UNIVERSITÀ
DI PAVIA

Department of Earth and Enviromental Sciences
Laurea Magistralis in
Agri-food Sustainability

Plant Microbial Fuel Cells: indoor and outdoor experimentation to enhance electrical performances

Supervisor:
Prof.ssa Silvia Paola Assini

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Experimental thesis by
Mariam Mahmoud

Academic Year 2024/2025

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ABSTRACT (ENGLISH)

The thesis addresses the study of Plant Microbial Fuel Cells (PMFC) as new technologies for the sustainable production of electricity from renewable sources.
In particular, we focused on the production of PMFCs with different characteristics to verify whether these can help improve the efficiency of electricity production in systems.

Microbial Fuel Cells (MFC) are based on the ability of bacteria to degrade organic compounds in an anaerobic environment, releasing electrons that generate electricity. These cells consist of an anode compartment and a cathode compartment separated by a proton exchange membrane. PMFCs are a version of this system in which the main feature is the presence of a plant as an additional component. In this version, the roots of the plants release organic substances through their leachate, thus feeding the microorganisms present in the soil. This exploits the phenomenon whereby the microbial populations present in the soil around the rhizosphere degrade organic substances to produce electrons; when the electrons complete the circuit, electricity is produced.

This thesis proposes a PMFC prototype that consists of separating the MFC from the plant. The pot containing the plants is located at the top and the plant is watered regularly; the drainage water filters into the container below, which contains the cells. In this way, the plant provides nutrients to the bacterial colonies in the soil through the nutrient-rich leachate derived from the rhizodeposits.

In particular, the cells set up indoors were constructed with different characteristics concerning the presence of the *Trichoderma* fungus and the presence of a membrane.
The *Trichoderma* fungus was tested with two different inoculations: in one case, it was inoculated into the plant's soil and therefore in contact with the plant's roots, while in the second case, it was inoculated only into the soil present in the cell.
Sets were also prepared with and without membranes to assess their influence.
A total of 72 cells were constructed, divided into 6 sets:

- control set
- control set with membrane
- set with *Trichoderma* inoculation in the plant
- set with *Trichoderma* inoculation in the plant and presence of membrane
- set with *Trichoderma* inoculation in the soil inside the cell
- set with *Trichoderma* inoculation in the soil inside the cell and presence of membrane

The results showed significant differences between the configurations. In particular, the sets with *Trichoderma* obtained better results, demonstrating how it can be evaluated as an additive in the construction of cells and can thus improve their performance. The sets with membranes achieved higher values overall, showing that this may also influence activity.

Two types of experiments were conducted for the outdoor trials:
- set up with different plants to assess which of them performed best
- set up with gold cathodes wires to assess whether the change in material influenced activity

Among the plants, *Allium angulosum* showed the highest values, indicating how the different characteristics of plants can influence cell function.

As for the cathode material, the gold cathode wires achieved the highest values. This is very important information, as in outdoor setups it is necessary to take into account how the rapid oxidation of the cathode can be a problem and how finding a more resistant material is therefore a good strategy.

ABSTRACT (ITALIAN)

La tesi affronta lo studio delle Plant Microbial Fuel Cells (PMFC) come nuove tecnologie per la produzione sostenibile di energia elettrica da fonti rinnovabili.
In particolare, ci siamo focalizzati sulla realizzazione di celle con caratteristiche diverse per verificare se queste possano aiutare a migliorare l'efficienza di produzione di energia elettrica dei sistemi.

Le Microbial Fuel Cells (MFC) sono celle a combustibile microbiche che si basano sulla capacità di batteri di degradare composti organici in ambiente anaerobico, liberando elettroni che permettono di generare corrente. Queste celle sono composte da un comparto anodico ed uno catodico separati da una membrana a scambio protonico. Le PMFC rappresentano una versione di questo sistema in cui la caratteristica principale è la presenza di una pianta come componente aggiuntivo. In questa versione sono le radici delle piante a rilasciare sostanze organiche tramite il loro percolato alimentando così i microorganismi presenti nel suolo. Viene così sfruttato il fenomeno per cui le popolazioni microbiche presenti nel suolo intorno alla rizosfera degradano le sostanze organiche per produrre elettroni; quando gli elettroni completano il circuito viene prodotta elettricità.

Nella presente tesi è stato proposto un prototipo di PMFC che consiste nella separazione della pianta della MFC. Il vaso contenente le piante si trova nella parte superiore e la pianta viene bagnata regolarmente; l'acqua di drenaggio filtra nel contenitore sottostante, che contiene le celle. In questo modo la pianta fornisce nutrienti alle colonie batteriche del suolo attraverso il percolato ricco di sostanze nutritive derivato dai rizodepositi.

In particolare, le celle allestite in *indoor* sono state costruite con delle caratteristiche diverse che riguardano la presenza del fungo *Trichoderma* e la presenza di una membrana.
Il fungo *Trichoderma* è stato testato con due inoculi diversi: in un caso è stato inoculato nel terreno della pianta e quindi a contatto con le radici della pianta mentre nel secondo caso è stato inoculato solo nel terreno presente nella cella.
Sono stati preparati anche set con assenza o con presenza di membrana per valutarne l'influenza.
In totale sono state costruite 72 celle divise in 6 set:

- set di controllo
- set di controllo con membrana
- set con inoculo del *Trichoderma* nella pianta
- set con inoculo del *Trichoderma* nella pianta e presenza di membrana
- set con inoculo del *Trichoderma* nel suolo all'interno della cella

- set con inoculo del *Trichoderma* nel suolo all'interno della cella e presenza di membrana

I risultati hanno evidenziato differenze significative tra le configurazioni. In particolare i set con presenza di *Trichoderma* hanno ottenuto risultati migliori dimostrando come questo possa essere valutato come additivo nella costruzione delle celle e possa in questo modo migliorarne le performance. I set con membrana nel complesso hanno raggiunto valori più alti mostrando come anche questa possa influenzare l'attività.

Per le sperimentazioni *outdoor* sono stati condotti due tipi di esperimenti:
- allestimento di celle con diverse piante per valutare quale tra esse avesse le performance migliore
- allestimento di celle con catodo in oro per valutare se il cambio di materiale influenzasse l'attività

Tra le piante *Allium angulosum* ha mostrato i valori maggiori indicando come le diverse caratteristiche delle piante possano andare ad influenzare il funzionamento delle celle.

Per quanto riguarda il materiale di catodo invece ad avere raggiunto i valori maggiori è stato il catodo in oro. Questo è un dato molto importante in quanto nell'allestimento outdoor bisogna tenere in considerazione come la veloce ossidazione del catodo possa rappresentare un problema e come quindi trovare un materiale più resistente sia una buona strategia.

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1. INTRODUCTION

Human society is presently facing two major challenges: environmental pollution and shortage of resources, both resulting from rapid urbanization and industrialization. Another threat is also the fast-growing human population, which is expected to reach 10 billion people in 2057 (Hoang et al., 2022). The shift to clean energy is crucial for tackling issues such as climate change, environmental pollution, and ensuring long-term energy security. Reliance on fossil fuels has caused significant environmental harm, making the move toward sustainable sources - such as solar, wind, bioenergy, and bioelectricity - urgently necessary. As global energy demand continues to rise, innovative solutions are required to integrate economic development with environmental responsibility.

In this context, Plant Microbial Fuel Cells (hereafter PMFCs) emerge as a promising technology, supporting the United Nations Sustainable Development Goals, particularly SDG 7 (affordable and clean energy) and SDG 11 (sustainable cities and communities) (Brugellis et al., 2025; Chong et al., 2025).

Microbial fuel cells (hereafter MFCs) are technologies that employ bacteria as the catalysts to oxidize organic and inorganic matter and generate electricity. During this process, the bacteria from these substrates produce electrons that are transferred to the anode (negative terminal) and then flow to the cathode (positive terminal) connected by a conductive material containing a resistor or operated under a load.

MFCs are being constructed using a wide range of materials in a very increasing diversity of configurations. These systems can operate under different range of conditions that include differences in temperature, pH, electron acceptor, electrode surface areas, reactor size, and operation time (Logan et al., 2006).

The performance of MFCs can be improved by changing the electrode materials, by enhancing bacterial adherence, optimizing electron transport, and modifying the electrode surface (Chakma et al., 2025).

MFC technology is promising, efficient and environmentally friendly approach in sustainable bioelectricity generation and in different applications like wastewater treatment.

Recently, MFCs have found application in different sectors like for example in wastewater treatment, microbial solar cells, bioelectricity generation, industrial chemicals recovery and pollutant removal, sensors, hydrogen production, bioremediation, and energy recovery (Shah et al., 2019).

1.1 - HISTORICAL NOTES

The original idea of using microorganisms to generate electricity was conceived and attributed to Potter M. C., a professor of botany at Durham University in 1911. He managed to generate electricity from the degradation of organic compounds using *Saccharomyces cerevisiae* and found out that the electrical effects are an expression of the activity of the microorganisms and are influenced by temperature, concentration of the nutrient medium, and the number of active organisms present. Unfortunately, the poor results obtained discouraged further research (Potter, 1997).

A major advancement came in 1931 thanks to Barnet Cohen, who designed experimental microbiological cells that were able to generate a total voltage of over 35 volts, even though the current remained very low, at just 2 milliampere (Shah et al., 2019; Cohen, 1931).

In the 1980s and 1990s, researches conducted by Robin M. A. and later by Bennetto and his team helped to explain how Microbial Fuel Cells work, introducing the use of mediators to facilitate the transfer of electrons between microorganisms and electrodes. This innovating approach led to the development of so-called "analytical MFCs," which are still used today in various laboratory applications (Benetto, 1990).

Since the 2000s, interest in this technology has grown rapidly, along with better understanding of how electrons are transferred in these systems.

Numerous research groups have studied MFCs as a tool for wastewater treatment, building systems from tiny lab-scale setups to large industrial plant. One of the first experiments dates back to 2007, when the Advanced Water Management Center at the University of Queensland, Australia, tested a 1 cubic meter system at the Foster's brewery in Yatala. The goal was to transform the wastewater produced by the brewing process into purified water, carbon dioxide, and electricity, showing the environmental and energy potential of microbial fuel cells (Shah et al., 2019).

Over the course of time, many configurations and modifications were done in the MFC technologies and by this way PMFC was purposed with the idea of incorporating a plant at an anode region as the source of substrates for bacteria (Nitisoravut & Regmi, 2017).

1.2 - OPERATION AND FEATURES OF MFCs

Microbial fuel cells can have different compositions depending on the materials used and the type of stratification. They are comparable to batteries and can be constructed in such a way
that the flow of protons occurs upwards or horizontally depending on the structure of the cell.
MFCs generate bioelectricity through the redox reactions of electrogenic bacteria. They consist of two main compartments: anode compartment with anode electrode and anolyte and cathode compartment with cathode and catholyte. Between the two compartments there is an ion exchange membrane (Shah et al., 2019).

MFCs exploit the ability of specific microorganisms present oxygen-free (anaerobic) environment as biological catalysts to break down organic compounds. This reaction allows the production of electrons and protons, which are used in the process of generating electricity (Antonopoulou et al., 2010).
In the anode compartment, bacteria break down organic material, releasing electrons and hydrogen ions. The protons migrate through the membrane towards the cathode, while the electrons travel along an external circuit, creating an electric current (Rahimnejad et al., 2011).
In the cathode compartment, oxygen serves as the terminal electron acceptor, completing the circuit by reacting with electrons and protons to form water molecules.
It is essential that the anode environment remains oxygen-free, as the presence of oxygen would disrupt proper electricity generation (Sharma & Li, 2010). The potential difference established between the two compartments drives the electron flow and forms the basis for the production of electrical current, which can be used, for example, to power small devices. **Figure 1** illustrates the general structure of an MFC, highlighting the two compartments and the membrane that separates them.

The term "organic substrate" refers to the set of compounds present in the organic material used as fuel. The composition of these substrates varies depending on the type of waste employed; however in any case, their oxidation leads to the generation of protons and electrons.
When glucose is used as a substrate, the reactions that take place in the two compartments can be described as follows. Equation (1.1) describes the reactions that occur at the anode and cathode, while equation (1.2) illustrates the process that occurs in MFCs (Di Lorenzo et al., 2019).

Anode:

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$ (1.1)

Cathode:

$24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$

Overall reaction:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Electrical energy (1.2)

Thanks to their ability to generate electricity and contribute to the degradation of organic contaminants, MFCs are a promising technological approach for wastewater treatment, offering clear environmental and energy advantages.

However, the performance of these systems is still influenced by numerous factors, including the design of the cell, the materials of the electrodes, their active surface area, the microbial composition, the type of substrate employed. Operational variables such as the pH and conductivity of the solution also play a significant role, along with efficiency of electron transfer, the effectiveness of the membrane in proton exchange, the supply of oxygen, and the continuous supply of water (Logan & Regan, 2006).

