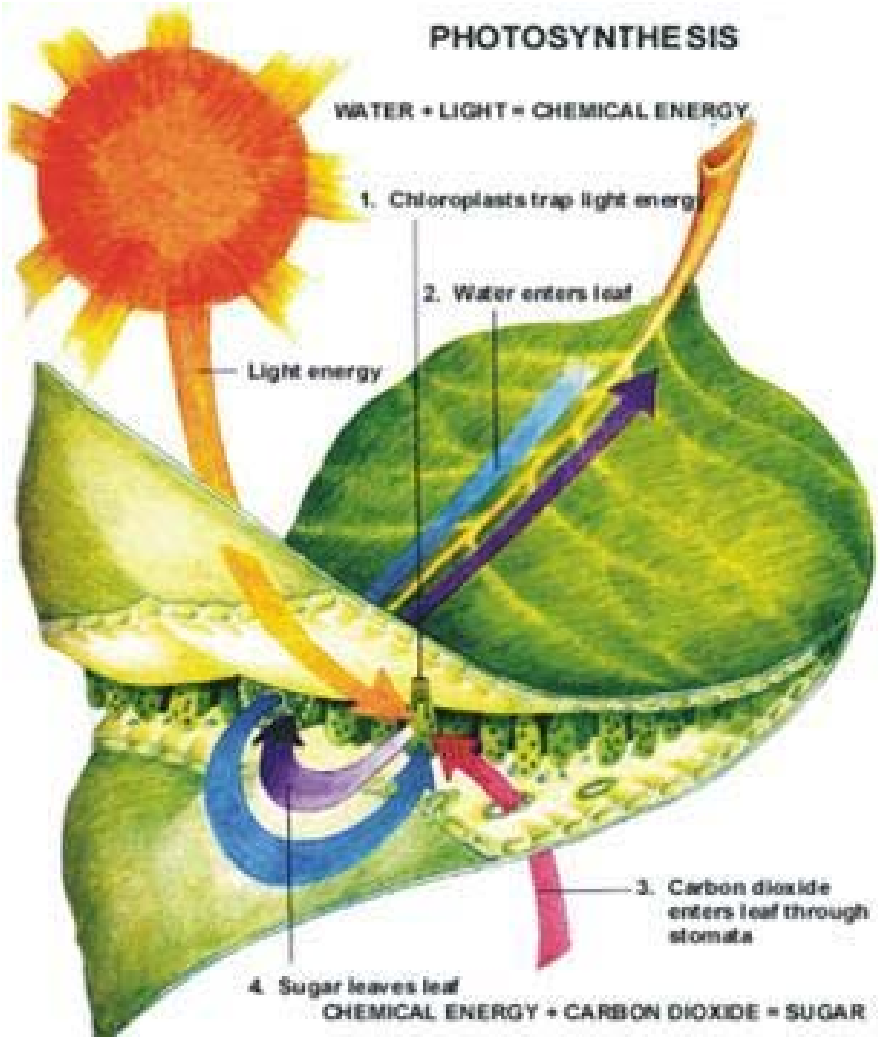


Figure 3. Photosynthesis is taken place on a leaf where the chloroplasts trap the light energy from the sun light while the absorbed water from the roots is carried to the leaves by the xylem. The same time carbon dioxide is obtained from the air that enters the leaves through the stomata and diffuses to the cells that are containing chlorophyll. Finally CO_2 is been used for the creation of sugars

Photosynthesis uses light energy and carbon dioxide to make triose phosphates (G3P). As we are going to see later on G3P is generally considered the first end-product of photosynthesis. It can be used as a source of metabolic energy, or combined and rearranged to form monosaccharide or disaccharide sugars. The most commonly used equation to describe the reaction of photosynthesis is:

$$\mathbf{6CO_2+6H_2O + light \rightarrow 6O_2 + C_6H_{12}O_6}$$

It is obvious that the synthesis of carbohydrates from CO_2 and H_2O is basically a reaction involving the reduction of CO_2. For this sequence energy and a provider of H^+ and electrons are needed. The energy comes from the sunlight. H^+ and electrons are derived from H_2O. Water in the presence of light and chlorophyll is been split into H^+ and OH^- in Photosystem II. O_2 and C that also are part of the synthesis come from CO_2. In the end the green pigment of chlorophyll is uniquely capable of converting the active energy of light into a latent form that can be stored (sugar) and used when needed.

Photosynthesis occurs in two stages. In the first stage, light-dependent reactions or photosynthetic reactions (also called the Light Reactions) capture the energy of light and use it to make high-energy molecules. During the second stage, the light-independent reactions (also called the Calvin-Benson Cycle, and formerly known as the Dark Reactions) use the high-energy molecules to capture and chemically reduce carbon dioxide (CO_2) to make the precursors of carbohydrates.

The photochemical reaction (light energy turns into chemical)

The light reactions occur in the thylakoid membrane of the chloroplasts. The light reactions take place in two clusters of pigment/protein complexes, known as photosystems I and II. Each photosystem possesses chlorophyll and several accessory pigments. These pigments help to make photosynthesis more efficient by absorbing different wavelengths of the light that comes from the sun.

When a photon gets in contact with a molecule of chlorophyll then an electron of chlorophyll is excited. The energy then is transferred from the antenna molecules to special

chlorophyll molecules where the photochemical reaction creates a chlorophyll cation and free electron. The electron transport chain makes it possible for this electron to be used for the reduction of NADP to NADPH with the help of a molecule of H^+ that was taken from splitting of H_2O.

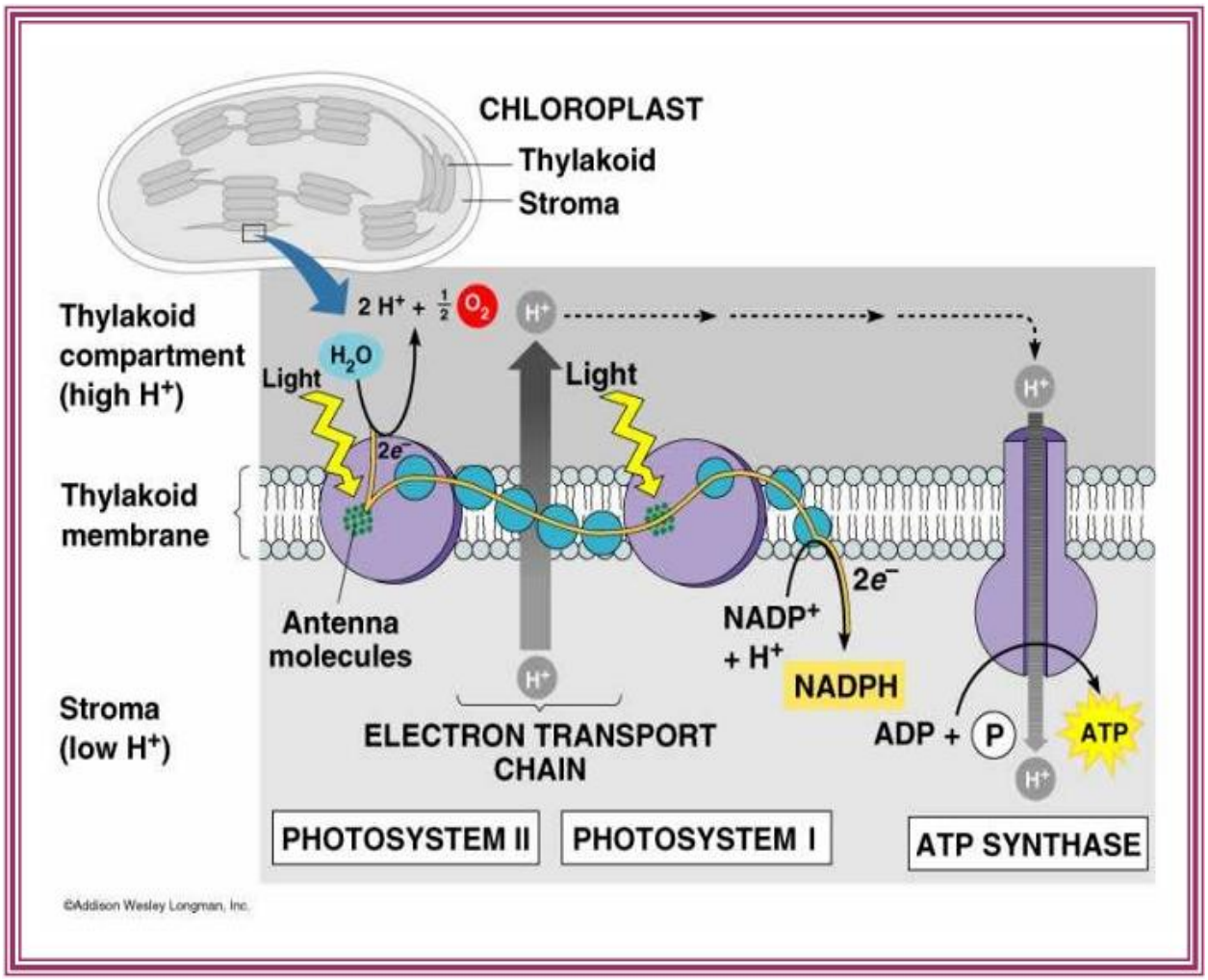


Figure 4. The light dependent reaction. The transportation of electrons where ATP and NADPH is been created.

When light hits photosystem II, electrons gain more energy and are carried via a chain of electron-carrying proteins to photosystem I. When the light hits second photosystem, the electrons are moved again to a molecule of energy-rich NADP. The electrons needed to replace those removed from photosystem are provided by photosystem II. The H^+ produced by the splitting of water, supplemented with additional ions from the surrounding stroma, create a proton gradient which provides enough energy to create several molecules of energy-packed ATP. Along with the NADPH produced by the electron transport, the ATP will be used immediately in the biochemical reaction leading to the reduction of CO_2 to carbohydrate (dark

reactions).

The biochemical reaction (CO_2 into carbohydrate)

This light independent reaction or "dark reaction" occurs in the stroma of the chloroplast. The stroma is a thick, syrupy fluid surrounding the thylakoid membranes. In this reaction CO_2 is bound to a compound known as ribulose 1, 5 bisphosphate**.** When CO_2 enters the cycle, as we can see in Figure 5, a series of steps catalyzed by enzymes takes place. ATP provides the energy for these reactions, while NADH is the reducing agent, attaching hydrogen to form the final product Glyceraldehyde-3P. In this process ADP and $NADP^+$ are been formed. After 3 turns of this circle with help from 18 ATP and 12 NADH the 3 molecules of CO_2 are transformed into a 3 carbon molecule Glyceraldehyde-3P. Two molecules of Glyceraldehyde-3P can be converted into glucose, a 6 carbon sugar which is a molecule with great importance for life's energy purposes as we are going to see later.