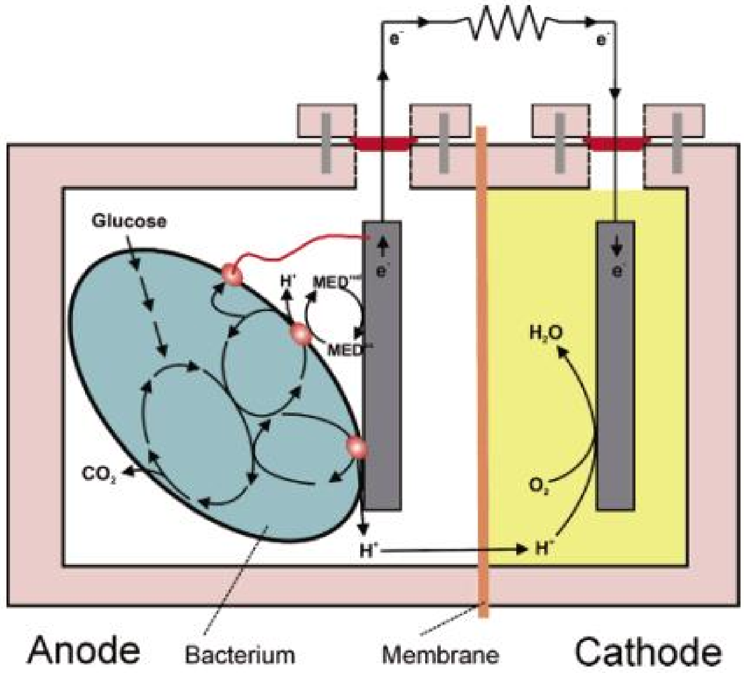


Figure 1: Schematic representation of a microbial fuel cell (Logan et al., 2006)

1.2.1 - ANODE

The anode compartment is one of the main parts of the system; it operates under anaerobic conditions and all the essential factors for biomass degradation are guaranteed inside it.
It can be made of different materials, but carbon and metal-based materials are generally used.

The most important characteristics that the material used for the anode must have are: electrical conductivity; high mechanical strength; resistance to corrosion; developed surface area; biocompatibility; environmentally friendly and low cost (Santoro et al., 2017).

The microbial consortia, pH, anode material, mediators, and substrate employed in the anode chamber are significant factors for MFC performance.
In the anodic compartment there is the formation of a biofilm (thanks to the microorganisms attached to the anode surface) that acts as a catalyst for the oxidation process of organic molecules (Chakma et al., 2025).

1.2.2 - CATHODE

The cathode chamber in MFC function as an electron sink where oxygen is reduced to water (Chakma et al., 2025).
The cathode compartment consists of: catalyst, electrode, and oxidant. The oxidant generally used is air or pure oxygen, while in other cases redox couples may be added to the compartment to accelerate the reaction. Oxygen reacts with protons and electrons transported from the anode compartment to form water. Oxygen is the most suitable electron acceptor for an MFC due to its high oxidation potential, availability, low cost (it is free), sustainability, and the lack of a chemical waste product (water is formed as the only end-product). The choice of the cathode material greatly affects performance, and is varied based on application. (Logan et al., 2006).
The oxygen reduction reaction that occurs at the cathode is often the limiting reaction in MFCs (Santoro et al., 2017).
The choice of cathode material greatly affects performance, and the materials used are generally the same as those in the anode compartment.
The abiotic catalysts used in MFCs can be classified into three main categories based on their function and whether they contain platinum or transition metals. These categories are: platinum-based, carbon-based (metal-free), or platinum group metal-free with more complex electron transfer (Santoro et al., 2017).

1.2.3 - MEMBRANE

The membrane separates the anode and cathode compartments and has different purposes in MFCs. It separates the corresponding chemical reactions and avoid short-circuiting between the electrodes, it enables the recovery of the reaction product from the cathode compartment and it can result in the establishment of a pH gradient and improve electrolyte resistance (Chakma et al., 2025).

In an MFC, a membrane's performance is determined by its transport properties. An optimal separator/membrane need to prevent the unintended transport of other molecules and to do that it must have excellent proton transfer efficiency and selectivity. Other factors to take into consideration while selecting separators or membranes are proton conductivity and selectivity, ion exchange capacity (IEC), membrane hydrophilicity, oxygen permeability, thickness, resistance and membrane surface.

A variety of materials can be used as separators in MFC systems; membrane materials commercially include Nafion, Zirfon, and Ultrex and others (Chakma et al., 2025).

1.2.4 - MICROBIAL COMMUNITY

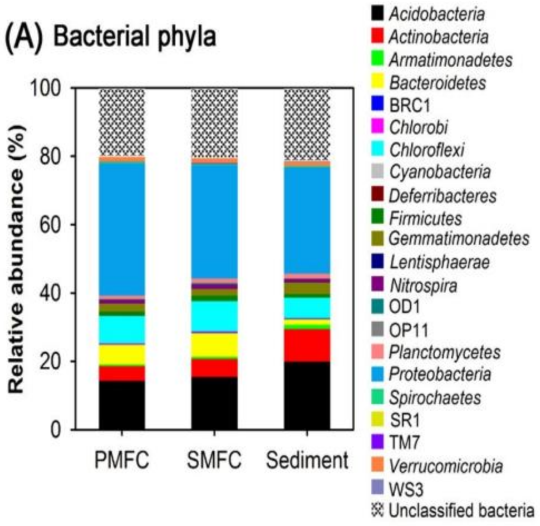
Microorganisms are the key components of microbial fuel cells. They decompose the organic matter present in fuels to generate useful protons and electrons. These organism can be both naturally occurring microorganisms and artificially cultivated through genetic engineering, taking into account their nature, activity, and stability.

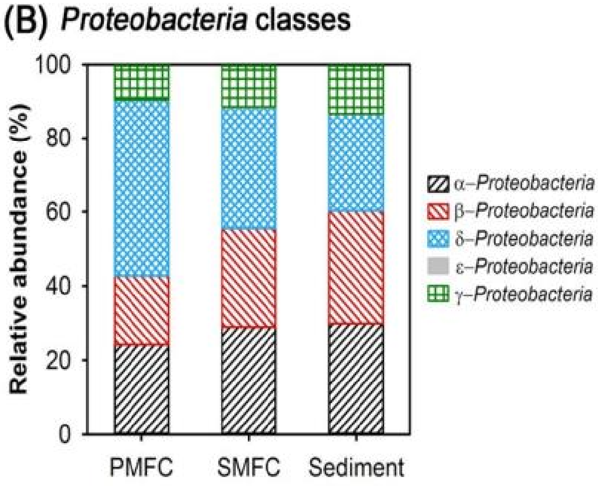
The formation of a biofilm on the surface of the anode is essential for efficient electron transfer in MFCs (Deng et al., 2012).

The phylogenetic diversity and heterogeneity of DNA in soil bacteria has been studied and according to some studies, a wide range of bacterial communities are capable of producing electricity in MFCs; Proteobacteria are one example. In particular a total of 22 phyla were observed (as shown in **Figure 2**), indicating extremely high biodiversity. *Proteobacteria* accounted for 31.1–38.7% of the total composition, followed by *Acidobacteria* (14.3–19.9%), *Chloroflexi* (8.1–8.9%), *Actinobacteria* (4.2–9.6%), and *Bacteroidetes* (1.7– 6.9%). The better the microbial community adapts to a system, the higher the chance of improved system performance is.

Archaea such as methanogens, hyperthermophiles, and thermophiles may also be present, and it has been shown that they coexist with microbial communities (Lu et al., 2015; Maddalwar et al., 2021).

The material to be degraded in MFCs is supplied in the form of wastewater rich in organic substances (Regmi et al., 2018).


(A) Bacterial phyla


(B) *Proteobacteria* classes

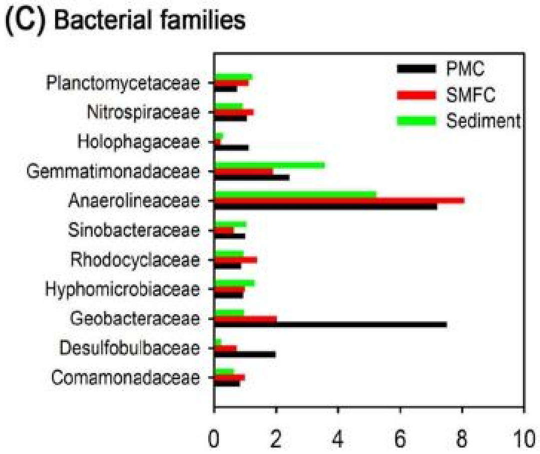

(C) Bacterial families

Figure 2: Taxonomic classification of bacterial DNA sequences from PMFC communities, SMFC control, and original sediment at the phylum level (A) and (C) and distribution at the class level (B) of the most dominant phylum of Proteobacteria (Lu et al., 2015)

1.3 - PLANT MICROBIAL FUEL CELLS

One limitation of MFCs is that they require a continuous supply of organic substrates is required to ensure the uninterrupted generation of electricity and it cannot always be implemented in practice. Plant microbial fuel cells (hereafter PMFCs), as a newer technology, largely eliminates this disadvantage of MFCs (Lepikash et al., 2024).

The main characteristic of PMFCs is that they have the plant as an additional component compared to the MFCs.

Plants are the primary producer in an ecosystem. They are autotrophs and utilize solar energy to produce biomass thanks to a pigment called chlorophyll present in the green part of its leaves. However, approximately 40% of this energy is consumed by the plants itself, while the remaining portion is exuded into the rhizosphere. PMFC exploits the phenomenon in which microbe populations present in the soil around the rhizosphere break down the organics to yield electrons and traps the electrons released by the microbes in the anode region. When the electrons pass through a load and reach a cathode completing the circuit, electricity, so called "bioelectricity", is produced (Nitisoravut & Regmi, 2017).

PMFC can be conceptualized as an open loop type of biosystem and can be divided into two major structures: the bio-control structure and bio-process structure. The bio-control structure, represented by the plant, receives the external input signal (in this case sunlight) to achieve voltage. The bio-process structure, consisting of the microbial population, produces the outputs (voltage) by processing material resources (root exudates). The outputs can be affected by variations caused by disturbances (Nitisoravut & Regmi, 2017).

PMFCs combine the principles of photosynthesis with bioelectrochemical systems, relying on microbial, electrochemical, material, environmental and engineering factors. The performance is governed by several factors such as plant species, operating conditions, inoculum properties and design parameters. Optimizing the operating conditions is essential for getting better power generation (Shaikh et al., 2021).

In the case of PMFC system, soil acts as membrane while operating as a single sediment configuration where distinct anaerobic and aerobic regions are developed based upon a vertical redox gradient (Greenman et al., 2024; Regmi et al., 2018).

By those principles PMFCs can generate electricity. Under favorable environmental conditions, a PMFC system can power up the load 24 hours per day without harming the plants. The interaction between the living plants and microbes serves as a source of bio-energy generator for the PMFCs, and this energy can be produced as long as the plants remain alive, and the soil supports microbe’s growth (Chong et al., 2025).

Different aspects need to be considered when choosing the plant for the PMFCs. The aspects that need to be taken into consideration are:

- strong survivability which is described as the ability of the plant to survive for long duration in an adverse growing condition;
- healthy growth rate measured as the relative increase in leaf area over time;
- roots growth rate;
- rhizodeposition rate which identified as the rate of material lost from plant roots;
- availability and adaptability of plant species;
- plant’s ability to generate a high amount of organic matter from its root exudates;
- high rate of photosynthesis (Chong et al., 2025)

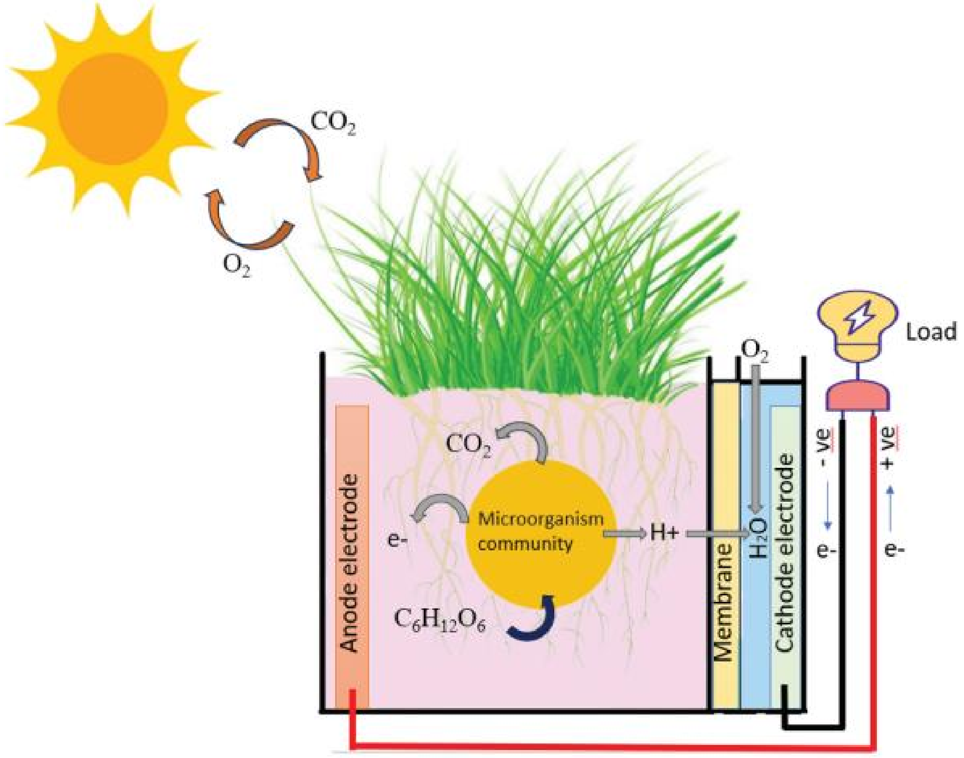


Figure 3: Schematic representation of a plant microbial fuel cell (Chong et al., 2025)

A critical factor to take into consideration is the plant's ability to generate a high amount of organic matter from its root exudates which is directly influenced by the rate of photosynthesis that should be high to accommodate such conditions (Apollon et al., 2021; Chong et al., 2025). Understanding the photosynthetic pathways of plants is therefore essential for selecting plants to be used in a PMFC system (Nitisoravut & Regmi, 2017).