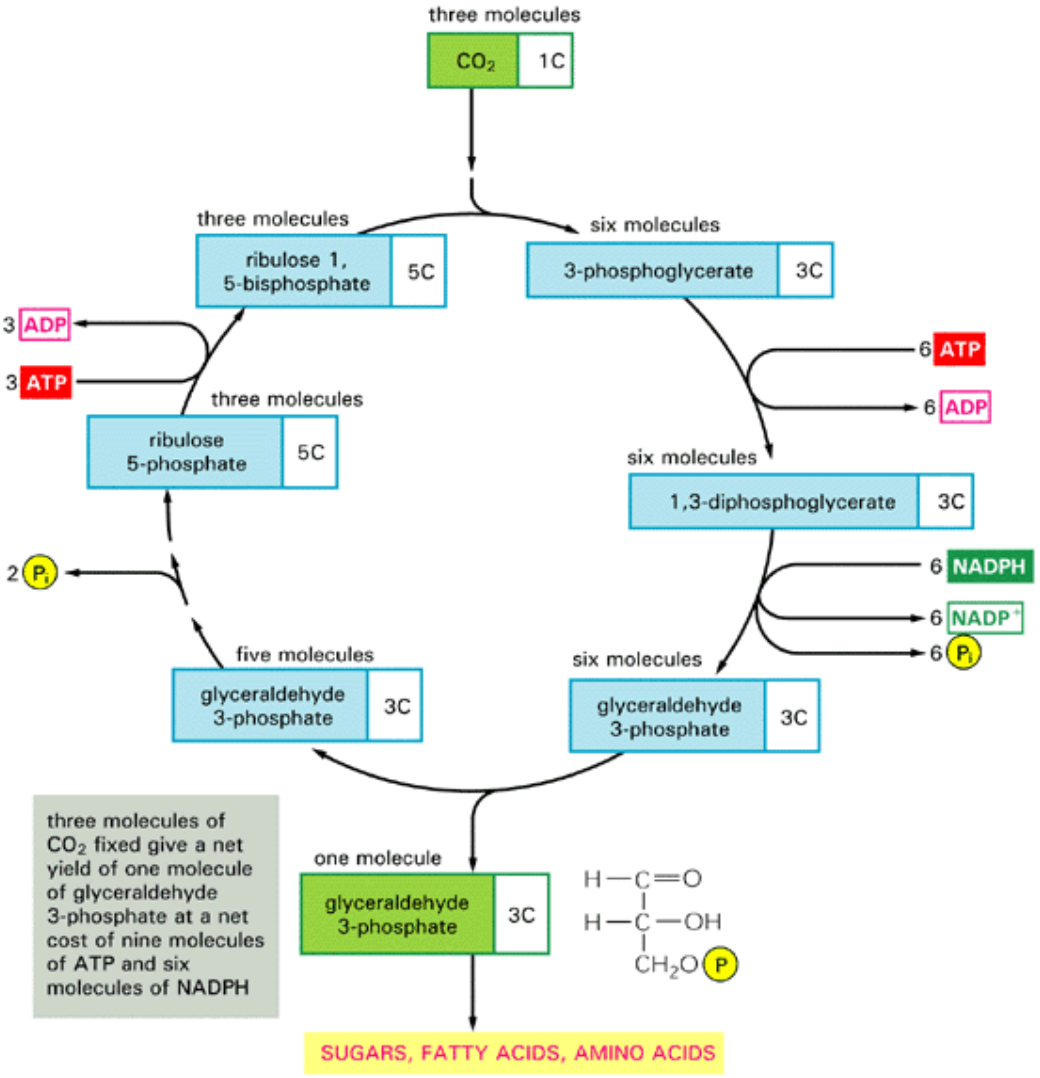


Figure 5. Calvin's Circle. Two of these "turns" as described above are needed to create a molecule of sugar.

The plant produces sugars and organic acids for storage of energy. As mentioned above first solar energy is turned into chemical energy (ATP and NADPH) through the photochemical reactions and then the ATP and NADPH are used to reduce CO_2 into sugars and other carbohydrates.

The rhizosphere and root products

Plant roots exude an enormous range of potentially valuable small molecular weight compounds into the rhizosphere. Some of the most complex chemical, physical, and biological interactions experienced by terrestrial plants are those that occur between roots and their surrounding environment [Bais 2006].

The rhizosphere is the volume of soil affected by the presence of root of growing plants. A multiple of compounds are released in the rhizosphere of the plant most of which are organic compounds and are normal plant constituents derived from photosynthesis (like the sugars we saw previously) and other plant processes. The relative and absolute amounts of these compounds differ from plant species, age of plant, cultivars and environmental conditions [Pinton et al. 2007].

Carbohydrates are stored in several parts of the plant including the root system. In annual plant species 30 to 60% of the photosynthetically fixed carbon can be transferred from the leaves to the roots and a considerably proportion of this carbon up to 50% is released into the rhizosphere [Pinton et al. 2007]. This process is called rhizodeposition and its products rhizodeposits. The term "rhizodeposition" has been used to describe the carbon root loss and this material generates the rhizodeposition effect [Lynch et al. 1990].

This rhizodeposition is affected by many factors like light intensity stress factors, temperature, nutritional status of the plants etc. Finally the root system release multiple types of organic compounds into the soil which include: root exudates (sugars, organic acids, phenols, and carboxylic acids) released passively from the root cells, secretions (polymeric carbohydrates and enzymes) released actively by the root cells , gases or excretions (ethylene , CO_2 etc), and root debris (dead cell materials) [Pinton et al. 2007].

Exudates in the form of sugars are soluble connections of C, H and O. They are categorized as monosaccharides, disaccharides and polysaccharides depending on the number of their monosaccharide on their molecule. In Figure 6 we can see common monosaccharides like those roots are releasing in the substrate.

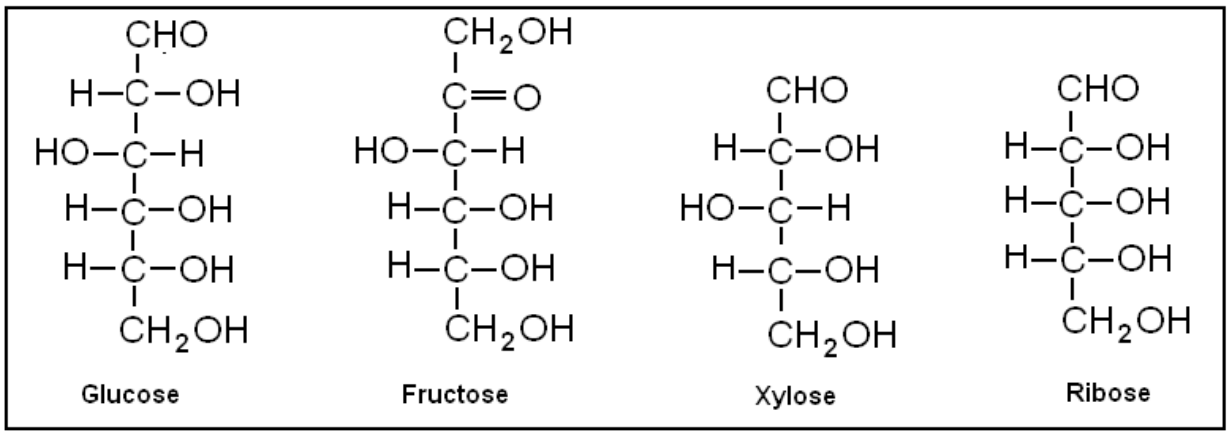


Figure 6. Chemical structure (fisher) of glucose, fructose, xylose and ribose

The organic acids contain a carboxylic group some contain two or contain a ketonic

group, the so-called ketonic acids. In principal all contain 2-6 molecules of carbon. It can be found at the cytoplasm and the vacuoles of the cells and they have a great role in the metabolism of cells because they are precursors in the creation of carbohydrates, fatty acids or amino acids.

Organic acids such us citric, malic and other have been proposed to be involved in many processes operating in the rhizosphere including nutrient acquisition and metal detoxification, alleviation of anaerobic stress in roots, mineral weathering and pathogen attraction. The content of root exudates in organic acids is highly variable and dependable on plant species, plant age and physiochemical environment of the plant. However the mechanisms that allow the organic acids to release from the roots, only recently started to be elucidated [Jones 1998].

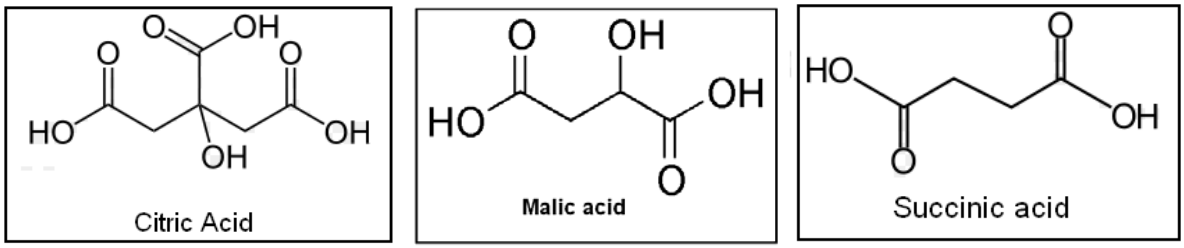


Figure 7. Chemical structure (chain) of some relevant organic acids.

Root products are representing a vast array of organic compounds. Of these, exudates represent a proportion that is likely to have a direct effect on microbial assimilation. Later on we will give an explanation of the importance of rhizodeposits and their role in the microbial fuel cell.

"Bacteria can use the rhizodeposits from plants producing electrical energy via the microbial fuel cell"

Bacterial relations with the plants

Bacteria are unicellular microorganisms ubiquitous at every habitat on earth, growing in soil, wastes, seawater and deep in the Earth's crust. They are vitally needed in recycling of nutrients and, in general, many important steps in nutrient cycles depend on bacteria. There are three types of Bacteria based on the kind of energy and the source of carbon they use for growth: the Phototrophic that use sunlight as source of energy, the Lithotrophic that use inorganic

compounds and the Organotrophic that use organic compounds, like carbohydrates. Organothrophic bacteria are capable of feeding from glucose (the main type of sugar found in the environment), fructose (found in fruit), sucrose (found in sugar cane), and xylose (found in wood and straw). In other words the last bacteria described are fed with the same carbohydrates that plants excrete from their root system previously described as rhizodeposits.

Since there are mutually beneficial interactions between plants and micro-organisms the bacteria must have a positive interaction with the plant roots. Root-microbe communication is an important process that characterizes the underground zone. Some compounds identified in root exudates have been shown to play an important role in root-microbe interactions [Walker et al. 2003] .The importance of the interaction between plants and bacteria in relation to the plant - MFC system is found when the largest fractions of rhizodeposits which are small molecules are efficiently synthesized by the plant and efficiently metabolized by the bacteria [Strik et al. 2008]. As we explained bacteria use the compounds of photosynthesis that are released in the rhizosphere of the plants as substrates for their own metabolism. If bacteria use the rhizodeposits for energy then what are the possibilities for us to use the bacteria for the same reason?

The MFC (microbial fuel cell) system

The MFC system is an emerging technology where biodegradable substrates are turned from wastewater or crops (exudates) into energy, CO_2 and water [Venkata et al. 2007]. In general micro-organisms use the energy for their metabolism and development. A part of that energy is possible to be harvested as electric current with the help of the MFC system. This happens when electrochemically active bacteria in MFC act like some kind of bio- catalysts using biomass for their metabolism releasing molecules of hydrogen while the same time are sending electrons to the anode compartment due to potential difference producing electrical energy [Allen and Benetto 1993].

The idea of using micro-organism to produce electric current was first conceived by M. C. Potter in 1912. Nineteen years later Barnet Cohen created a series of microbial half fuel cells that were capable of producing over 35 volts, when connected together, though only with a current of 2 milliamps. DelDuca in later years used *Clostridium butyricum* producing hydrogen with fermentation of glucose with the use of bacteria reactants at the anode in a hydrogen and air fuel cell. Unfortunately his system was unstable due to the nature of hydrogen production from

the micro-organisms. Suzuki later resolved this issue and the final design concept of an MFC came into existence a year later with work once again by Suzuki. We can see a simple model of a microbial fuel cell in Figure 8.

The idea was picked up again and studied later years in more detail first by M.J. Allen and then by H. Peter Bennetto both where from King's College London. It was Bennetto who saw the possibilities of using the method for generation of electricity for developing countries. It is now known that electricity can be produced directly from the degradation of organic matter in a microbial fuel cell, although the exact mechanisms of the process are still to be fully understood [Allen and Benetto 1993].