Photosynthesis is the biological process by which plants synthesize food and it takes place mainly in the leaves, where the green part of plants, called chlorophyll, captures light energy (Rahman et al., 2025).

Plants suitable for PMFCs use are C3 and C4 type of plants (differ from one another in their photosynthetic pathways) which are governed by Calvin cycle. Calvin cycle is a series of chemical reactions which help the plant to convert carbon dioxide from the air into glucose during photosynthesis (Chong et al., 2025).

C3 photosynthesis is the most common pathway. A C3 type plant experience loss of some energy during photosynthesis because of photorespiration. This happens when the enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase enzyme) reacts with oxygen, which reduces the amount of carbon that can be fixed and releases some of it as carbon dioxide.

The term C3 comes from the fact that the first stable product formed in the Calvin cycle is a 3-carbon molecule called 3-phosphoglycerate (Chong et al., 2025).

C4 plants are more efficient because they convert CO_2 into a 4-carbon sugar compound, which is then moved into the stroma (the inner part of the chloroplast). There, the compound releases CO_2, which is used in the Calvin–Benson cycle to continue the photosynthesis process (Greenman et al., 2024).

C4 plants would be expected to have a higher rate of rhizo-deposits of exudates at the rhizosphere that act as substrate to fuel (Chong et al., 2025) and consequently a higher power output when integrated into a PMFC (Greenman et al., 2024).
Another important characteristics of the C4 plants is that they are adapted to fry and hot atmosphere which make them suitable for the use in a wider range of environments (Greenman et al., 2024).
C4 plant also has the highest rate of solar energy conversion and have a better yield in power generation when utilized in PMFC technologies (Chong et al., 2025).
The C_4 photosynthetic mechanism help to enhance plant productivity, and thus, engineering the C_4 cycle into C_3 crops such as rice (*Oryza sativa*) would be a promising approach (Krone et al., 2025).

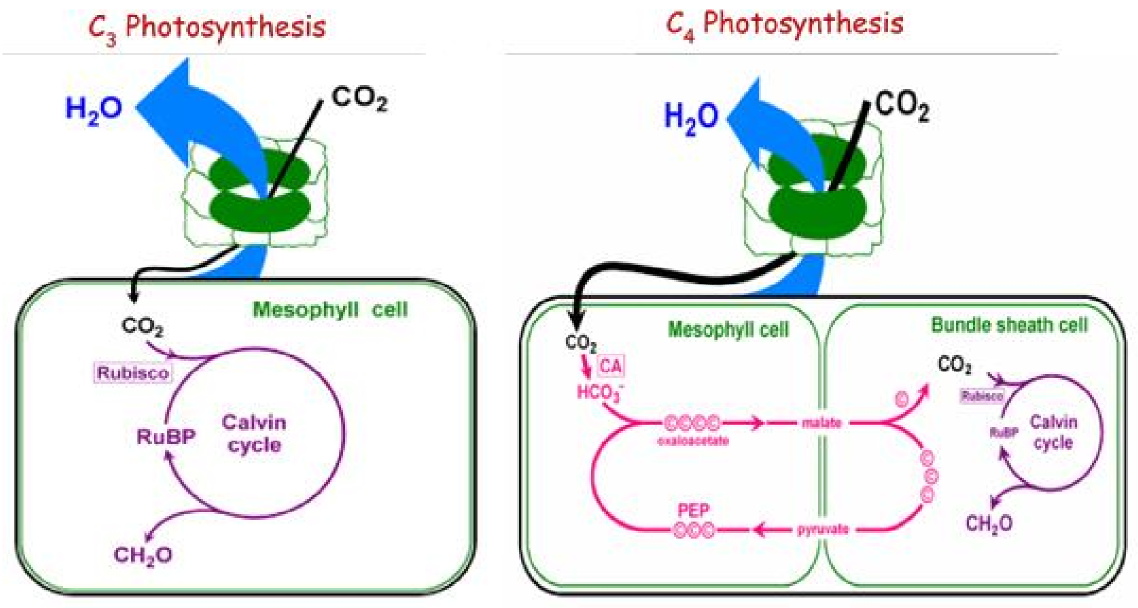


Figure 4: A schematic diagram of C3 and C4 photosynthesis (Wang et al., 2012)

1.4 - *Trichoderma*, A POSSIBLE ALTERNATIVE

An aspect of the experimentation conducted in this thesis was to evaluate the application of *Trichoderma* in PMFCs. For this reason, it is important to understand its main characteristics.

The taxonomy of the *Trichoderma* genus is complex and constantly evolving. It is a genus of fungi belonging to the Ascomycota division, the Hypocreaceae family, and the Hypocreales order (Adnan et al., 2019).
Trichoderma is a genus of mostly asexual (the teleomorphic forms are *Hypocrea*) filamentous fungi, widespread around the world and in fact it is a dominant component of the mycobiome of various soil ecosystems (such as farmland, prairie, forests, salt marshes, and deserts) in all climatic zones (Tyśkiewicz et al., 2022).

Fungi of the *Trichoderma* genus are extremely widespread in nature, thanks to their ability to adapt to very different environmental conditions and their natural competitiveness with other microorganisms. One of the characteristics that makes them particularly interesting is their ability to degrade cellulose, which is why they are easily found in environments where decomposing plant material is present (Poveda et al., 2020).

The optimal conditions for growth have been studied in different research and it has been found that they are a pH between 4.6 and 6.8 and temperatures between 25 and 30°C (Singh et al., 2014).

Trichoderma species can easily grow in any type of rhizosphere soil and can survive there for several months. The existence of *Trichoderma* in any soil makes it a dominant biocontrol agent because it suppress the population of other competitive micro-organisms (Adnan et al., 2019).

Recent research has highlighted the innovative potential of species of the genus *Trichoderma* not only in the field of biodegradation, but also in the production of sustainable electricity within PMFCs. The work of Rojas-Flores et al. (2024), highlights the fungus's ability to degrade a small amount of plastic waste, in particular 12 low-density polyethylene, thus generating electricity using potato waste as a substrate.
The integration of *Trichoderma sp.* into PMFCs therefore opens up new prospects for bioenergy and sustainable waste management.

1.5 - *Spathiphyllum lanceifolium* FOR INDOOR EXPERIMENTATION

For the indoor experiment conducted in this study *Spathiphyllum lanceifolium* was used in PMFCs. The choice of this plant was made thanks to studies, like the one from Kwon&Park (2021) that have investigated the utility of this ornamental plant as a PMFC to produce voltage and current.

The species *Spathiphyllum lanceifolium*, a perennial herbaceous plant native to the tropical areas of Central and South America, was selected for the PMFC indoor system.
The choice was motivated by a series of characteristics that make it particularly suitable.
S. lanceifolium has a dense and extensive root system, capable of ensuring a large contact surface with the rhizosphere microflora. This characteristic is fundamental as it promotes interactions between the plant roots and the electrogenic microorganisms in the substrate, which are responsible for transferring electrons to the external circuit in the PMFC.
S. lanceifolium adapts well to conditions of high soil moisture and partial submersion, characteristics frequently found in PMFC systems, which require a moist or water-saturated substrate.

Figure 5: *Spathiphyllum lanceifolium*, as a plant-microbial fuel cells (PMFCs)

1.6 - OUTDOOR PLANTS

Part of the experimentation was carried outdoor. In particular, in this case, the plants used for the experiment were *Carex remota L., Lythrum salicaria L.,* and *Allium angulosum L.*.
These plants were chosen for their botanical characteristics (root types and life form) and, above all, because they are native to the area.

PMFCs performances appears to be significantly influenced by both life forms and root architecture (Brugellis et al., 2024)so they should be taken into consideration when choosing the plants.
Root types can contribute to the bioelectrical performance of plants because it reflects the extensiveness and complexity of the root system, around which microbial community develops.
For example, plants with fibrous root systems appeared to be not recommended for PMFCs.

Selecting the appropriate plant species can significantly enhance the bioelectrical performance of the cells. Ideal criteria to take into consideration for plant selection include plant hardiness, growth rate, the microbial community at the rhizosphere, the extensiveness of the root system, adaptability, etc. (Brugellis et al., 2024; Kabutey et al., 2019; Nitisoravut & Regmi, 2017). For instance , plant species with C4 photosynthetic pathways are generally preferred in PMFCs application because they exhibit high rates of solar energy conversion and high photosynthetic efficiency, resulting in increased rhizodeposition that serves as a substrate for microbial oxidation (Brugellis et al., 2024; Kabutey et al., 2019).

According to the Raunkiær system (Oxford At The Clarendon Press, 1934), life forms categorize plants based on the location of their growth point during adverse season, which determines their ability to survive harsh conditions. Depending on the region of location, the adverse season can be the cold winter or the dry summer.

In Europe, the growth point during the unfavorable season usually corresponds to winter buds. According to Raunkiær classification of life forms they include: Epiphytes which grow attached to other living plants; Phanerophytes with the growth point in the unfavorable season at least 50 cm above ground level, often on stems; Nanophanerophytes with the growth point in the unfavorable season between approximately 25 to 50 cm above ground level; Chamaephytes with the growth point in the unfavorable seasons up to about 25 cm above ground; Hemicryptophytes with the growth point in unfavorable season at or just below the soil surface; Therophytes which survive adverse season as seeds; Cryptophytes or Geophytes with the growth point in the unfavorable season below ground level; Helophytes with the growth point with have winter buds below water while their flowering parts remain above water; Hydrophytes that are aquatic plants (Brugellis et al., 2024).

Life forms reflects the plant's life cycle and their biomass persistence over one or more years: Therophytes are annual species that survive for only few months each year; Hemicryptophytes are herbaceous perennial species that last for several years, typically maintaining vegetative parts throughout the year; Geophytes/Helophytes/Hydrophytes are herbaceous perennial species that persist for several years but generally retain vegetative parts during only part of the year; Chamaephytes are perennial herbaceous/lignified species; Nanophanerophytes and Phanerophytes are woody perennial species, both persisting for several years and maintaining green biomass during all or part of the year depending on whether they are evergreen or deciduous.

These differences in biomass growth and persistence can influence bioelectrical performance in PMFCs experiments. For example the work of Brugellis et al., 2024 focused on the botanical issues crucial for ensuring a well-functioning system, which also serves as a mirror to the functionality of the technology (Brugellis et al., 2024).

Another important point is the choice of native plants for PMFCs in outdoor applications with a view to sustainability and biodiversity conservation. If the choice falls on non-native species a risk assessment of their invasion potential must be conducted. In fact the choice of species should consider not only their electrical performance but also their implications for biodiversity to enhance the sustainability of PMFCs.

According to Perkiomen Watershed Conservancy (*Benefits of Native Plants*, s.d.) native plants are species that naturally grow in a particular region, ecosystem, or habitat without human intervention or interference.

The use of native plants can give different advantages to the environment. For example they can provide habitat resources to the local pollinators and birds as well as other wildlife. They also are more adapted to the region of cultivation and this give them more stability and resistance since they may have developed resistances to local pests (so they require little or no pesticide use) and root systems that help them to increase tolerance to drought and reduce fertilizers need (*Benefits of Native Plants*, s.d.).

Native species in fact are considered to be better adapted to the local environment, which means they grow more efficiently and require less maintenance and also to provide better habitat resources for other native species.

Positive effects of native species are consistently reported across multiple classes of animal taxa, different biodiversity measures, and scales of measurement.

If we think about using PMFCs also in urbanized spaces, it is important to take into consideration the use of native species. In fact increasing the richness, cover or density of native plants in urban green spaces has been repeatedly linked to increases in animal biodiversity.

It is likely that planting native species serves to increase overall biodiversity in urban green space (Berthon et al., 2021).

Figure 6: Pictures of the native plant species chosen for the outdoor test: *Allium angulosum (a), Lythrum salicaria (b), Carex remota L. (c)*

1.7 - POSSIBLE APPLICATIONS

PMFCs are very promising for their ability to produce electricity directly from living plants, being in this way an alternative source of renewable energy.