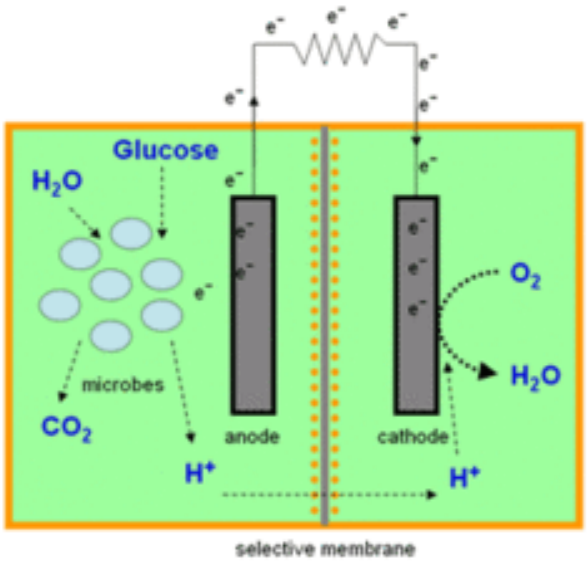


Figure 8. Model of a simple MFC – system where we can see an anode compartment, a cathode, a membrane of ion-exchange end an electrical circuit which connects the two compartments into a sealed chamber (cell) containing solution and substrate (glucose) for the bacteria.

Recently in the May of 2007 the University of Queensland which is located in Australia, has completed a prototype of MFC with the cooperation of the Fosters Brewing Company. They have managed to create a 10 litre design with the ability to convert the brewery waste water into CO_2, clean water, and electricity. After the success of the last prototype, plans are in effect to produce a 660 gallon version for the brewery, which is calculated to produce 2 kilowatts of power totally. While this amount of energy production is negligible, an additional production of clean water is created, an important benefit in Australia, which is experiencing its worst drought the last 100 years [Destries 2007].

Electricity production with Plants and MFC

When micro-organisms consume a substrate such as sugar in aerobic conditions they produce carbon dioxide and water. However when oxygen is not present they produce carbon dioxide, protons and electrons [Allen et al. 1993] as described below:

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$$

Microbial fuel cells can use inorganic mediators to tap into the electron transport chain of cells and steal the electrons that are produced or instead of mediators with the help of micro-organism such as *Rhodoferax ferrireducens* which can deliver directly the electrons to the electrode [Chauldhuri et al. 2003]. As mentioned above the micro-organisms release electrons under anaerobic conditions, but if oxygen was present into the microbial fuel cell then they would collect all the electrons.

For the purpose of using plants in combination with the MFC there has to be the possibility for their roots to develop into anaerobic environment. Afterwards instead of adding carbohydrates for the bacteria to use as a substrate, as it should be done in a conventional MFC system, the plant is constantly realising exudates that bacteria can use for their metabolism, generating flow of electrons as described above. In order to turn this into a usable supply of electricity this process has to be accommodated in a fuel cell which offers the ability to generate a useful current with a complete circuit [Benetto 1990].

The plant grows normally into the chamber on a substrate which can be rockwool with added nutrient solution. The solution that surrounds the substrate and the micro-organisms, are mixed together inside the chamber which is sealed to stop oxygen entering and forcing the micro-organism to use anaerobic respiration.

An electrode is placed in the solution that will act as the anode as described previously where the roots of the plant are naturally growing inside the chamber and the rest of the plant above. The anode will serve the purpose of accepting later on the electrons released from the bacteria becoming the electro-generic, negatively charged electrode. In the second chamber of the MFC there is another solution and electrode. This electrode, called the cathode is positively charged and is the equivalent of the oxygen sink at the end of the electron transport chain, only

now it is external to the biological cell. The solution is an oxidizing agent that picks up the electrons at the cathode. The two electrodes are connected with a wire which will make possible the transportation of electrons and the production of current. For production of electricity there has to be potential difference between the anode and the cathode, which is first created in the anode compartment by the bacteria .Of course electrons can also flow with the use of a resistor to the cathode. The two chambers are connected with an ion-exchange selective membrane. This last feature allows the protons produced, as described in figure 9 to pass from the anode chamber to the cathode chamber and the molecules of H can form water to restore balance.

When the system is functioning properly the bacteria start to accumulate carbohydrates and release electrons creating potential difference (Volt) between the anode and the cathode compartments, then flow of electrons (Ampere) is created leading to the production of electrical energy (Watt) [Bennetto 1990]. The current generated may be detected with a microammeter while the transformation of energy from the oxidation into light or heat may be demonstrated by lightening a small bulb.

It is notable that in the absence of any regeneration to the substrate the system will eventually run out of oxidizable fuel and the flow of electric current will cease. When a continuous supply of fuel is maintained regeneratively, the device becomes a *fuel cell* which can support the electricity production of long periods [Benetto 1990].

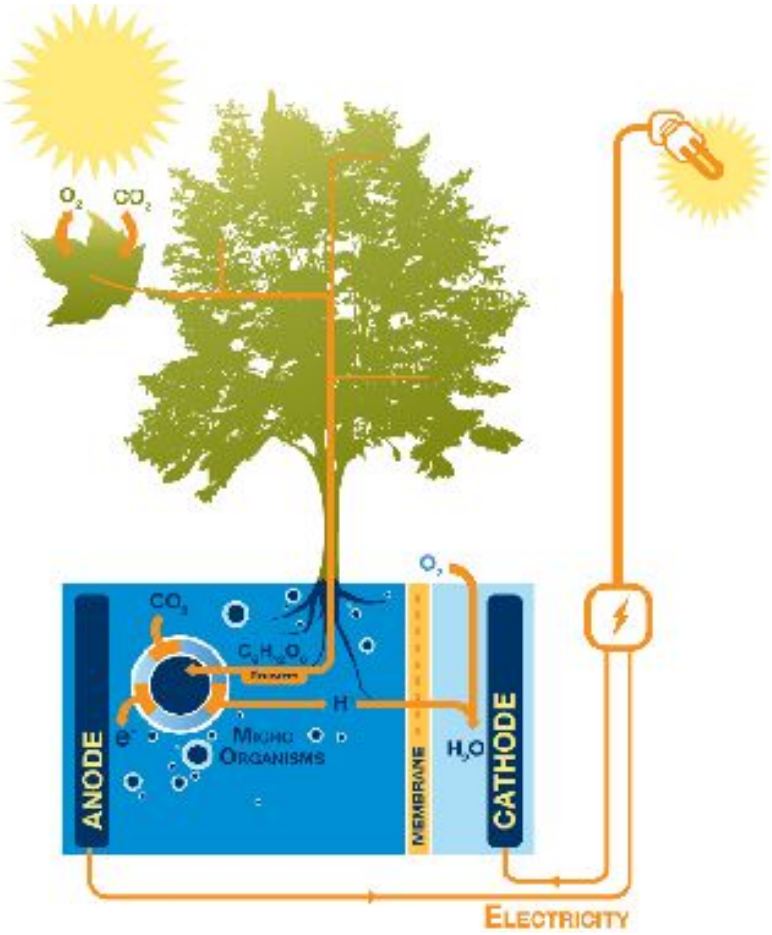


Figure 9. Model of a plant microbial fuel cell producing electricity and driving with energy a light bulb. Carbon dioxide is fixed and released as rhizodeposits (e.g. root exudates) by the plants and are utilized by micro-organisms that return the carbon dioxide into the atmosphere. The micro-organisms use the anode as electron acceptor for gaining metabolic energy. These electrons flow due to the potential difference from the anode through an electrical circuit with a load or a resistor to the cathode. Hence, electricity is generated which can be used, for example, driving a light source. To remain electro neutrality, protons are transported through the membrane into the cathode where oxygen is reduced with the protons and electrons to form water.

The Plant – MFC system (fig. 9), as been presented by previous studies [Logan et al. 2006], has been characterized by certain advantages towards other systems described in the beginning. It functions nondestructive for the plants producing energy in situ [Schamphelaire et al. 2008], it has potential implementation in salt marshes wetlands and poor soils without competition of the food or conventional bio-energy production, which makes it an additional bio-energy supply, it has an estimated minimum potential electricity production of 21 GJ ha -1 year -1 (5800 kWh ha -1 year -1) in Europe and finally is a carbon neutral and combustion- emission free operation which gives to it a huge environmental advantage [Strik et al. 2008]

Part II

Effects of anaerobic root growth and nutrient limitation to photosynthesis and exudation rate of tomato and reed mannagrass plants.

Principles summary and aim of study

As we have seen the Plant-MFC system is based on two principle ideas that can be found in natural processes of life. The energy provided by sun in the coming formations is been accepted by the plants and stored though the mechanism of photosynthesis after several reactions in the form of carbohydrates which constitutes a chemical form of energy that is used from the plants to survive, part of which is being released to the rhizosphere from the roots of the plant.
The electrochemically active micro-organisms that form symbiotic relations with the plants in the rhizosphere when sealed under anaerobic environment start to use the carbohydrates released from the plant providing the anode with electrons which are turned into electric energy due to the flow which is created from the potential difference.

To make the Plant-MFC a viable technique, the efficiency of the processes needs to be optimised, the stimulation of exudation is essential. In previous studies it has been found that that deficiency of phosphate and iron is increasing exudation of organic acid anions in some species [Kamilova et al. 2006]. It is possible to stimulate the exudation of citrate from toxicity of aluminium into white lupins [Wang 2007]. Malic and citric acid appear to be the primary components that are released by roots due to phosphate deficiency [Jones 1998, Hoffland 1989]. Other studies have been made in root excretion of carboxylic acids and protons in phosphorus deficient plants [Neumann 1999] which lead to the conclusion that stimulation of root products is a possible fact that needs more study to be fully understood leading eventually to the increase of the exudate production.

The aim of this study is first to investigate if limitation of phosphate can improve exudation by stimulating the organic compounds production and release on the substrate from the plant. This will lead to higher amount of resources for the metabolism of the bacteria which afterwards will have more impact in the electrons released from the micro-organisms. Secondly we investigate the photosynthetic response of the tomato and reed manna grass plants which is affected by the condition of the plant that grows under anaerobic root environment and finally how this is related to the exudation of sugars and organic acids.

Materials and Methods

Plant anaerobic growth conditions and treatments

In this study eight plants of reed manna grass (Glyceria maxima) and four plants of tomato (Lycopersicon esculentum) were chosen to grow in a controlled environment in specially constructed closed boxes made of polyethylene. Those boxes where sealed for keeping the air outside of the box in order to keep an anaerobic environment. For the root system to grown anaerobically in the box special sealing material (non-toxic) was placed on the surface of the box to securely separate the root system from the atmospheric air (see fig. 11) .The idea was to mimic the anaerobic conditions that are necessary for a well-functioning anode compartment of an MFC [Strik et al. 2008]. By the term "anaerobic" we describe conditions of low oxygen supply (0-5%) which modify the internal processes of the substrate and do not necessarily imply a complete absence of oxygen molecules. The boxes where implied with nitrogen gas.

Tomato was chosen because it is considered a plant that exudates sugars and organic acids in high amounts and also is a widely used plant in traditional agriculture and also as an experimental subject, although we expect that the tomato plants will respond negatively to the anaerobic root conditions. In addition this plant will give us the option to cross results with previous studies [Kamilova et al. 2006] related to the amounts of exudates we expect to get from our plants which will give a more complete understanding of the processes involving release of organic compounds into the substrate in anaerobic conditions.

Reed manna grass was chosen because is one of the few native species that can efficiently grow in anaerobic riverbank sediments. In addition, previous studies show that electricity production is possible with reed manna grass plants in microbial fuel cells proving the principle of electrons flow with the use of exudates from plants [Strik et al. 2008]. It will be interesting to compare a naturally grown in wet-lands specie with a widely used rich vegetative plant such as the tomato as potential candidates for the Plant – MFC system combination.

Figure 10. To the left picture of Lycopersicon esculentum plant and to the right picture of Glyceria maxima plant growing naturally in local territories (Netherlands)

When the plants reached the first month of growth the examination of released exudates started. The plant roots where sealed into anaerobic root conditions and we begun to collect samples from the nutrient solution that surrounds the substrate inside the boxes in order to measure the possible exudates.

The substrate we used for the plants to grow was rockwool. This substrate type is easy to control and in addition has become widely used the recent years in growing vegetables such as tomatoes etc. An additional reason that we used stone wool is that it contains aluminium ions that may lead to high organic acid content in root exudate, as explained previously [Kamilova et al. 2006].

Rich nutrient solution was allowed to pass to the box through another container (see fig.9) which volume was 5 litres max. When we accomplished 3 weeks of notices and measurements related to the releases of the plants in the substrate we started a deficiency on phosphorus to see how this would affect the plant. By limiting phosphate we aimed to increase the exudation rate as mentioned on literature [Kamilova et al. 2006]. The nutrient solution was renewed at the second and the fourth week for preventing any other nutrient limitation that could have affected the rate of exudation in our plants. The renewing of nutrients took place after the

sample collections.

All tomato plants had their flowers removed constantly to prevent fruit development and to keep their energy for growth and storage instead. The aim was to have the plants produce sugars that wouldn't be used for fruiting but in contrast to be used for root growth and root products facts that could possibly have an instant effect on the amounts of exudates released from the plants to the rhizosphere.

The temperature inside the greenhouse was between 18 and 23^{o}C during day and 15 to 17^{o}C during night. The pH of the substrate where the plants were grown was 6 to 6.5 and the relative humidity of the environment was 70-75%.

Root exudates collection

As mentioned above when the plants reached the first month of growth we started to collect samples from the nutrient solution that surrounds the substrate inside the boxes in order to measure the possible presence of exudates.

For the collection of the liquid samples were used syringes of 10 ml attached to needles of 12 cm that could reach the lowest levels of the box. The syringes were attached to filters to keep the samples clean from debris and any kind of material that could prevent the good functioning for the subsequent HPLC measurements. We used the needle aiming the core part of the box located at the center of the rockwool substrate through the specially placed sealed membranes on the boxes as we can see in Figure 11. After collecting the solution with the syringe we injected it on vials of 10 ml. Finally we stored all the vials containing the samples from the substrate at -18^{o}C in a refrigerator.

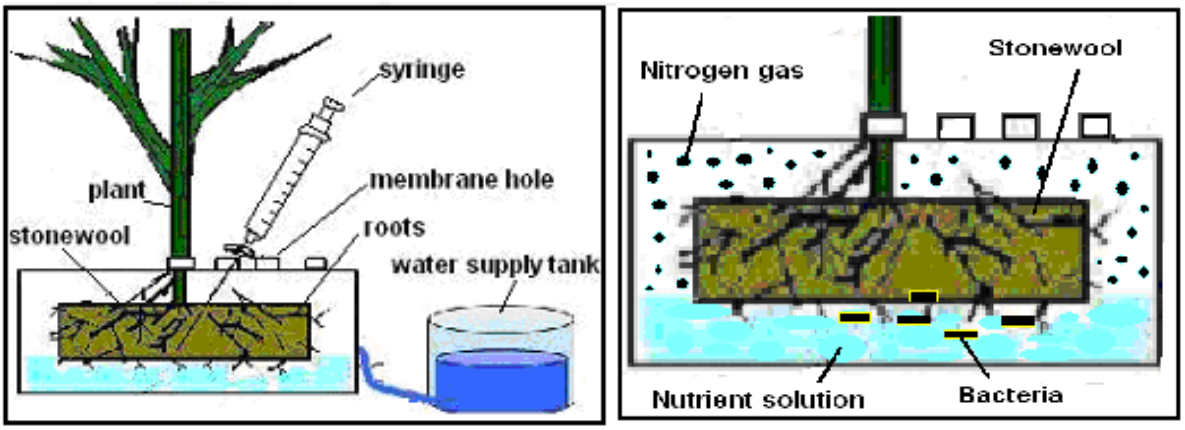


Figure 11.**To the left:** Illustration of exudates sample collection. We can see the sample collection process aiming

the core part of the substrate through membranes that do not allow oxygen exchange. **To the right**: illustration of the anaerobic box contents, nitrogen gas to an estimated level of 95% approximately, symbiotic micro-organisms, stone wool substrate and given nutrient solution.

The freezing conditions were applied to prevent bacterial activity from feeding with the exudates while inside the vials. We expect that the bacteria would stay immobilized until the HPLC measures start to determine the levels of exudates in the samples.

For the examination of released exudates from the plants, the collection of exudate samples was scheduled to be taken every week. The total sample collections were six , the three first collections where taken before the phosphorus limitation, to measure the amounts of possibly released exudates with a given rich in all nutrients solution and the other three after the limitation, to examine if the phosphate deficiency would increase the amounts of sugars and organic acids released in the substrate. Two samples were taken from each plant to a total of eight samples from the tomato plantation and sixteen from the reed manna grass per week.

Photosynthesis measurements

The photosynthesis measurements were carried with the ADC LCpro+ portable photosynthesis system. LCpro+ is a device that provides full automatic and independent control of the environmental conditions within the leaf chamber. It can utilize the microclimate and display the photosynthetic and respiration response of leafs.

The LCpro+ is functioning by passing air through a leaf chamber with a constant flow rate. The leaf alters the composition of the air by photosynthesis, respiration and transpiration depending on the microclimate. An infrared gas analyzer measures the CO_2 and H_2O concentration of the incoming air and in the outgoing air. This device uses specific software for the analysis and microclimate control enabling the system to generate automatic experiments such as light response curves displaying accurately the current photosynthetic and transpiration response of the plant calculated and expressed in μmol H_2O /m^2/s for transpiration and in μmol CO_2 /m^2/s

To begin the photosynthesis measurements we attached the chamber on the device (see fig 12) and started a pre-set illumination sequence that was selected in the software then the device was ready to automatically give results.

We attached the leaves of the tomato plants to the chamber at the different highs of 20 cm

and 70 cm from the base. This was because higher and lower leafs of the same plant may have differences in their photosynthetic and transpiration rates. The results were calculated in average per plant.

Figure 12. To the left picture of ADC LCPro+ console and to the right picture of the leaf chamber while measuring leaves of tomato plant.

The LCpro+ device was used only at the beginning and end of the experiment.

High performance liquid chromatography (HPLC)

For the determination of the quantity of sugars and organic acids contained in the samples we collected from the nutrient solution we had to prepare an amount of samples to be tested with the HPLC method.

The High performance Liquid Chromatography (or High pressure liquid chromatography, HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phases, the molecules being analysed, and the solvent(s) used.

For the quantification of the organic acids and sugars we used the ion – exchange method which was carried with a Shodex RI-71 series high performance liquid chromatography (HPLC) system. Organic acids were separated using a column suitable for organic acid and sugars

analysis. The mobile phase was 1.25 mM H_2SO_4 at a flow rate of 0.4 ml/min. The wavelength of UV detector was set at 512 nm. The temperature of the water bath containing the reaction coil was at 70 ºC and the reaction time was approximately 1 min.

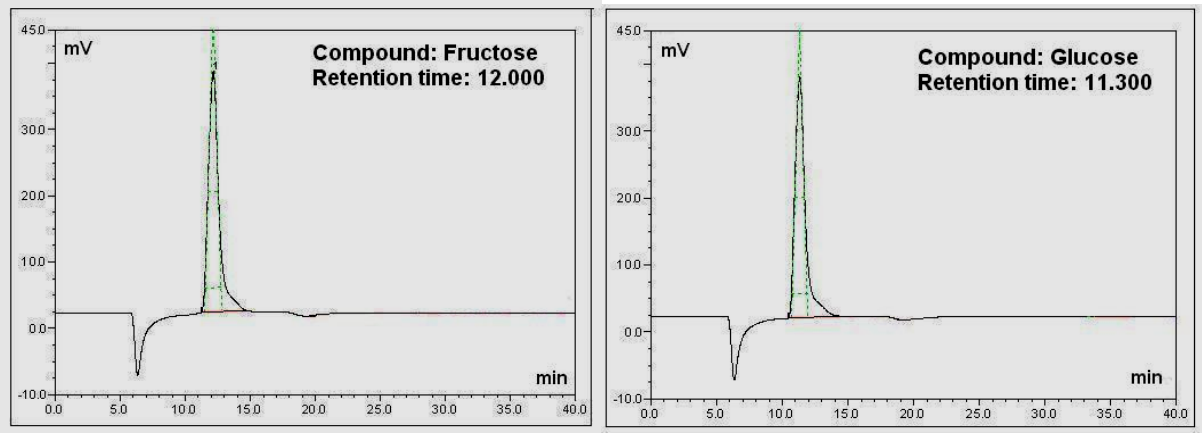
Preparation of samples and quantitative determination of sugars and organic acids

The collected samples were let in room temperature to restore. We prepared the samples of reed manna grass and tomato by taking 1 ml of the collected samples with accurate pipette and injecting it into vials of 1 ml that are used specifically for HPLC tests. Afterwards the 1 ml samples were placed in the HPLC system to be tested.

To compare the samples with known concentration samples and determine the quantity of exudates we prepared standard solutions based on reported exudates results in previous studies [Kamilova 2006, Hoffland 1988]. The sugars were: fructose, glucose, ribose and xylose and the organic acids: citric, succinic and malic. We dissolved 500 mg/L for each sugar and organic acid and used 1 ml. One 1 ml sample was prepared for each standard with known concentration of 500mg/L (0.05%) and was placed into the HPLC system to be tested.

The identification of exudates was made by comparing the retention time (min) of the standard solutions and the retention time of the exudates samples. By ‘match’ we mean that both have the same retention time. If the retention time of the standard solution matched the retention time of the sample then we could recognize it as the same compound.

The results for the standard solutions made of sugar compounds are presented below in figure 13 and the standards made of organic acids are presented in figure 14.



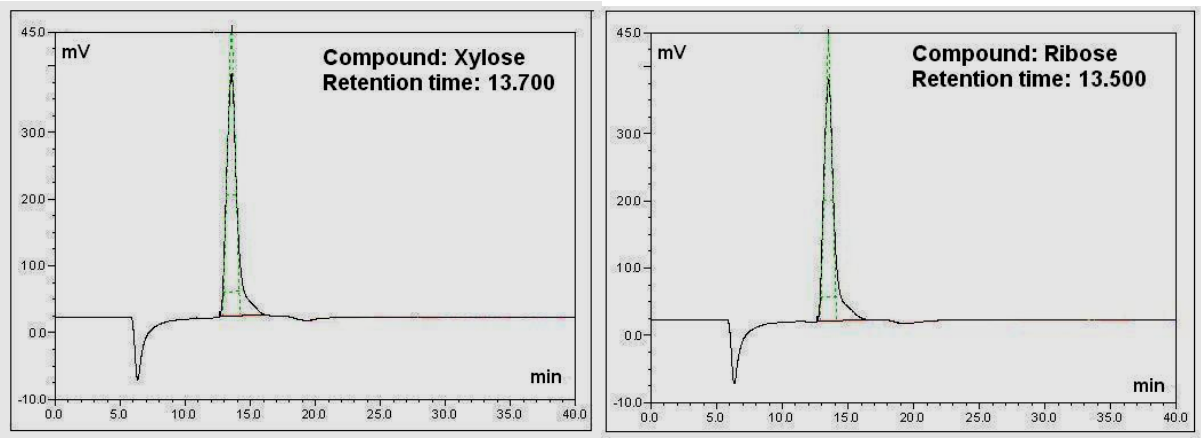
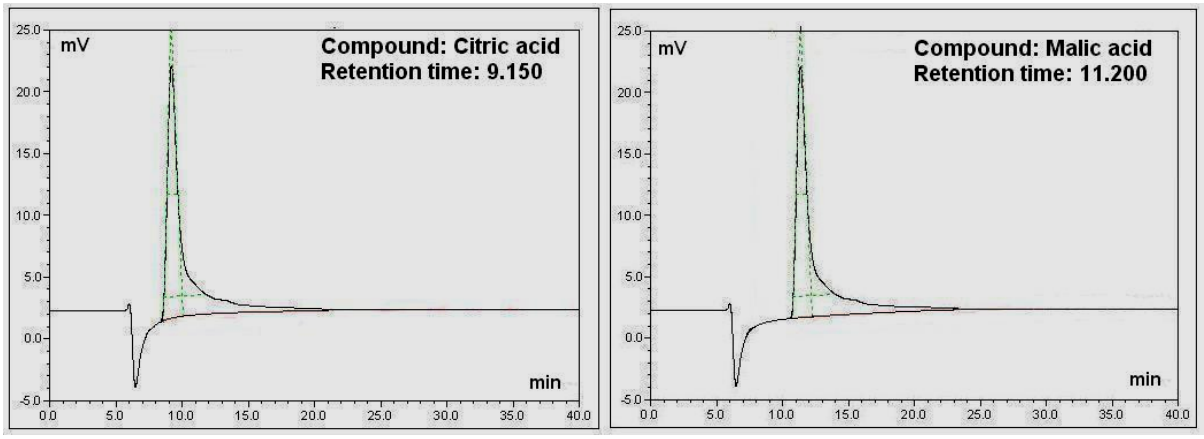


Figure13. Peak results for fructose , glucose , xylose and ribose with known concentrations of 0.05% analyzed on HPLC, we can also see the retention time of the compounds.