They can be implemented in different applications like for wastewater treatment, biosensors, bioremediation of polluted sites, to power ultra-low power remote devices, a local power supply to charge portable devices, powering camera traps in remote areas and serve as a biosensor for monitoring plant health in smart greenhouses (Chong et al., 2025; Lepikash et al., 2024).

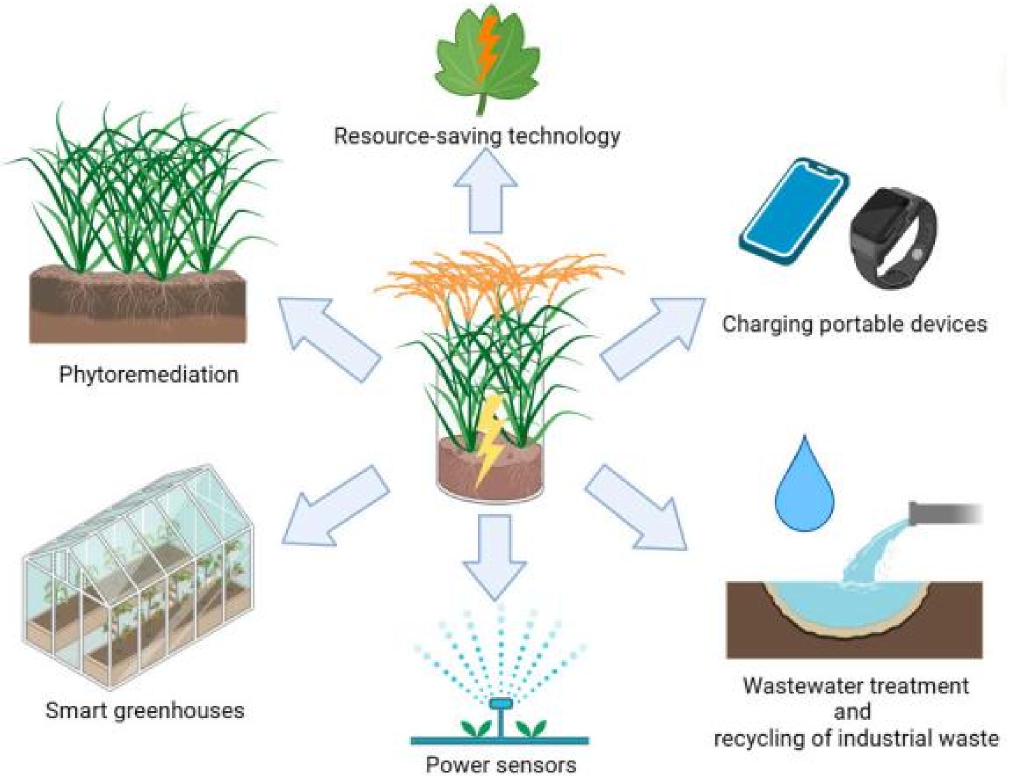


Figure 7: Possibilities of PMFC application (Lepikash et al., 2024)

PMFCs have emerged as a very promising alternative technology for powering ultra-low power remote devices, in particular sensors for various environmental monitoring and Internet of Things (IoT) applications. IoT system rely on sensors that continuously monitor and transmit information about their environment or the object’s status, enabling smart, energy-efficient operations and real-time decision-making. When deployed outdoors, such on farms, these sensors can be integrated and powered by PMFC (thanks also to their low-power needs) as its renewable energy.

The integration of PMFCs with remote devices or wireless sensor nodes offers different advantages by providing a self-powered solution (eliminating in this way the need for external power sources or frequent battery replacements) and it can offer a continuous and sustainable source of energy, ensuring uninterrupted operations of the devices.

PMFCs can function as biosensors thanks to their ability to detect variations in metabolic activities. In particular they can be used as indicators for environmental changes since this can change their ability to produce electricity. The biosensing mechanism can be divided into two categories which are: exudate-based sensing and microbial community dynamic sensing.

For the exudate-based sensing can happen that changes in the exudates of the plant (that the plant root exude) can modulate the microbial metabolic activities since the microbial communities uses them as carbon source. This can directly impact the electricity generation and so the electrical signal measured can be correlated to the environmental changes which influence the plant health and the rate of changes on exudates production.

For the microbial community dynamic sensing they can be used to detect changes in the microbial communities since this can be influenced by external analytes like pollutants that can change their composition.

With their biosensing capability PMFC can be applied for detection of environmental changes, plant health, soil water content, soil health, and pollutants (Chong et al., 2025).

PMFCs can be used for wastewater treatment applications thanks to different abilities of plants and microbes. Many plants can act as biofilter because they possess the ability to absorb and accumulate heavy metals (like lead, cadmium, and arsenic) from their environment (phytoremediation). Certain plants can also absorb and sequester radionuclides like cesium and strontium

Besides that, different microbes within the PMFC are capable of biosorption and bioaccumulation of heavy metals by sequestering or transforming metals, making them less bioavailable and harmful; for example some bacteria can convert metal ions into less toxic or even nontoxic forms.

Thanks to the use of these plants and the presence of these microbes PMFCs can be a very promising option in this sector (Chong et al., 2025).

PMFCs can be used also for bioremediation of polluted sites. In particular the plants in the PMFCs, through their root systems, enhance the degradation of pollutants by providing a favorable environment for microbial activity and pollutant uptake.

They can be used for phytoremediation of chromium, zinc and cadmium. To improve phytoremediation we should encourage plant development and stress tolerance (Chong et al., 2025).

PMFCs can be implemented in agricultural fields by using energy production via cells connected to crops such as rice to power sensors for monitoring field conditions. In particular as cited before we can use the energy produced to power sensors used in remote sensing or to monitor the environmental conditions.
Some studies have investigated the use of PMFCs in agriculture fields like for example Kouzuma et al. (2014) that have investigated the microbial electricity generation in rice paddy fields.

In a paddy-field MFCs system, graphite felt is used as an anode material and the anode is set in the rice paddy soil prior to the planting of rice. The cathode, also made of graphite felt, is placed on the water surface. The contact between rice roots and the anode is crucial because rhizosphere microbes oxidize photosynthesized organic compounds excreted from the roots generating in this way electrons that are immediately transferred to the anode. In these systems electricity generation is dependent on the photosynthetic rate of rice plants; so we have an increase during the daytime and decrease at night (Kouzuma et al., 2014).

Different plant species that are important as food cultures have been investigated in PMFC systems such as rice, tomato, bean, and maize. Also, aromatic and culinary herbs have been studied, like fenugreek and mustard greens, lemongrass, garlic chives, and sweet basil, as well as water spinach.

A promising opportunity is also the integration of these systems into vertical farming systems that has been demonstrated for garlic chives, lemongrass, and tomatoes. These approaches is increasingly being seen as the future of agriculture because it can contribute to more sustainable agricultural practices by eliminating the need for land exploitation and intensive resource use (Rusyn et al., 2025).

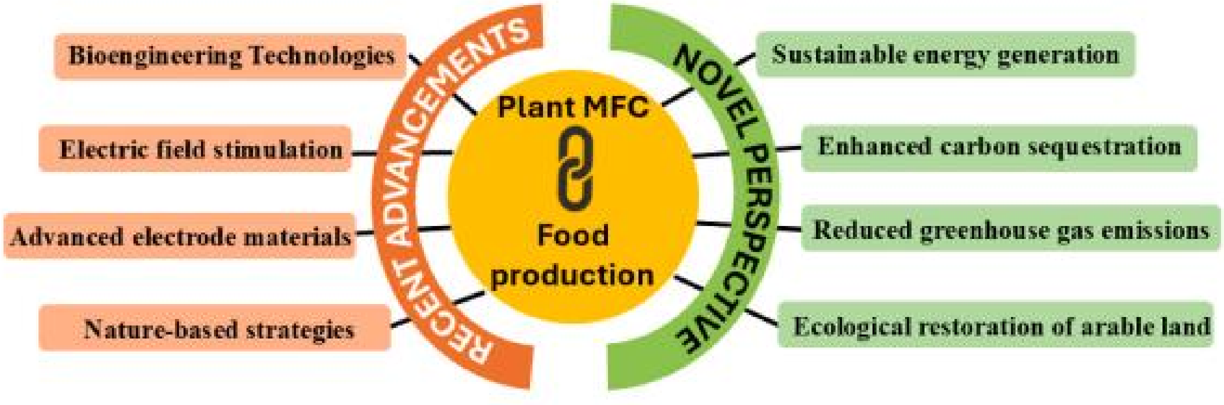


Figure 8: Advancements and novel perspectives for PMFCs (Rusyn et al., 2025)

1.8 - CHALLENGES

PMFCs seems to be a very promising sustainable source of energy but they face several challenges that should be taken into consideration. Addressing these challenges is crucial for enhancing the performance and efficiency of these systems, further research and studies are needed to optimize them and realize the full potential of PMFC technology.

These technologies nowadays are still considered by many as insufficiently powerful, however this perception is largely based on findings from laboratory studies where often they have limited materials or conditions that restrict the optimization of power output. New materials, enhanced for example with additive nanoparticles are already demonstrating significant improvements in power output performance and electrical activity which shows that these technologies are not yet fully exploited and can be improved (Greenman et al., 2024).

One of the most significant challenge faced by PMFCs is their relatively low power output compared to other renewable energy source which limits their application for powering electronic devices or larger-scale energy needs. This low power output condition of PMFCs is influenced by different factors like for example the choice of electrode materials, plant species, environmental conditions and system design. The selection of those factors directly impacts the electrochemical efficiency, cost, environmental sustainability and overall PMFCs performance.

Plant selection and growth present a critical challenges that impact the performance and efficiency of these systems since they play a crucial role in the bioelectric parameters and electricity generation of PMFCs. Also, the type of material used as substrate is important because it affects the vitality and growth of the plant which directly impacts the system.

A big challenge for the large-scale implementation of PMFCs is the cost, in fact the high electrode costs significantly limit large-scale implementation (Chong et al., 2025).
In order to address the high cost of electrode materials used in MFCs and to lower total costs, researchers are seeking more affordable alternatives such as carbon-based electrodes and biocatalysts (Chakma et al., 2025).

Another factor that can impact PMFC performance is controlling microbial species and diversity in the substrate of PMFCs. The challenge is in understanding and managing microbial diversity in PMFCs substrate due to the intricate relationships between plant diversity, substrate complexity, and microbial community (Chong et al., 2025).

Another challenge is the fact that PMFCs require a startup phase which can range from days to weeks in which the cells cannot provide power since the microbial colony need to mature biofilm. This can influence the power production and the time evolution (Doglioni et al., 2024).

Other factors to take into consideration are the environmental variables which can influence the production power. Attention should be paid con the light conditions and the irrigation period since those could increase or decrease the power generation. Regular watering in natural light, humid conditions, and sunny weather enhances photosynthesis which increase organic matter formation in plants that supports energy generation in PMFCs (Rahman et al., 2025).

More studies should be conducted in order to limit those challenges and try to make the use of PMFCs sustainable from an economic and operational point of view.

2. AIM OF THE WORK

The research group with which this thesis was carried out is focusing on the development of Plant Microbial Fuel Cells (PMFCs), with the aim of achieving energy performance levels that allow low-power electronic devices, such as environmental sensors or microLEDs, to function.

The minimum energy performance considered acceptable for powering an energy harvesting system is at least 600 mV of voltage and a current between 0.500 and 0.600 mA per cell.

What makes these systems so interesting is their flexibility of use. From a sustainable point of view the use of these types of systems in agriculture appears to be very promising.

Several studies (like the one from Rusyn et al. 2025 and Yadav et al., 2020) have demonstrated the usefulness of PMFCs and how they can be integrated into both traditional and modern agriculture (such as vertical farming). By using the electricity produced by these systems exploiting agricultural plants, it is possible to power ultra-low power remote devices, in particular sensors for various environmental monitoring and IoT applications. It is also possible to use those systems as biosensors for environmental monitoring.

The energy efficiency of PMFCs can be influenced by several factors, including the plant species used, the type of substrate, and the presence of beneficial microorganisms.

The objective of this thesis is to evaluate the effect of different configurations on the electrical performance of PMFCs. We have developed indoor and outdoor experiment.

In the indoor setup we wanted to evaluate in term of electrical performance:
- the difference between the absence and presence of the membrane in the MFC;
- the difference between PMFC inoculated and not inoculated with *Trichoderma* in the plant soil or in the MFC soil.

In the outdoor setup we wanted to evaluate in terms of electrical performance:
- the difference between PMFCs whose plants have different traits, in particular root type and life form;
- the difference between classic PMFCs with copper cable cathodes and PMFCs with gold-part cable cathodes.

3. MATERIALS AND METHODS

3.1 - INDOOR MATERIALS

In this experiment, we tested a new PMFC prototype in which the plant is detached from the MFC. The pot containing the plants is on the top, drainage water percolates to the container below, which contains the cells. By this way the plant provides nutrients to the soil bacterial colonies through the nutrient-rich leachate derived from rhizodepositions.