As we can see after the analysis on the HPLC detector, every standard sample revealed a "peak" that could be identified at certain time (retention time). By knowing the retention time of the standards we can identify the possible peaks from our exudate samples if both match the same retention time.

As an example, after those measurements we know that the retention time of fructose standard sample as appeared by the HPLC is 12.000 (12 minutes). By knowing this we can expect that if our exudate samples contain fructose then it will have the same retention time so a peak will be formed at the twelfth minute. If a peak appears that isn't matching any of the retention times then we know that this compound isn't one of our standards. Since we know that these compounds are the most commonly released by the roots we expect them to appear before other compounds.



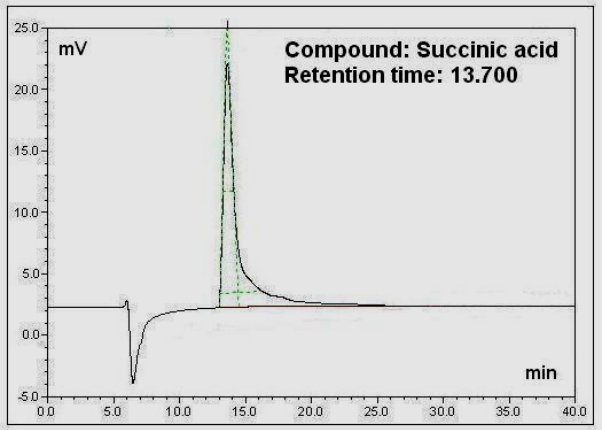


Figure14. Peak results for Citric acid, Malic acid and Succinic acid with known concentrations of 0.05% analyzed on HPLC, we can also see the retention time of the compounds.

The determination of quantity was made comparing the height (Height mV) and the area covered (area mV per*min) among the standard solutions that we can see in table 1. (The Height and the area covered are automatically displayed from the HPLC device). By knowing that all standards had a concentration of 0.05 % we could compare the results and calculate the concentrations of the tested samples.

Table 1. Height mV and Area mV*min of standard solutions with known concentration 0.05%.

Standard solution	Height (mV)	Area (mV*min)	Concentration (%)	Retention time(min)
Fructose	36,211	33,986	0.05	12.000
Glucose	36,062	32,331	0.05	11.300
Xylose	40,840	44,442	0.05	13.700
Ribose	36,897	43,567	0.05	13.500
Citric acid	20,789	30,390	0.05	9.150
Succinic acid	25,367	31,021	0.05	13.700
Malic acid	28,797	31,670	0.05	11.200

An example: For calculating fructose concentration we know that the peak reached 26,211mV of height and covered an area of 33,986 mV*min which equals to 0.05% concentration or 500 mg/L. If we find fructose in our exudate samples then we will have to compare the area covered (which if based on previous studies we could expect an amount from 10 to 300 mg/L for our plants). If the area covered of our exudate sample is 11,328 mV*min then the concentration is 1/3 of our standard sample or 0,016% or 160 mg/L. In this example we

would expect to see a peak or a mV of High divided by three also, compared to our standard sample's peak on figure 12.

Preparation for Organic material oxidization (COD) measurements

The determination of organic material was carried out with the COD method (chemical oxygen demand). In this method organic material is oxidized by potassium dichromate in acidic conditions and a catalyst (Ag^{+}). By the addition of Hg^{2+} the catalyst is protected from sedimentation with Cl^{-}. The reduced quantity of chromate can be determined photometricaly and is related to the COD of the sample meaning that the amount of organic material within the samples is expressed in mg/L. This method will show the possible increase of organic material a fact which will mean that there is increase of organic compounds released from the plant after the limitation, plus that it might detect organic material that the HPLC method cannot detect.

Totally 20 samples were prepared for COD measurements. We took 2 ml of the sample with accurate pipette and injected it into standard made chromate solutions. The mixture was sealed and shaken for the chemical reaction to take place faster. The vials were placed into oven for two hours for the reduction of the organic material and then let out to retain room temperature and be ready for measuring with photometer.

Samples were chosen from the second week, the fourth week and the sixth week. Two samples were also measured from the nutrient solutions as standards (a nutrient solution with all the nutrients and a sample without phosphorus) to compare.

Results

Plant condition and consequences of anaerobic root growth

On the first week of the experiment, one month after the planting, the tomato plants

seemed well, with normal growth, color and healthy root system. The reed manna grass plants were shocked after the re-planting but that was normal.

On the second week of our experiment symptoms of purple coloration on the tomato leaves were observed. The reed manna grass plants were still looking shocked.

After the third week bursting of the epidermis was noticed for the tomato. Although the root system was examined and its condition seemed to be good, the tomato plants seemed to have difficulties. The growth of the plants also seemed to be slowing down. From the other hand the reed manna grass plantation was now looking completely adjusted to the anaerobic environment and growing naturally.

At the fourth week all tomato plants in general looked bad, with pale green color and "depressed" appearance while one of the plants suffered from wilting and started to become yellow. The plant No 3 also seemed to decline. Discoloration to the leaves and dryness was noticed. The roots though where still looking good in all plants. The reed manna grass plantation was in good condition.

The fifth and sixth week our tomato plants started to have serious problems of anoxia and growth stop forcing us to remove two of the tomato plants. Several symptoms of imbalance caused in the plants where also noticed such as dark leaves and downward curving leaves, bursting of epidermis, adventitious root formation on the lower stem, shoots on the leaves,10-20 shoots at previously pinched shoots on the stem and adventitious roots bursting through the epidermis higher on the stem, and eventually up to the top. These symptoms are presented below (Figure 15). Plant No 3 was totally dry at the sixth week and plant No 4 followed. Increase of ethylene release from the shoots was measured for the two plants.

The reed manna grass plants were all having normal growth and until the sixth week of our experiment the plants showed no difficulties in adjusting into the anaerobic root environment.

Figure 15. Pictures of subsequent observations when keeping the tomato plants in anaerobic root environment and pinching all shoots and trusses. From left to right we can see: 1.Dark leaves and downward curving leaves. 2. Bursting of epidermis. 3. Adventitious root primordia on the lower stem. 4. Shoots on the leaves. 5. Multiple shoots at previously pinched shoots on the stem. 6. Adventitious roots bursting through the epidermis higher on the stem, eventually up to the top.

Photosynthesis results

The photosynthesis and transpiration measurements show a reduction from the start of the experiment to the end of the 6-week period.

The first measurements taken on the leaves the show a photosynthetic response starting from 0 and gradually advances to 25 μmol /m^2/s of CO_2 intake (the device increases PAR automatically calculating the photosynthesis respond and giving the curve later on) for all plants

in average as we can see at the light response curve below (Fig 16). The transpiration of the leaves starts from 2 and reaches 4 μmol /m^2/s of water evaporated at the maximum light intensity of 1000 μmol PAR/m^2/s (Fig 17) again for all plants in average.

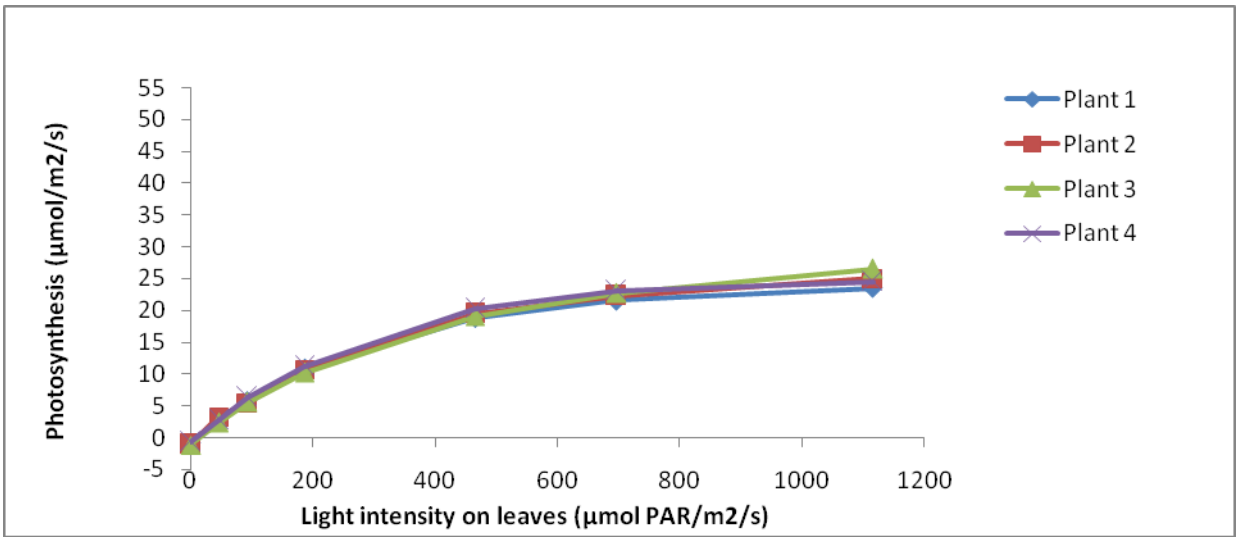


Figure 16. Effects of light intensity to the photosynthesis (CO_2 intake) of tomato leaves at the begin of experiment

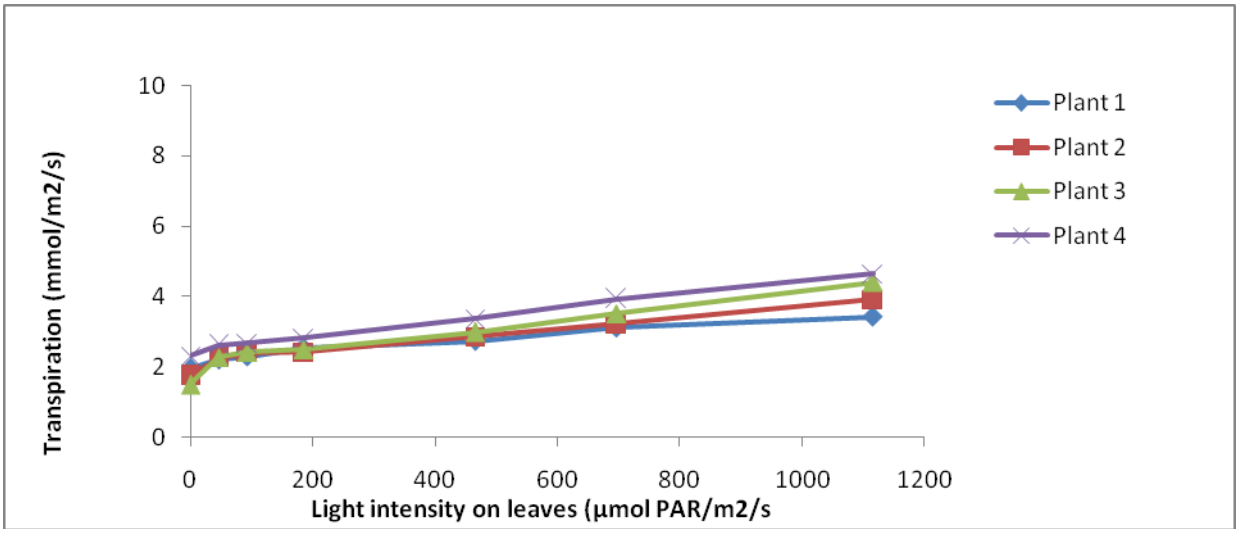


Figure 17.Effects of light intensity to the transpiration (H_2O evaporated) of tomato leaves at the begin of experiment

The second measurements taken the sixth week on the leaves for the first and the second plant show a photosynthetic response that start from 0 and gradually advances to 17 and 20 μmol /m^2/s of CO_2 intake as the light intensity reaches 1200 PAR (Fig 18). Tomato plant 4 shows a maximum level of 10 μmol /m^2/s at 500 PAR and then a decrease follows. Plant 3 was constantly at 3 μmol /m^2/s in average until the end of the measurements. The transpiration on plants 1 and 2 starts from 1 μmol/m^2/s of H_2O evaporated and reach almost 2 in average for both plants. Plant 4

again shows some transpiration at a level of 1 μmol/m^2/s at all light intensities but plant 3 reacts very weak to light at a rate below 0,5 μmol/m^2/s. Plants 3 and 4 show very weak performance in both levels at the sixth week. As mentioned above the photosynthesis and transpiration levels were lower than the first measurements

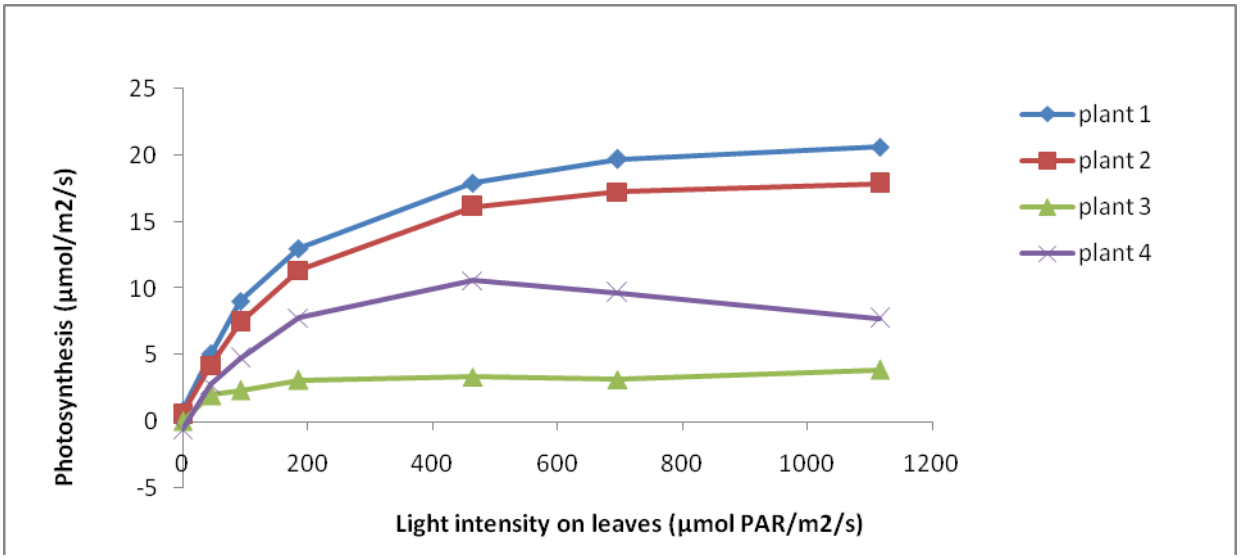


Figure 18. Effects of light intensity to the photosynthesis (CO_2 intake) of tomato leaves at the end of experiment

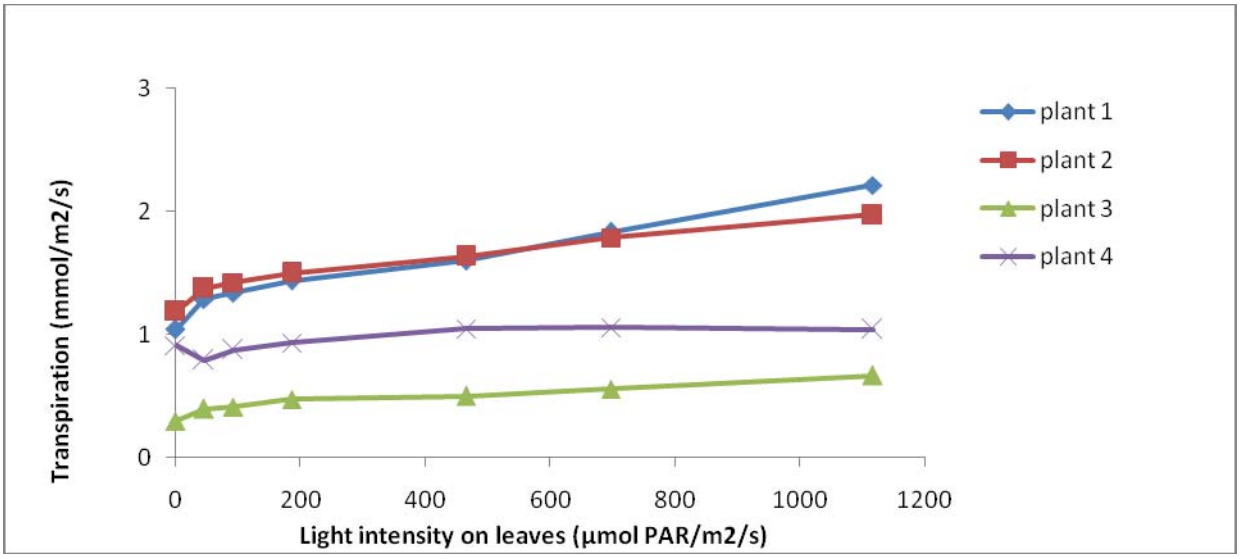


Figure 19.Effects of light intensity to the transpiration (H_2O evaporated) of tomato leaves at the end of experiment

Exudation results

The results from HPLC show no presence of exudates detected on our samples for the total period of the experiment.

The samples collected at the first week seemed to have no peaks that could be identified

as sugar or organic acid. This continued for the total period of the experiment.

At first we can also see the results from the nutrient solution and the solution without the phosphate that we used for the plant's deficiency (fig 20).

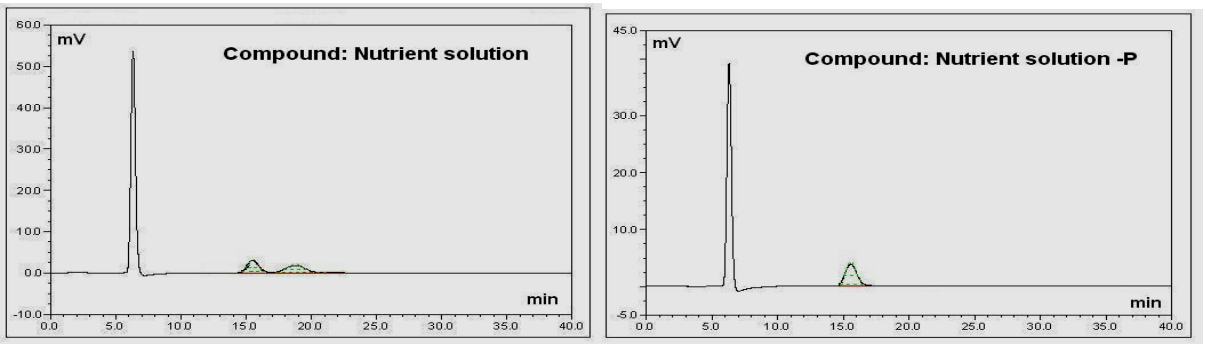


Figure 20. From the left to the right we can see peak results of Nutrient solution sample and peak result of Nutrient solution without phospahte that was analyzed on HPLC. The first peak is an HPLC formation that shows the relative height of the peak (mV).

The results from HPLC are presented in the graphic forms below bellow (fig 21) where representative samples are shown for each week's collection (since the total samples where 144 and an equal number of HPLC results we had to choose a representative sample for each plant).

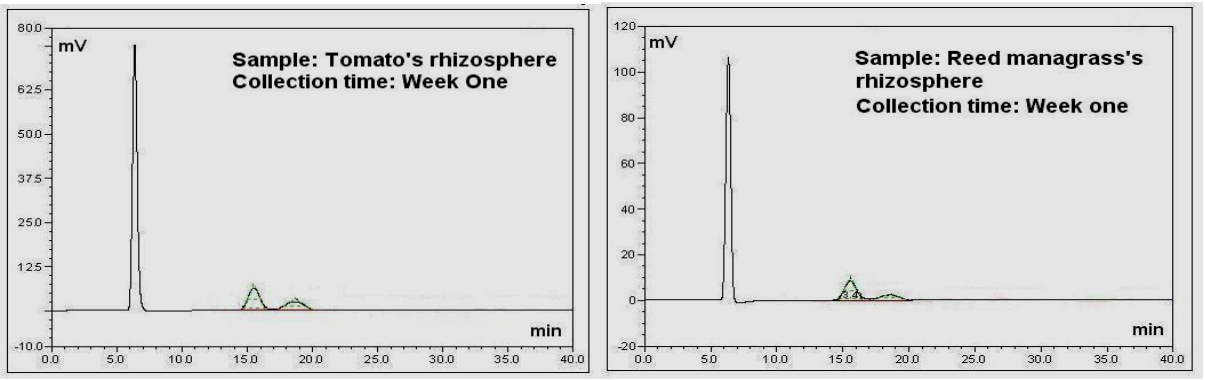


Figure 21. From the left to the right we can see peak results of tomato and reed manna grass samples taken from the rhizosphere of the plants at the first week. We can see that the compounds identified match the retention time of the compounds of the nutrient solution.

As we can see there are no other peaks in our results from the HPLC identified. The peaks that are present in these figures (fig 21 to fig 26) are matching the nutrient solution's peaks excluding the possibility of these peaks to be any of the suggested organic acid or sugar that have been used in the standard sample preparations.

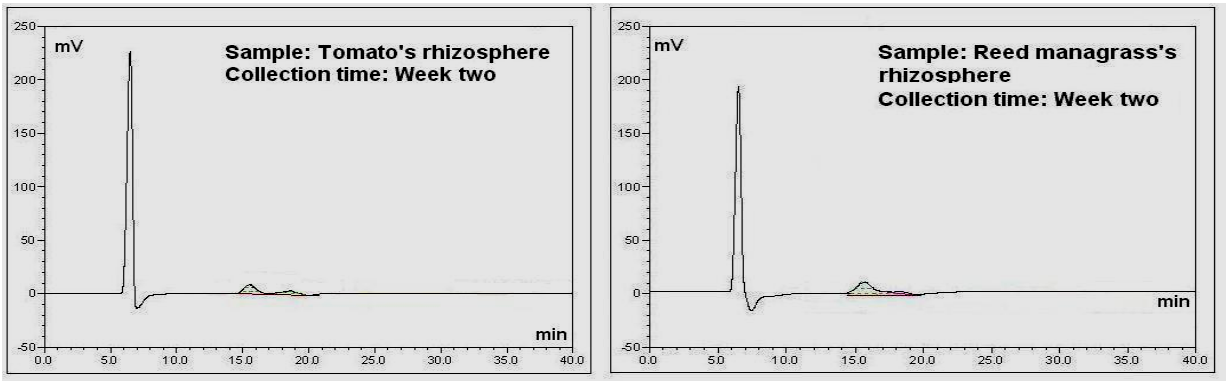


Figure 22. From the left to the right we can see peak results of tomato and reed manna grass samples taken from the rhizosphere of the plants at the second week.