A key characteristic of this prototype is the separation between the rhizosphere zone and the Microbial Fuel Cell, allowing a more accurate control of bioelectrochemical processes. This is highly advantageous for the maintenance of the prototype and allows the MFCs to retain their potential thanks to the nourishment provided by the plant.

Figure 9: Novel prototype of PMFC

The basic structure of the cells is composed by the following elements: anode compartment (electrical cable wrapped in two layers of aluminum foil), separation element between the two electrodes (soil, carbon pellet and nonwoven polyethylene fabric) and cathode compartment (electrical cable wrapped in carbon felt). The cells were constructed on 18/11/2024 and 19/11/2024.

To build the cells we used 8 cm x 8 cm x 4 cm derivation box and stratificated the layers in it. The aluminum foil anodes measured 8 cm × 8 cm (surface area 64 cm^2) and were approximately 2 mm thick. The carbon felt cathodes measured 8 cm × 8 cm (surface area 64 cm^2) and were approximately half a centimeter thick. Carbon pellet layers were approximately 5 mm thick. Nonwoven polyethylene fabric was used as a membrane due to its low cost and easy availability.

Figure 10 illustrates the materials we used.

Figure 10: Cells components: derivation box, anode, non-woven fabric, carbon pellet, cathode and soil

The electrodes were made from standard 1.5 mm electrical cables, stripped and placed in a folded aluminum sheet for the anode or stapled to carbon felt for the cathode, as shown in **Figure 11**.

These materials were chosen because they are good conductors that collect the electrons produced by the electrochemical reactions.

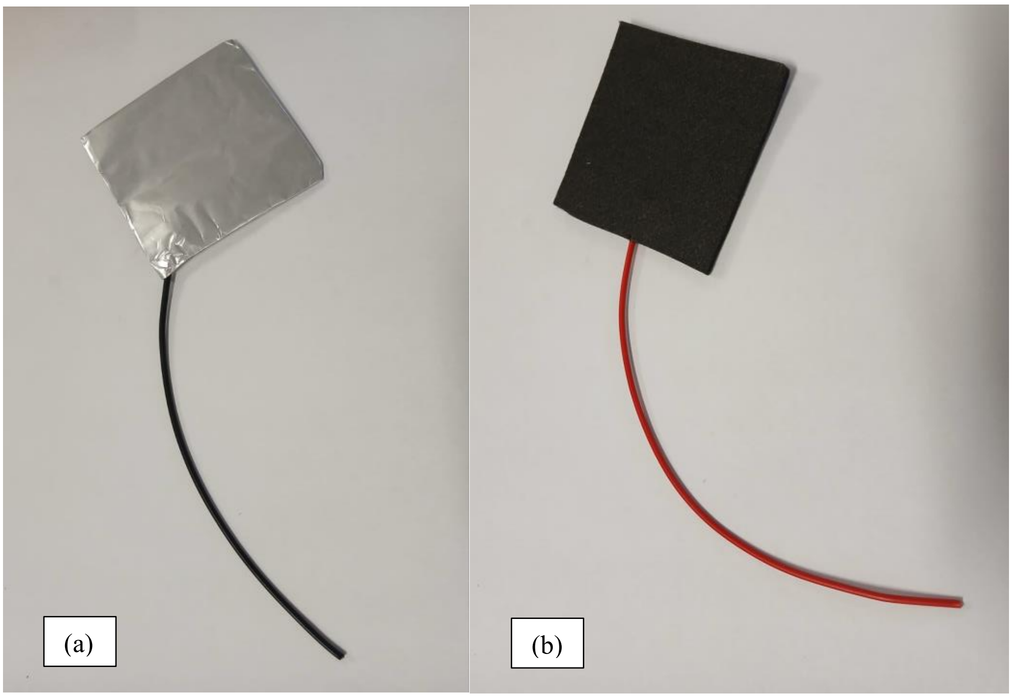


Figure 11: Electrodes used for the construction: aluminum anode (a) and carbon felt cathode (b)

The non-woven fabric is a sheet that acts as a separator between the electrodes and is made of polyethylene microfibers. It is a 100% recyclable component and is characterized by being waterproof and air permeable.

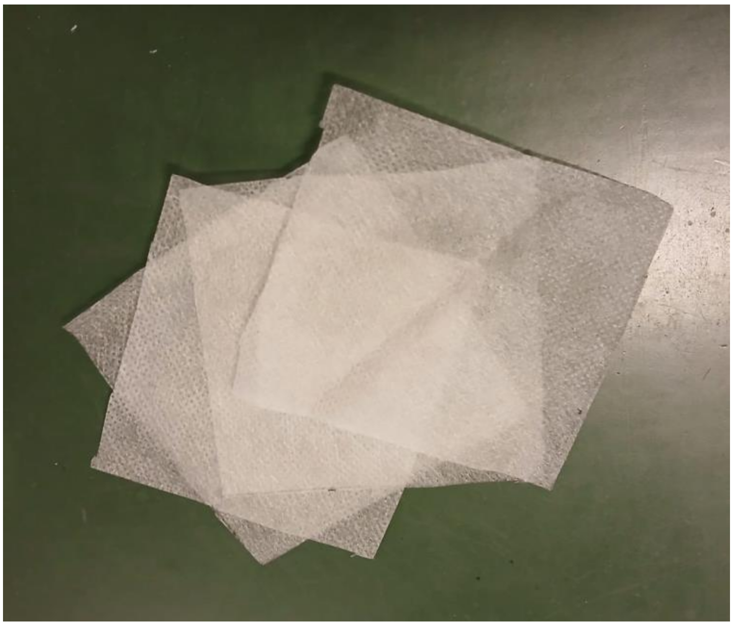


Figure 12: Nonwoven polyethylene fabric

Carbon pellet is activated carbon in 4mm granules. They are mainly used to limit emissions of most organic solvents into the atmosphere, to try to eliminate odors, volatile substances, or decomposing products.

Figure 13: Carbon pellet

In particular we have constructed 72 cells, divided in 6 sets (12 cells for each set) according to the different layering methods.

Soil Control (SC) cells were constructed using the following layers:

- 30 g C pellets
- C felt cathode
- 100 g universal potting soil
- Aluminum foil anode (Al)

Membrane Control (MC) cells were constructed using the following layers:

- 30 g C pellets
- C felt cathode
- Nonwoven polyethylene fabric
- 100 g universal potting soil
- Aluminum foil anode (Al)

All SC 1-12 and MC 1-12 cells were watered with 6 plants of *Spathiphillum lanceifolium* in trays of 4 cells each. The difference between the sets is that the MC sets contains the nonwoven polyethylene fabric as addition in the separation element.

Trichoderma Plant-Membrane (TPM) cells were constructed using the following layers:

- 30 g C pellets
- C felt cathode
- Nonwoven polyethylene fabric
- 100 g universal potting soil
- Aluminum foil anode (Al)

Trichoderma Plant-Soil (TPS) cells were constructed using the following layers:

- 30 g C pellets
- C felt cathode
- 100 g universal potting soil
- Aluminum foil anode (Al)

All TPM 1-12 and TPS 1-12 cells were watered in trays containing 4 cells per plant, with 6 plants of *Spathiphillum lanceifolium* whose soil was inoculated with *Trichoderma asperellum* suspension on 11/14/2024 at a rate of 200 ml per kg of soil, i.e., approximately 170 ml per 850 g pot. The difference between the sets is that the TPM sets contains the nonwoven polyethylene fabric as addition in the separation element.

Trichoderma Cell-Soil (TCS) cells were constructed using the following layers:

- 30 g C pellets
- C felt cathode
- 150 g soil inoculated with Trichoderma suspension
- Aluminum foil anode (Al)

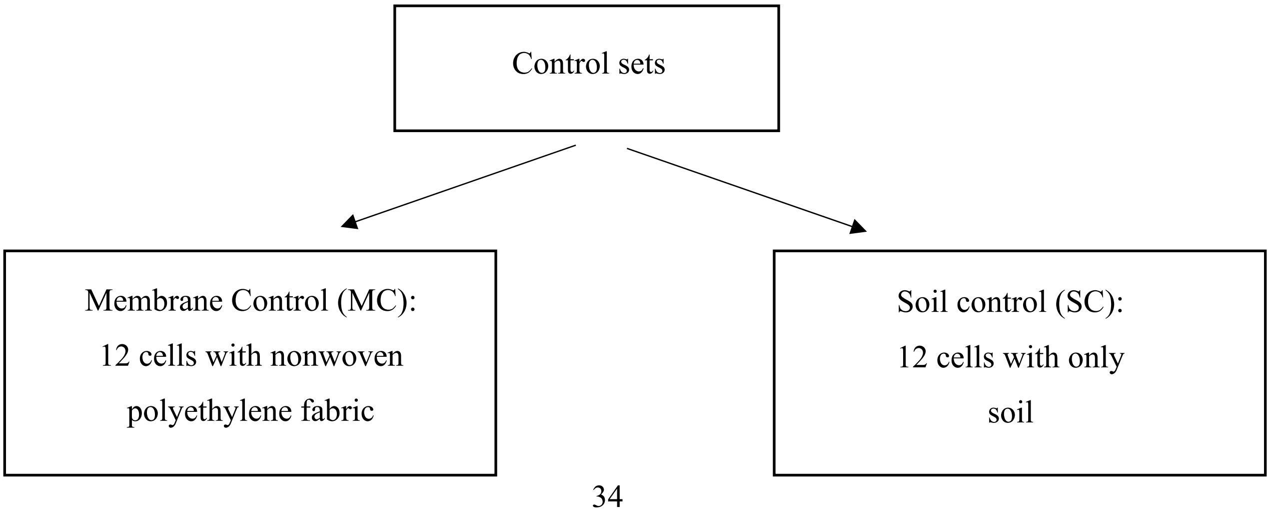
Trichoderma Cell-Membrane (TCM) cells were constructed using the following layers:

- 30 g C pellets
- C felt cathode
- Nonwoven polyethylene fabric
- 150 g soil inoculated with Trichoderma suspension
- Aluminum foil anode (Al)

All TCM 1-12 and TCS 1-12 cells are watered in 4-cell trays per plant with 6 plants of *Spathiphillum lanceifolium*. The soil present in the cells (universal potting soil) was inoculated with *Trichoderma asperellum* suspension on 11/11/2024 at a rate of 200 ml per kg of soil. The difference between the sets is that the TCM sets contains the nonwoven polyethylene fabric as addition in the separation element.

Figure 14: Trays containing the constructed cells

In summary:



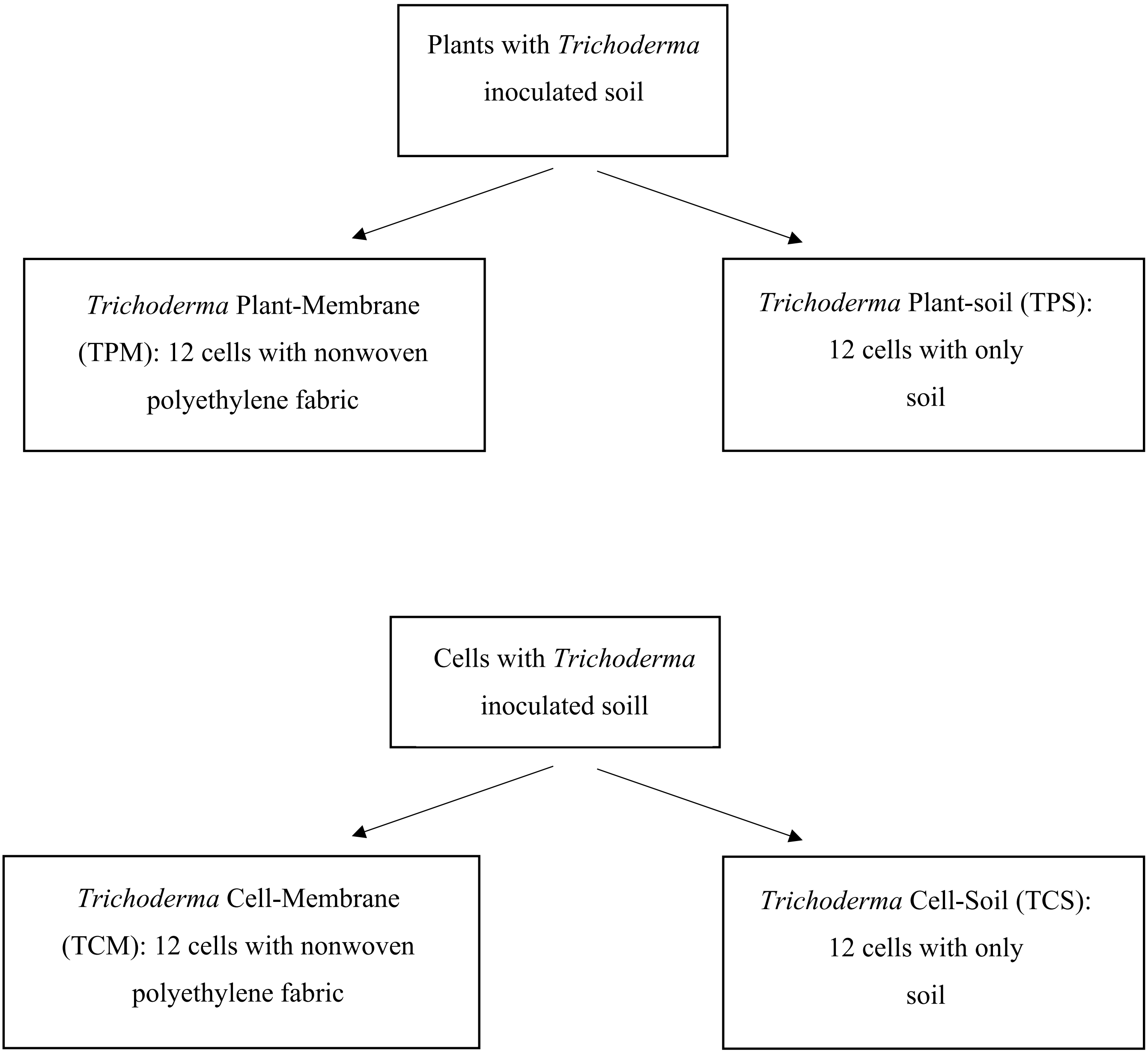


Figure 15: Schematic diagram of the laboratory setup

From an environmental point of view, *Spathiphillum lanceifolium* plants were grown under controlled conditions, with exposure to indirect natural or artificial light. Direct light was avoided to prevent damage to leaf tissue. Irrigation was carried out regularly, keeping the substrate constantly moist but without prolonged waterlogging; the average frequency was once a week. The plants were kept within an optimal temperature range of 20–27°C.

Figure 16: PMFCs with *Spathiphyllum lanceifolium*

3.2 - INDOOR DATA COLLECTION AND STATISTICAL ANALYSIS

The electrical output measurements of the PMFCs were taken using a Keithley 2000 digital multimeter (**Figure 17**). In particular, the following parameters were collected: open circuit voltage (mV), short circuit peak current, and short circuit current after 5 s (mA).

This monitoring was carried out from November 20, 2024, to April 24, 2025, once a week. The objective was to monitor the energy output of each PMFC over time, evaluating electrical performance.

The Power figure of merit generated was calculated using the following formula:

Power (mW) = Voltage (mV) × Peak current (mA).

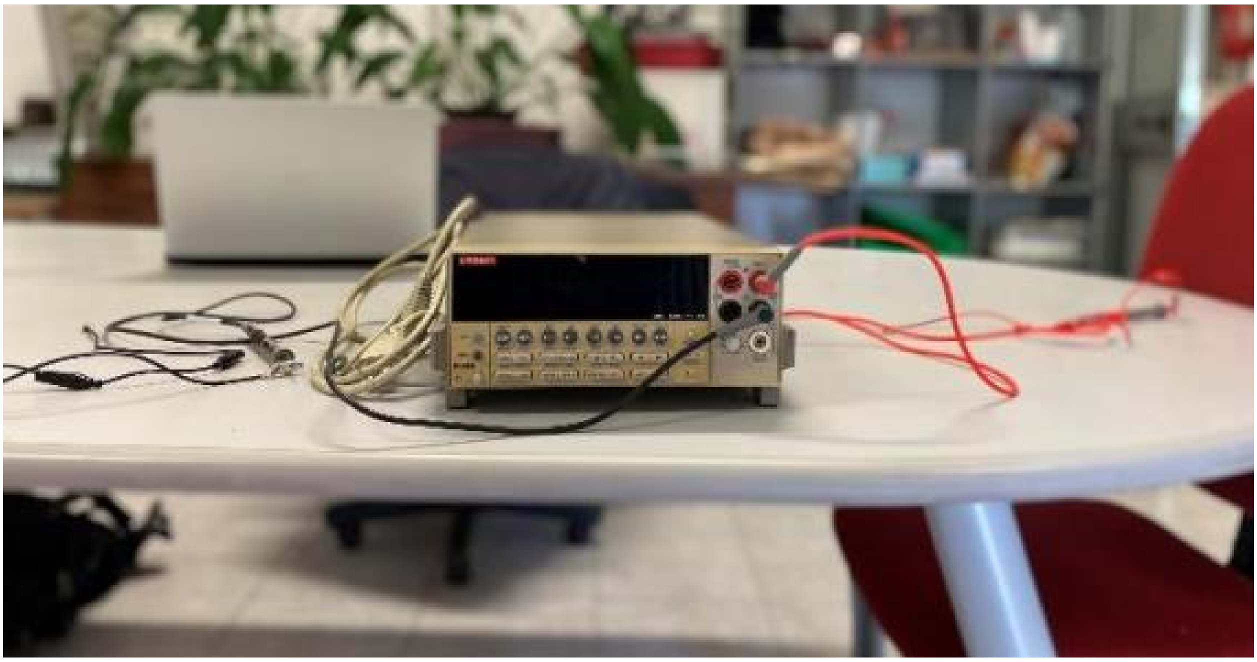


Figure 17: Keithley 2000 digital multimeter

The data collected were initially recorded in a paper notebook and then transcribed into an Excel spreadsheet, where the averages of the values for each parameter collected for each cell during the experimental period were calculated.

The distributions of the averages obtained were imported into PAST 4.09 software in order to perform detailed statistical analyses.

The first step was to determine whether the distribution was normal or non-normal. In the case of a normal distribution, a one-way ANOVA test was performed, followed by Tukey's post-hoc test, to identify significant differences between the groups.

In the case of a non-normal distribution, the non-parametric Kruskal-Wallis test was used, followed by Dunn's post-hoc test.

Finally, statistically significant differences between the different PMFCs were also visualized using graphs generated directly by the PAST software, thus facilitating the interpretation of the results.

3.3 - OUTDOOR MATERIALS

In this part of the experiment the PMFC were located in Cascina Cassinino- Sommo- Pavia.
To evaluate the best plant species for outdoor application, 36 MFCs were wetted with percolate from plants having different traits. The choice of the species was based on the results of previous literature analysis, native species were selected in line with a biodiversity prospective.
The plants used for the experiment are: *Carex remota L.* , *Lythrum salicaria L.* and *Allium angulosum L.*.

N° of cells	Plant species	Life form	Root type
12	*Allium angulosum L.*	Geophyte	Bulbous
12	*Lythrum salicaria L.*	Hemicryptophyte	Fasciculated
12	*Carex remota L.*	Hemicryptophyte	Adventitious

Table 1: Experimental design and plant traits corresponding to each plant species

The cells were constructed with the following layering (from the bottom): aluminum anode, all-purpose soil, carbon felt cathode, and carbon pellets.

Figure 18: The 48 PMFCs of the Outdoor experimentation

To test the improvements in durability and resistance to oxidation, 12 cells were constructed using a gold terminal at the end of the cathode cable (the section inserted into the carbon felt).
The set was wet with percolate from *Allium angulosum L.* in order to compare it with the classical copper cathodes set.

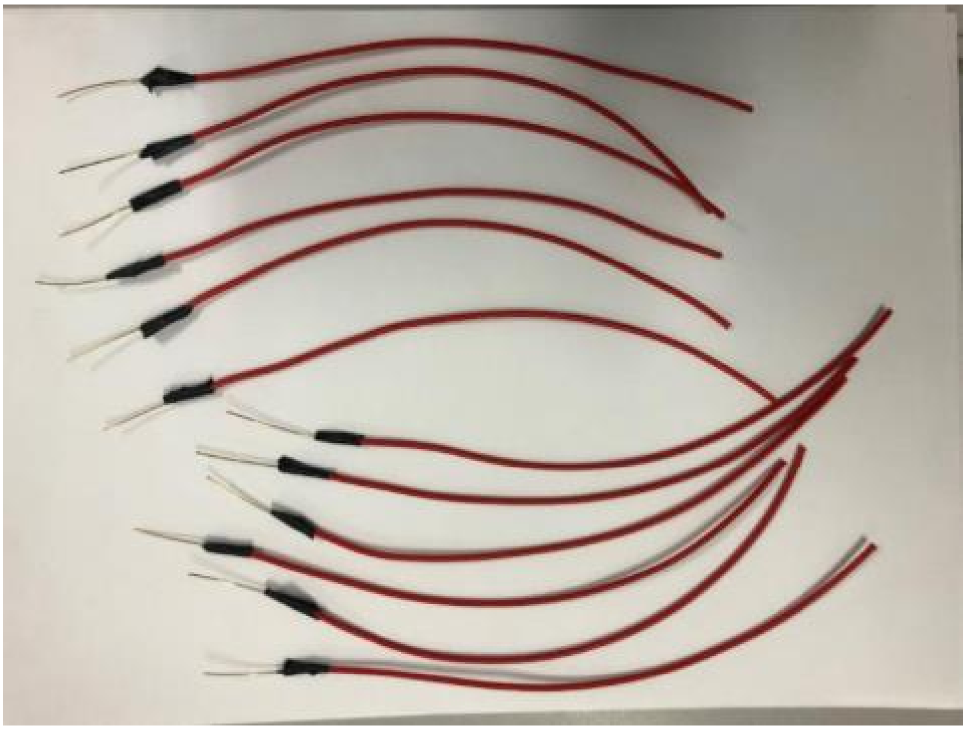


Figure 19: Cathodes constructed with gold wire ends

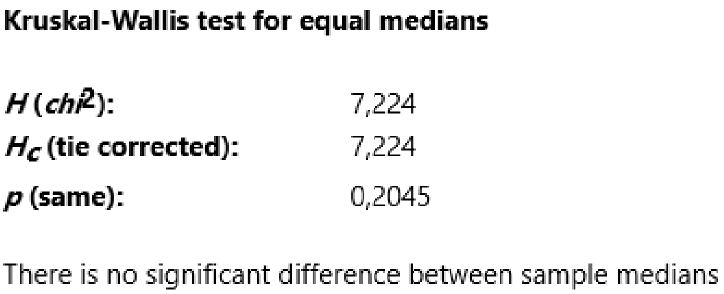
3.4 - OUTDOOR DATA COLLECTION AND STATISTICAL ANALYSIS

The electrical output measurements of the PMFCs were taken using AMPROBE 37XR-A. The means of the distributions of voltage, peak current, current after 5 seconds, and power were calculated for each MFC of each experimental setup. To compare two samples, the t-test was performed when the distributions were normal, and the Mann-Whitney nonparametric test for equal medians was used when the distributions were non-normal. To compare multiple samples, the ANOVA one-way for equal means was performed when the distributions were normal, and Kruskal-Wallis non-parametric test for equal medians followed by Dunn’s post-hoc test with raw values was performed when the distributions were non-normal.
All analyses were conducted using Past 4.09 software.

4. RESULTS

4.1 - INDOOR

For the voltage parameter the sets have a non-normal distribution. The non-parametric Kruskal-Wallis test is therefore performed, which does not return significance (**Figure 20**).



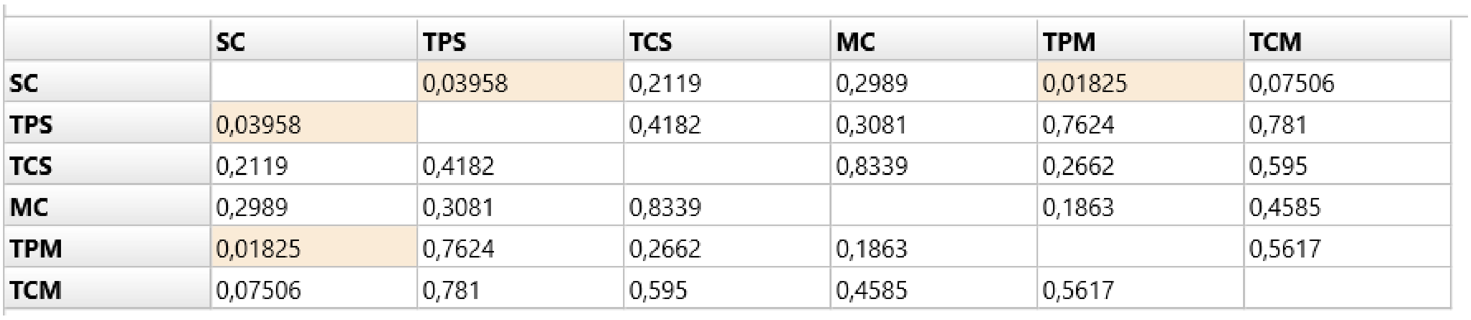
Kruskal-Wallis test for equal medians

H (chi^2): 7,224
H_c (tie corrected): 7,224
p (same): 0,2045

There is no significant difference between sample medians

Figure 20: Kruskal-Wallis test results for the indoor voltage parameter

The subsequent Dunn's post hoc test with raw p-values returns a significant difference between the SC set and the TPS and TPM sets (**Figure 21**).