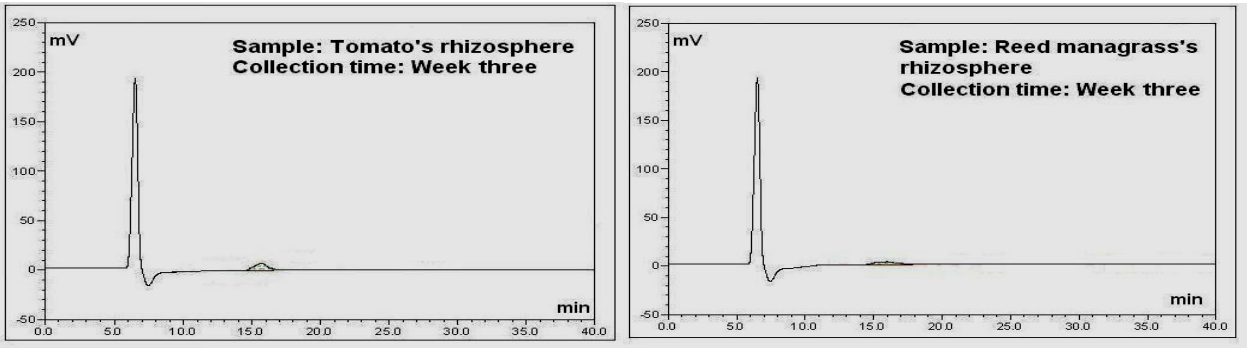


Figure 23. From the left to the right we can see peak results of tomato and reed manna grass samples taken from the rhizosphere of the plants at the third week.

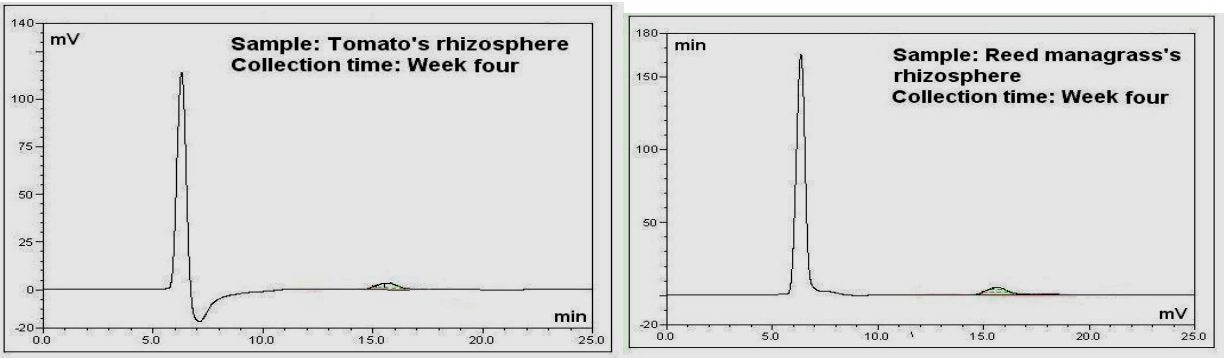


Figure 24. From the left to the right we can see peak results of tomato and reed manna grass samples taken from the rhizosphere of the plants at the fourth week.

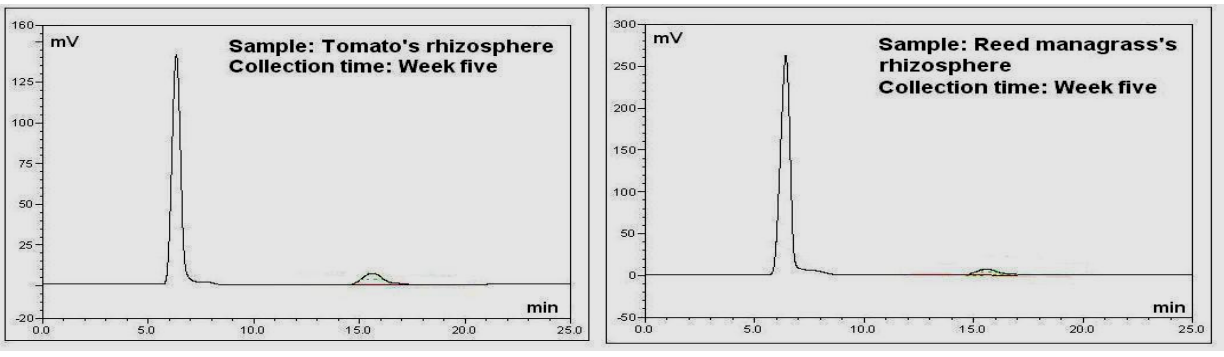


Figure 25. From the left to the right we can see peak results of tomato and reed manna grass samples taken from the rhizosphere of the plants at the fifth week.

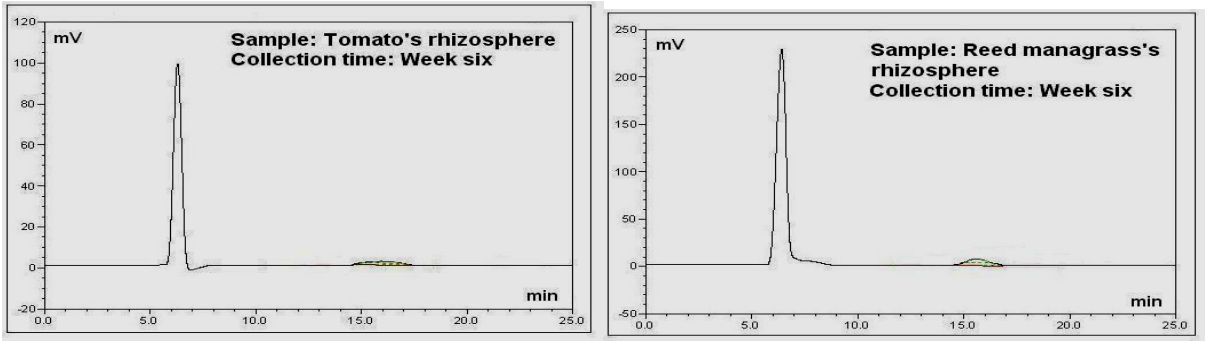


Figure 26. From the left to the right we can see peak results of tomato and reed manna grass samples taken from the rhizosphere of the plants at the sixth week.

As described in the Material and Method section we would expect a matching of the retention time between our exudate samples and of any of the used standards. Since the identification of any sugars or organic acids wasn't possible we couldn't detect the concentration based on the high and area covered between standards and exudates samples as it is shown the concentration of exudates was below our detection limit(see Table 2) .

Table 2. Alteration of sugars and organic acids composition (on average) for tomato and reed manna grass plants per week. (n.d.: not detectable.)

		Average Concentration (%) per plant						
Week	**Plant sample**	**Fructose**	**Glucose**	**Xylose**	**Ribose**	**Citric acid**	**Succinic acid**	**Malic acid**
1	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Organic material results (COD)

The COD method showed that in our samples there was no increase in the organic material compared to the nutrient solutions without exudates that could be oxidized showing presence of rhizodeposits.

Obviously there was already material in the nutrient solution that could be oxidized which revealed 189 mg/L in the standard solution and 190 mg/L for the -P solution. Compared with the results of the exudates samples we can see that the amount of material in the nutrient solutions was higher. Tomato showed 176 mg/L the second week in average, 145 mg/L the fourth week after the phosphorus deficiency and 158 mg/L on the sixth week at the end of the experiment. Reed manna grass showed 156 mg/L the second week then a little higher oxidized material in the fourth week's samples after the deficiency such as 162 mg/L and 143 mg/L at the end of the experiment. All samples showed a reduction in oxidized material compared to the Nutrient solution and –P.

Table 3.Alteration of average oxidized organic material in between week two, four and six.

Week	Sample	Average oxidized organic material(mg/L)
-	Nutrient Solution	189
	Nut. Solution -P	190
2(before deficiency)	Tomato	176
	Reed mannagrass	156
4(after deficiency)	Tomato	145
	Reed mannagrass	162
6(after deficiency)	Tomato	158
	Reed mannagrass	143

Discussion

On plant conditions

The aim of investigating the plant conditions was to determine if it is possible to grow plants of tomato and reed manna grass into anaerobic root environment and how this would effect the root production of organic compounds of the plants.

As we have seen above the condition of the tomato plants was gradually becoming worst every week suffering from anoxia and growth arrest. Especially from the third week onwards the tomato plants where having serious problems developing further.

In contrast the reed manna grass plants after their recovery form the re-plantation were growing well probably because the offered environment in the boxes was more natural for them to grow. Since between the two species of plants we used, there are other conditions are the same (phosphorus deficiency, growth conditions etc.) this could suggest that both the anaerobic root growth and the removal of flowers which only the tomato plants where treated with is causing the abnormalities to the tomato plants.

It seems more reasonable that the symptoms as presented in Figure 15 are associated with severe auxin or cytokinin overdosing as a result from the over-increase of high concentration of sugars inside the plant due to the removal of flowers and the unnatural for the plant root environment. It could suggest that the anaerobic root growth is causing negative condition for the roots to grow however the roots where in good condition in all the plants.

Furthermore, if the sugar over-increase is causing these symptoms to the plant, then these responses indicate that by removing the plant flowers the plant can not produce compounds in high rate, enough to prevent the over accumulation of the sugars, thus leading to the results of no exudation or low concentration exudation which is the opposite effect of stimulation.

Another reasonable explanation is that these symptoms are caused by waterlogged soil conditions that can rapidly damage the vegetative growth of tomato plants since the bottom part of the substrate (stone wool) was constantly in contact with nutrient solution. It was been found that injuries from water-logging include reduction in shoot extension, epinastic leaf growth, adventitious root production at the base of the stem and an overall reduction of the plants growth. Several of these responses are associated with changes in the production and translocation of hormones and specially ethylene. [Jackson 1979].

Using plants grown in nutrient solution it has been also found that low concentration of

O_2 in the root zone caused epinasty and elevated ethylene levels in the shoots. Since no ethylene was involved from the nutrient solution alone, this suggested that root O_2 deficiency can stimulate shoot ethylene production [Bradford et al. 1978]. This could be a reasonable explanation of the declination of plant 3 which was found in poor condition and plant 4 following after the fifth week.

On photosynthesis

The aim of measuring the photosynthesis was to determine and compare the photosynthetic rate in leaves of tomato plants that were grown under controlled conditions first with normal nutrient supply and then under limited phosphate.

Our results show that the photosynthetic rate as well as the transpiration of the leaves has decreased during the experiment if compared to the first measurements.

We can see that in the first measurement of photosynthesis and transpiration all plants are having the same response. In the last measurement we can see that plant 1 and 2 have similar response curve. At this point it has to be mentioned that plant 3 had difficulties of surviving from the fifth week onwards and therefore the photosynthesis may have been affected by its condition. Plant 4 also had difficulty gaining enough water and nutrients since the storage container that was providing the nutrient solution did not function well. That is probably an additional reason of lower photosynthetic activity and transpiration on plant 4 in week 6. Besides the technical problems in plant 3 and 4 we can see that all plants suffer from low photosynthetic response compared to the start of the experiment.

Although factors like chlorophyll concentration on the leaves, age of the plant or stomata state can affect the photosynthetic rate, it looks more reasonable to assume that the concentration of the sugars in the leaves affected the results (besides plant 4, which was also suffering from low water supply and plant 3 that was nearly dried out at the time of the measurement). It is known that high concentration of sugars in the leaf can inhibit photosynthesis. All the flowers of the plant were removed to minimize loss of sugars to the fruit. The phenotype of the plant indicates large amounts of sugar in the leaves. The fact that flower removal leads to an increase in leaf sugar content indicates that these sugars are not very efficiently transferred to the roots for exudation.