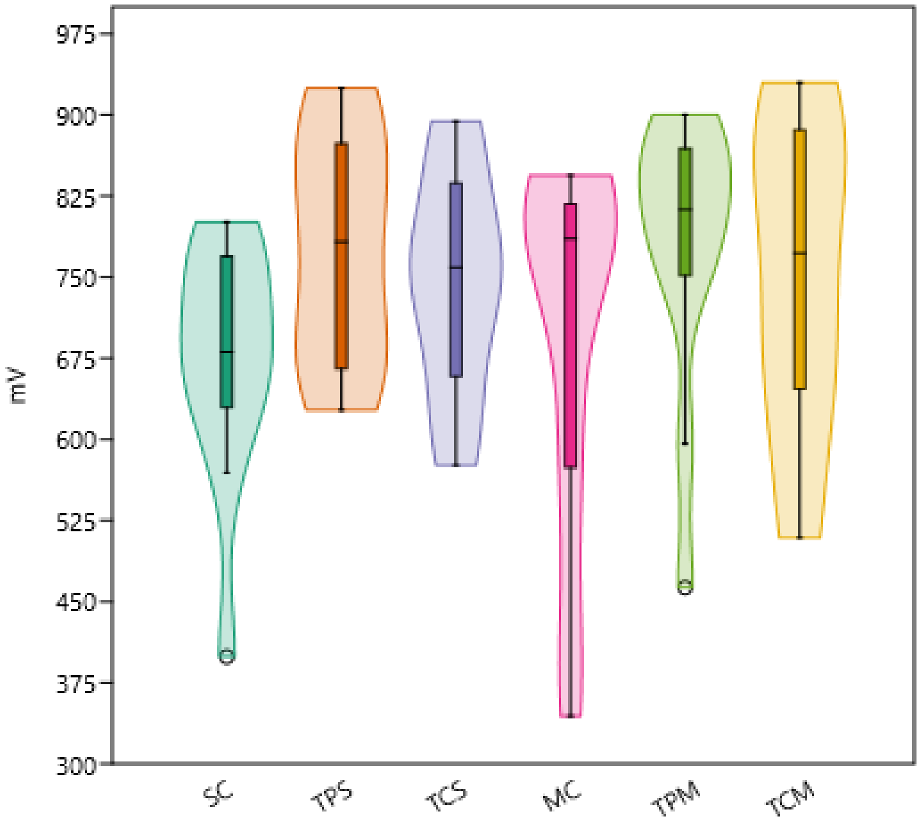
	SC	**TPS**	**TCS**	**MC**	**TPM**	**TCM**
SC		0,03958	0,2119	0,2989	0,01825	0,07506
TPS	0,03958		0,4182	0,3081	0,7624	0,781
TCS	0,2119	0,4182		0,8339	0,2662	0,595
MC	0,2989	0,3081	0,8339		0,1863	0,4585
TPM	0,01825	0,7624	0,2662	0,1863		0,5617
TCM	0,07506	0,781	0,595	0,4585	0,5617	

Figure 21: Dunn's post hoc test results for the indoor voltage parameter

SC: Soil Control; TPS: *Trichoderma* Plant Soil; TCS: *Trichoderma* Cell Soil

MC: *Membrane* Control; TPM: *Trichoderma* Plant Membrane; TCM: *Trichoderma* Cell Membrane

The violin and box plots show the distributions of the mean voltage values (mV) for each group (**Figure 22**).

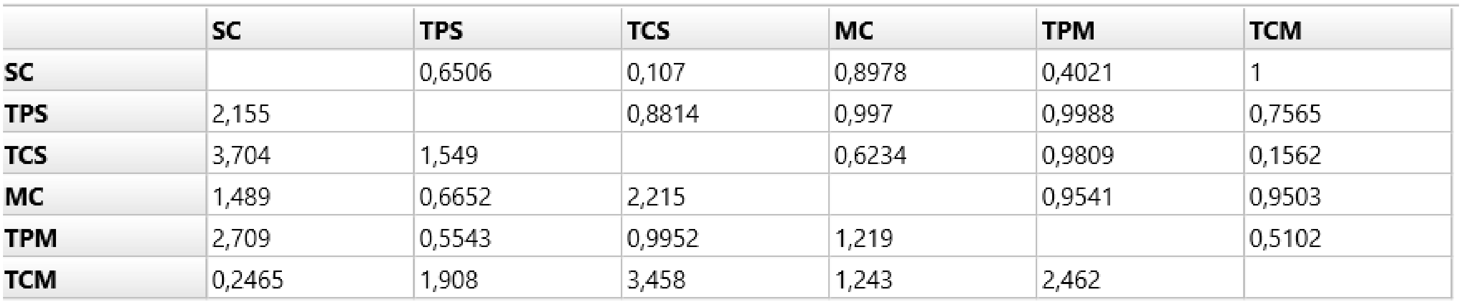


SC	Soil Control
TPS	*Trichoderma* Plant Soil
TCS	*Trichoderma* Cell Soil
MC	Membrane Control
TPM	*Trichoderma* Plant Membrane
TCM	*Trichoderma* Cell Membrane

Figure 22: Violin and box plots for the voltage indoor parameter

For the peak current parameter all sets have normal value distributions. ANOVA is performed, which returns no significant difference (p value = 0.08196).

Tuckey's pairwise test shows no significant differences (**Figure 23**).



	SC	TPS	TCS	MC	TPM	TCM
SC		0,6506	0,107	0,8978	0,4021	1
TPS	2,155		0,8814	0,997	0,9988	0,7565
TCS	3,704	1,549		0,6234	0,9809	0,1562
MC	1,489	0,6652	2,215		0,9541	0,9503
TPM	2,709	0,5543	0,9952	1,219		0,5102
TCM	0,2465	1,908	3,458	1,243	2,462	

Figure 23: Tuckey's pairwise test results for the indoor peak current parameter

SC: Soil Control; TPS: *Trichoderma* Plant Soil; TCS: *Trichoderma* Cell Soil

MC: *Membrane* Control; TPM: *Trichoderma* Plant Membrane; TCM: *Trichoderma* Cell Membrane

The violin and box plots show the distributions of the mean peak current values (mA) for each group (**Figure 24**).

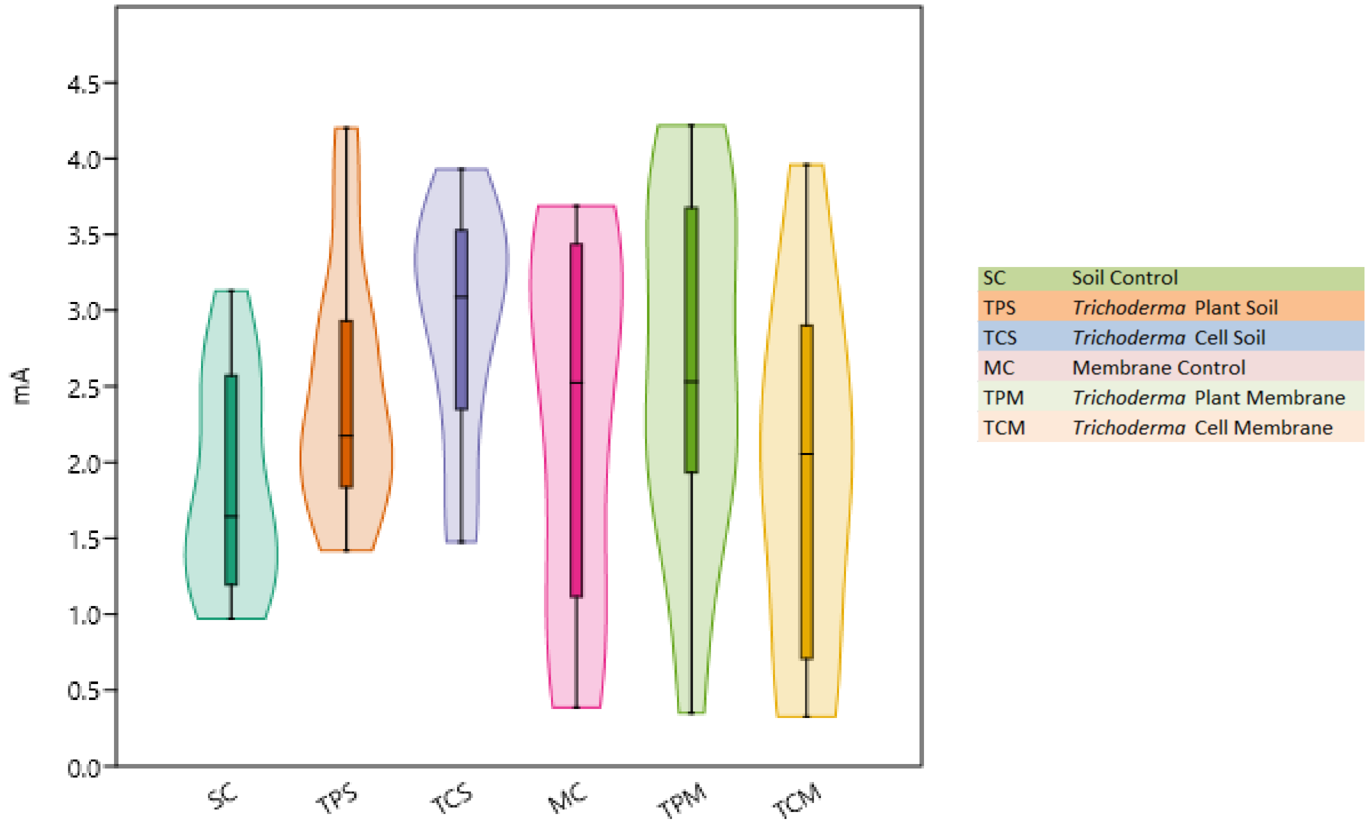
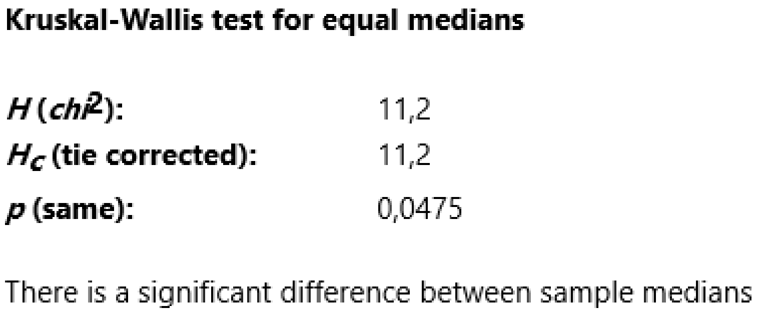


Figure 24: Violin and box plots for the indoor peak current parameter

For the current after five seconds parameter the TCS set (*Trichoderma* inoculated in the cell) has a non-normal distribution. The non-parametric Kruskal-Wallis test is performed, which returns a significant difference between the samples (**Figure 25**).



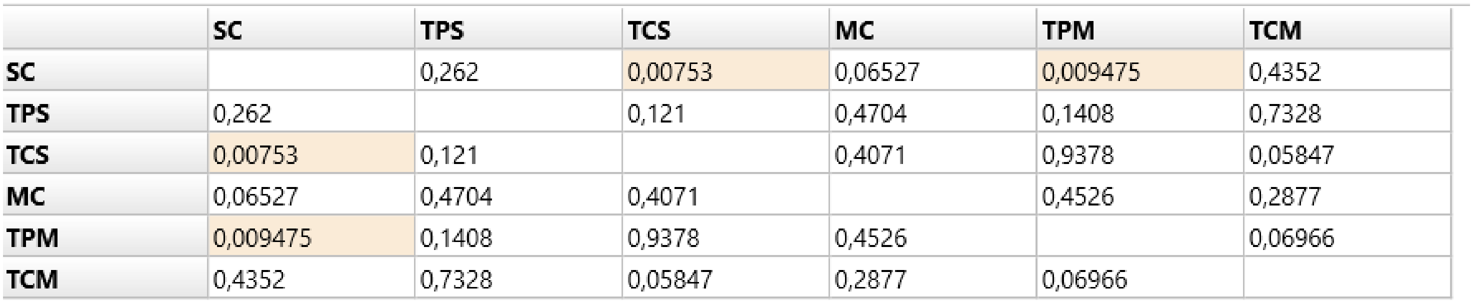
Kruskal-Wallis test for equal medians

H (chi^2):	11,2
H_c (tie corrected):	11,2
p (same):	0,0475

There is a significant difference between sample medians

Figure 25: Kruskal-Wallis test results for the indoor current after five seconds parameter

Dunn's post hoc test with raw p-values shows a significant difference between the SC set and the TCS and TPM sets (**Figure 26**).



	SC	**TPS**	**TCS**	**MC**	**TPM**	**TCM**
SC		0,262	0,00753	0,06527	0,009475	0,4352
TPS	0,262		0,121	0,4704	0,1408	0,7328
TCS	0,00753	0,121		0,4071	0,9378	0,05847
MC	0,06527	0,4704	0,4071		0,4526	0,2877
TPM	0,009475	0,1408	0,9378	0,4526		0,06966
TCM	0,4352	0,7328	0,05847	0,2877	0,06966	

Figure 26: Dunn's post hoc test results for the indoor current after five seconds parameter

SC: Soil Control; TPS: *Trichoderma* Plant Soil; TCS: *Trichoderma* Cell Soil

MC: *Membrane* Control; TPM: *Trichoderma* Plant Membrane; TCM: *Trichoderma* Cell Membrane

The violin and box plots show the distributions of the mean current after 5 second values (mA) for each group (**Figure 27**).