Another reason that could lead to the decrease of photosynthesis is the anaerobic

conditions in the root zone. Although anaerobic conditions are necessary for a well-functioning anode compartment of an MFC, this is not a suitable environment for the tomato plant. It is mentioned in the literature that stressful conditions lead to stimulation of exudation but what if the stressful state of the plant has a negative impact in the photosynthesis of the plant assuming reduce of the fixed carbon? In that situation the plant exudation could be less in total.

Another reasonable explanation is the effects caused by water stress or water logging inside the boxes where the plants where growing. It has been found that in hydroponically grown tomato plants that where subjected to moderate water stress in the nutrient solution the gas exchange was modified. A decrease of photosynthesis and transpiration was observed resulting in reducing of water use efficiency. One of the possible reasons for the reduction of photosynthesis is the structural damage to the thylakoids, which affects the photosynthetic transport of the electrons. [Zgallai et al. 2005]

On root exudation

In this study the aim was to determine the concentration of sugars and organic acids in our samples taken from tomato and reed manna grass plants over the experimental period of six weeks. By determining the amounts of exudates released we could investigate the effects of limiting phosphate into the stimulation of exudation.

As shown in the section Results, there is no presence of detectable amounts of glucose, fructose, xylose, ribose, citric acid, malic acid or succinic acid in our samples. These exudates are the main exudates we could expect since other studies have shown such results [Kamilova et al. 2006].

About the other peaks that were present in our samples, as seen on the HPLC results (fig 21 to fig 26), we can say that probably have no relation to the standard samples that we used for identification of sugars or organic acids because they are detected in different time. As we can see there are no other peaks in our results from the HPLC identified. If the peaks that are present in these figures (fig 21 to fig 26) are matching the nutrient solution's peaks the possibility of these peaks to be any of the suggested organic acid or sugar is excluded. We therefore conclude that these compounds are not exudate material from the plant.

Based on the concentrations of the used standards we conclude that if any of such exudates were present in our samples, then its concentration should be much lower than 0.05%. If that is the case then it was too low to determine. In previous studies [Kamilova et al. 2006] the

results on exudates analysis were around 5.85 mg/L for Glucose, 10.53 mg/L for fructose and 93,4 mg/L for citric acid which shows the difference from our results. Another example for succinic acid is the mg/L per tomato plant that was 61.5mg/L. The volume of water in our boxes was 5 L max. 60/5 is equal to 12 mg/L that could be our expected amount. Since we used bigger plants of total weight (215gr of dry weight in average in our plants to 12-20gr in previous studies) we could expect values of 60-200 mg/L per plant in the case of anaerobic grown root system, although younger plants are exudating considerably higher amounts [Kamilova et al. 2006]. In that case we would have identified the released root material.

A possible reason why we might not have detected any sugars or organic acids could be the bacterial activity. Organic compounds in root exudates are continuously metabolized by root-associated micro-organisms at the rhizosphere. Microbial activity results in quantitative and qualitative alterations of the root exudate composition due to degradation of exudate compounds and the release of bacterial metabolites [Pinton et al. 2007]. Other studies in the past have given results on exudation of fixed carbon from non-sterile, hydroponically grown wheat seedlings. It was found through the use of inhibitors 30% of the total tracer released from the roots was probably root exudate, the remaining fraction being carbon dioxide from root respiration. When no inhibitors were present the micro-organism population on the root was able to utilize all of the root exudate so that none accumulated in the root bathing solution [Minchin 1984].

Sterile conditions could offer the advantage of preventing the bacterial activity from possible metabolizing of the exudates in the samples. Under non-sterile conditions time is an important factor affecting the impact of microbial activity to the concentration of organic compounds inside the samples [Pinton et al. 2007].

On oxidized organic material

The results show that there was no increasing of the organic material that could be oxidized like sugars or organic acids on our samples. In contrast the samples lost organic material .The aim of this measurement was to detect organic material increase plus compounds that couldn't possibly be recognized by the HPLC method we used. We would expect an increase of organic material that can be oxidized due to exudates release but that was just an expectation.

In addition to the rest results, the COD analysis reveals that the nutrient solution had already organic material that could be oxidized by this method. As mentioned the tomato showed 176 mg/L the second week in average, 145 mg/L the fourth week after the phosphorus deficiency

and 158 mg/L on the sixth week at the end of the experiment. The decrease of organic material from the second to the fourth week can be explained as the loss of nutrients from the growing of the plants. The following increase of organic materials amount from the fourth to the sixth week can be explained by the renewing of nutrients in the solution we used inside the boxes to feed the plants. The assuming possibility for the plant to have shown an exudate increase has been proved false when we cross the results of COD with the results from HPLC. If there was any possible exudate increase we would have recognize it there as a peak with standard retention time. Another assumption could be that there was some root products which were simply not matching our standards used as most expected to be in the sample.

We can see the same happening for the reed manna grass plants. At the first measurement the second week we got 156 mg/L then a little higher oxidized material after the deficiency such as 162 mg/L in the fourth week's samples due to the renewing of nutrients into the solution and 143 mg/L at the end of the experiment. Again if we had some presence of compounds into our samples it would be recognized as peaks with certain retention time with the HPLC.

Conclusions

In this experiment we tried to create the possibility of stimulation of root exudation by limiting the phosphate to plants that where grown into anaerobic root environment. We can see from the results section that there were no exudates detected in our samples for both tomato and reed mannagrass, the photosynthetic response of the tomato was reduced compared to the first week and the total health of the plants has declined.

The most reasonable explanation is that the plant's rhizodeposits where most likely utilized by the microorganisms as substrates for their metabolic activities. A possible case scenario is that the absence of exudates might be caused by microbial activity in the sample between thawing and measurement. Since the expected values, should have been higher than our results, there must be a factor that is negatively affecting the rhizodeposition in our plants and it should be treated accordingly. It could be that there are either internal factors that prevent the rhizodeposition or either external factors that reduce the amount of exudates after rhizodeposition maybe while the sugars or organic acids are accumulating in the substrate. This could suggest the use of various strategies like application of antibiotics or becteriostatic compounds for root pretreatment to prevent the biodegradation during root exudate collection.

The tomato plants at the end of the experiment were showing low photosynthetic rate, low transpiration and bad health, most probably due to accumulation of sugars in the leaves which reduces the photosynthetic rate or the substrate effects to the total health of the plant or the decrease of photosynthesis and transpiration due to structural damage to the thylakoids, which affects the photosynthetic transport of the electrons.

The tomato plants suffered from the anaerobic root growth which is not their natural environment in combination with the nutrient limitation and the possible water logging damage of the roots inside the box which leads to the conclusion that the total health of the plant was affected negatively, limiting its life. It is not clear if the previous conclusion suggests that those conditions have a positive or negative impact on the release of organic acids and sugars in the substrate of tomato plants. Furthermore we can conclude that plants that don't adjust in such conditions naturally are hard to be used in MFC systems without treatments that would expand their vitality.

Plants like reed mannagrass which can grow an anaerobically naturally, can be used for the application of MFC systems due to the potential of the plant to withstand the environmental conditions. Of course further studies on the efficiency of exudates between plants have to be done in order to determine which plants offer optimization of exudate releases to be used in the MFC systems. In the future perspectives and applications we should follow pretreatments that will prevent the plants from excreting ethylene gas from the shoots (like plant 3 and 4) thus destroying the plants vitality.

To maximize the production of exudates certain strategies should be followed. Improvement is possible by: better management of the vitally of plants balancing the total health and potential of rhizodeposition to stressful techniques, optimizing the plant photosynthetic and growing conditions thus increasing the photosynthetically fixed carbon, selection of species with higher adjustability and exudation dynamics.

Literature

1. Allen, R.M. Bennetto, H.P. 1993. Electricity production from carbohydrates. Microbial fuel cells. Vol 39: 27–40.
2. Annual energy review 2007. Energy Information Administration Vol 1: 439

3. Bais H.P. Weir T.L. Perry J.G. Gilroy S. Vivanco J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57: 233–266.
4. Bennetto, H. P. 1990. Electricity Generation by micro-organisms. Biotechnology Education. Vol 1 , No 4: 163-168.
5. Bishop J.K.B. Rossow W.B. 1991. Spatial and temporal variability of global surface solar irradiance. Geophysical Research Letters. Vol 96: 16839-16858
6. Bradford K.J. Rilley D.R. 1978 .Effects of root anaerobiosis on ethylene production , epinasty and frowth of tomato plants. Plant Physiology Vol 61: 506 – 509.
7. Chauldhuri S.K. 2003. Electricity generation by direct oxidation of glucose in mediator-less microbial fuel cells. Nature Biotechnology 21: 12-29.
8. Chow J. Kopp R.J. Portney P.R. 2003. Energy resources and global development. Science 302: 1528–1531.
9. Destries. M. 2007. Fosters brewery to develop fuel cell that run on beer. Associated Press 1200: 331-332
10. Hoffland E. Findenegg G.R. Nelemans J.A. 1989 . Solubilization of rock phosphate by rape. II Local root exudation of organic acids as a response to P-starvation. Plant Soil 113: 161-165.
11. Jackson M.B. Gampbell D.J. 1979. Effects of benziladenine and giberelic acid on the responses of tomato plants to anaerobic root enviroments and to ethylene. New phytologists Vol 82: 331- 340.
12. Jones D.L. 1998. Organic acids in the rhizosphere – a critical review. Plant and soil 205: 25-44.
13. Kamilova, F. 2006. Organic acids, sugars, and L-Tryptophan in exudates of vegetables growing on stone wool and their effects on activities of rhizosphere bacteria. MPMI Vol. 19, No. 3: 250–256.
14. Lynch J.M. Whipps J.M. 1990. Substrate flow in the rhizosphere. Plant and Soil 129: 1–10.
15. Logan B.E. Hamelers H.W.M. Rozendal R. Schroder U. Keller J. Freguia S. Aelterman P. Verstraete W. Rabaey R. 2006. Microbial fuel cells: methodology and technology. Environmental Science and Technology 40: 5181–5192.
16. Minchin P.E.H. McNaughton G.S. 1984. Exudation of recently fixed carbon by non-sterile roots. Journal of Experimental Botany 35: 74–82.

17. Neumann G. Römheld D. 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil 211: 121–130.
18. Pinton R. Varanini Z. Nannipieri P. 2007. The rhizosphere: Biochemistry and organic substrates at the soil-plant interface. Vol 2: 447.
19. Rabaey, K. Verstraete, W. 2005 .Microbial fuel cells: novel biotechnology for energy generation. Trends in Biotechnology 23: 291–298.
20. Salisburi, F. Ross, L. 1991. Plant physiology. Wadsworth publishing company, California. 4: 682.
21. Schamphelaire L.D. Bossche L.V. Dang H.S. Höfte M. Boon N. Rabaey K. Verstraete W. 2008 . Microbial fuel cells generating electricity from rhizodeposits of rice plants. Environmental science and technology 42: 3053-3058.
22. Strik, D. Hamelers, B Snel, J. Cees, J.N. Green Electricity production by collaboration of living plants and bacteria in a fuel cell. International Journal of energy research 1: 112-119.
23. Venkata, M.S. 2007. Bioelectricity production by mediator-less microbial fuel cell under acidophilic condition using wastewater as substrate. Current Science Vol 92 No 12: 1720-1726.
24. Walker T.S. Bais H.P. Grotewold E. Vivanco J.M. 2003. Root exudation and rhizosphere biology. Plant Physiology 132: 44–51.
25. Zgallai H. Steppe K. Lemeur R. 2005. Photosynthetic, physiological and biochemical responses of tomato plants to glycol induced water deficit. Journal of integrative plant biology Vol 47 Issue 12: 1470-1478.