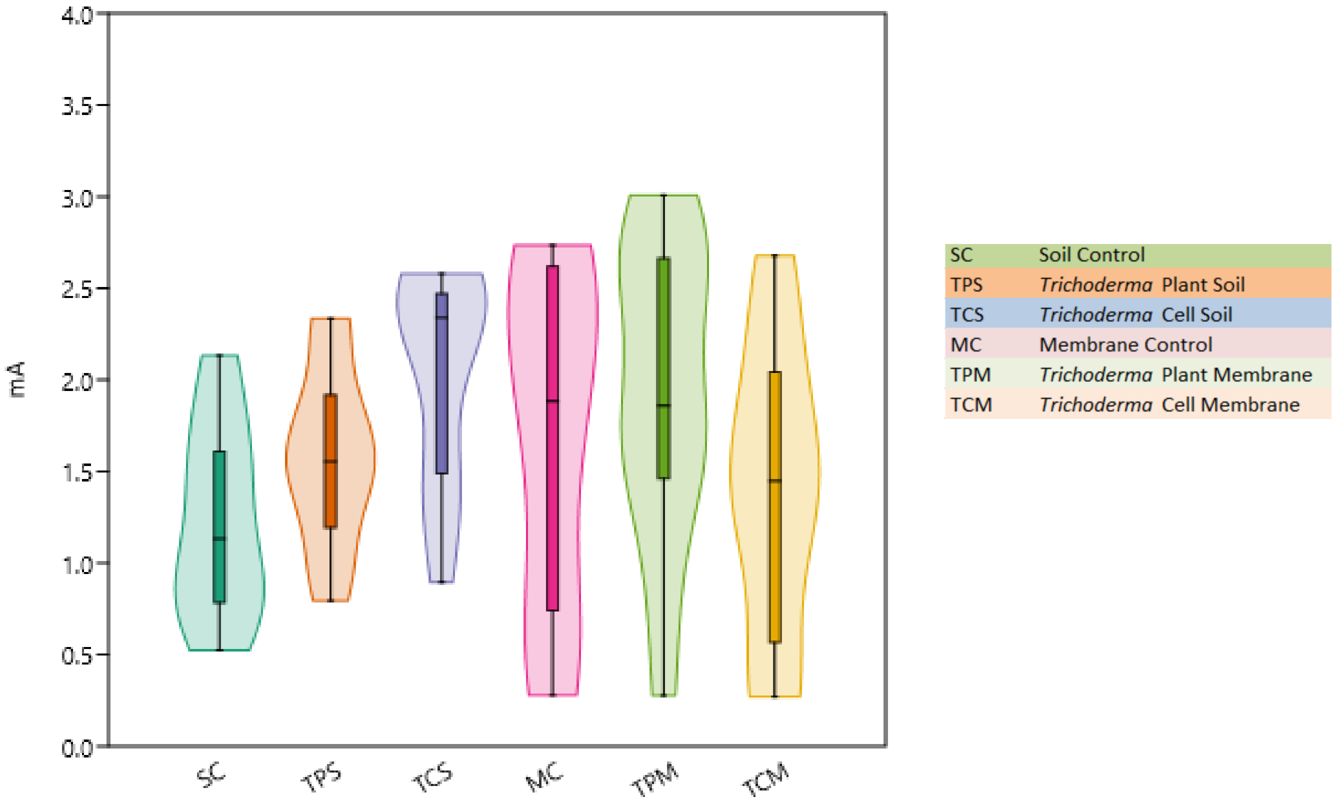
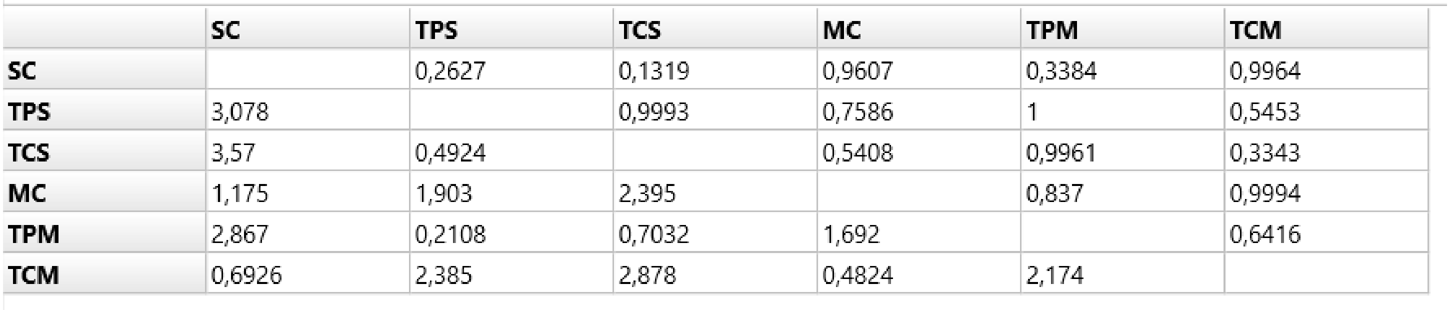


Figure 27: Violin and box plots for the indoor current after five seconds parameter

For the power parameter all sets have normal value distributions. ANOVA is performed, which returns no significant difference (p value= 0.07131).

Tuckey's pairwise test shows no significant differences (**Figure 28).**



	SC	TPS	TCS	MC	TPM	TCM
SC		0,2627	0,1319	0,9607	0,3384	0,9964
TPS	3,078		0,9993	0,7586	1	0,5453
TCS	3,57	0,4924		0,5408	0,9961	0,3343
MC	1,175	1,903	2,395		0,837	0,9994
TPM	2,867	0,2108	0,7032	1,692		0,6416
TCM	0,6926	2,385	2,878	0,4824	2,174	

Figure 28: Tuckey's pairwise test results for the indoor power parameter

SC: Soil Control; TPS: *Trichoderma* Plant Soil; TCS: *Trichoderma* Cell Soil

MC: *Membrane* Control; TPM: *Trichoderma* Plant Membrane; TCM: *Trichoderma* Cell Membrane

The violin and box plots show the distributions of the mean power values (mW) for each group (**Figure 29**).

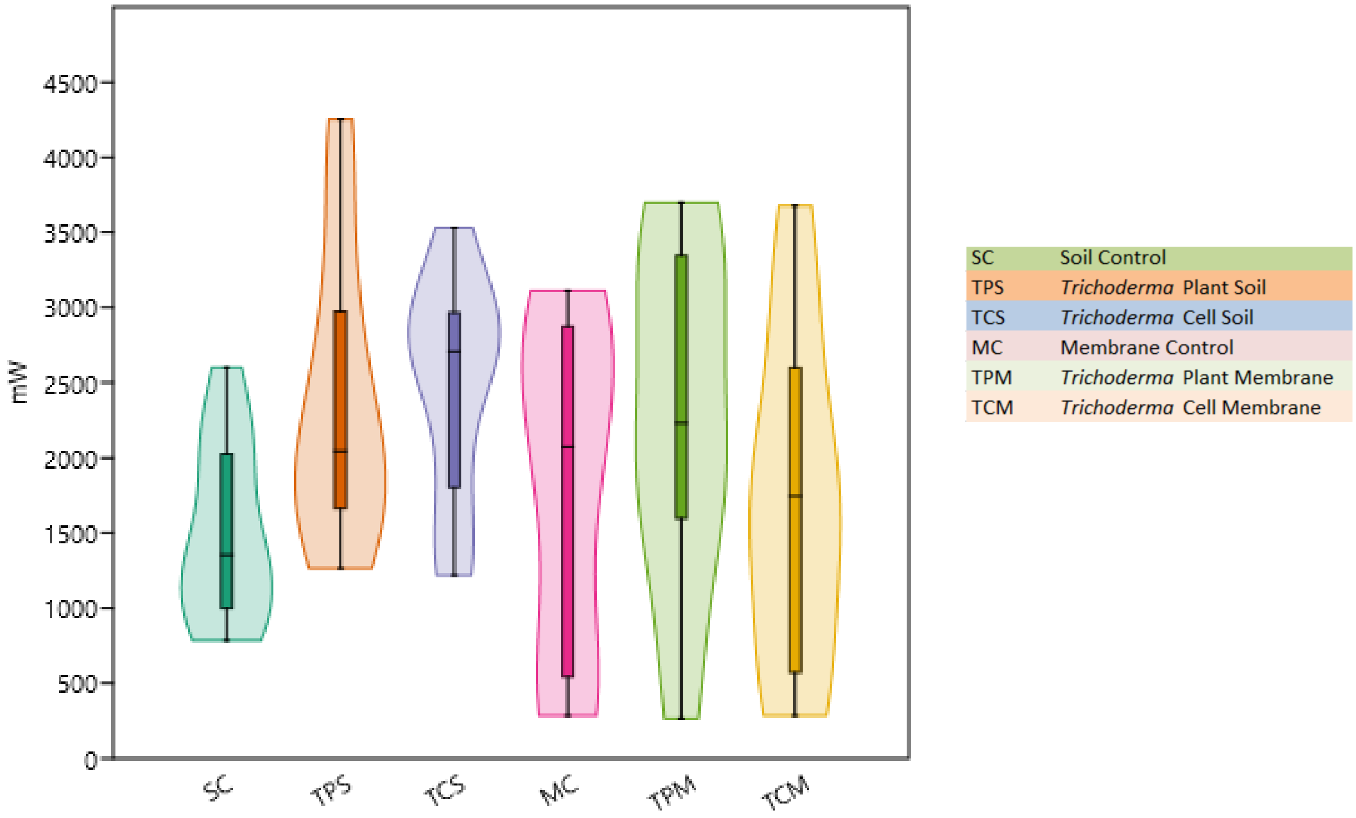


Figure 29: Violin and box plots for the indoor power parameter

		Maximum	Minimum	Median
Voltage (mv)	SC	801	399	681
	TPS	925	627	783
	TCS	894	576	759
	MC	844	344	786
	TPM	900	463	812
	TCM	930	509	772
Peak Current (mA)	SC	3.129	0.972	1.643
	TPS	4.199	1.419	2.175
	TCS	3.931	1.476	3.088
	MC	3.688	0.385	2.524
	TPM	4.219	0.351	2.531
	TCM	3.959	0.325	2.056
Current after 5s (mA)	SC	2.132	0.524	1.133
	TPS	2.334	0.794	1.554
	TCS	2.579	0.894	2.339
	MC	2.735	0.278	1.883
	TPM	3.007	0.277	1.859
	TCM	2.677	0.269	1.447
Power	SC	2600.751	784.505	1352.958
	TPS	4252.145	1263.257	2044.605
	TCS	3531.297	1217.391	2706.130
	MC	3112.342	286.920	2073.269
	TPM	3698.499	265.267	2230.489
	TCM	3678.138	285.427	1746.116

Table 2: Data of maximum, minimum and median collected for each set

SC: Soil Control; TPS: *Trichoderma* Plant Soil; TCS: *Trichoderma* Cell Soil

MC: *Membrane* Control; TPM: *Trichoderma* Plant Membrane; TCM: *Trichoderma* Cell Membrane

In **Table 2** there are the data of maximum, minimum and median measured for each set.

VOLTAGE: For the voltage parameter, the PMFCs treated with *Trichoderma* produced higher voltages than the control. In particular, treatment with *Trichoderma* in the cell and with the presence of a membrane (TCM) showed the absolute maximum value. In general, the lowest values were always given by the two control sets (SC and MC). Among these, the control with membrane (MC) has higher values. The highest values were achieved by the sets with *Trichoderma* in the plant (TPM and TPS) compared to those where *Trichoderma* is present in the cell.

PEAK CURRENT: For the peak current parameter, treatment with *Trichoderma* in the cell showed the highest median, while PMFCs with treatment in the plant reached the highest maximum value. Both are higher than the control.
The two sets with *Trichoderma* treatment in the plant (TPS and TPM) reached the highest maximum values, but even in this case, the set with the membrane (TPM) performed better.

CURRENT AFTER 5S: For the parameter current after 5 seconds, we have higher values in the PMFCs with *Trichoderma* treatments, especially in the case of the treatment in the cell, which shows a median value almost double than the control one. The highest maximum value was achieved by the set with membrane and *Trichoderma* in the plant (TPM), while the highest median value was achieved by the set without membrane and with *Trichoderma* in the cell (TCS).
The control set with membrane (MC) achieved higher values than the sets without membrane.
POWER: The power generated follows the same trend observed in the previous parameters. Both *Trichoderma* treatments produce higher power than control. The treatment in the plant shows the absolute maximum value, while the treatment in the cell shows the highest median value.

4.2 - OUTDOOR

4.2.1 - TESTS AMONG PLANT SPECIES WITH DIFFERENT TRAITS

For the voltage parameter the distributions of mean values were normal. ANOVA indicates no significant difference between sample means [*p* (same) = 0.4663].

The violin and box plots show the distributions of the mean voltage values (mV) for each group (**Figure 30**).

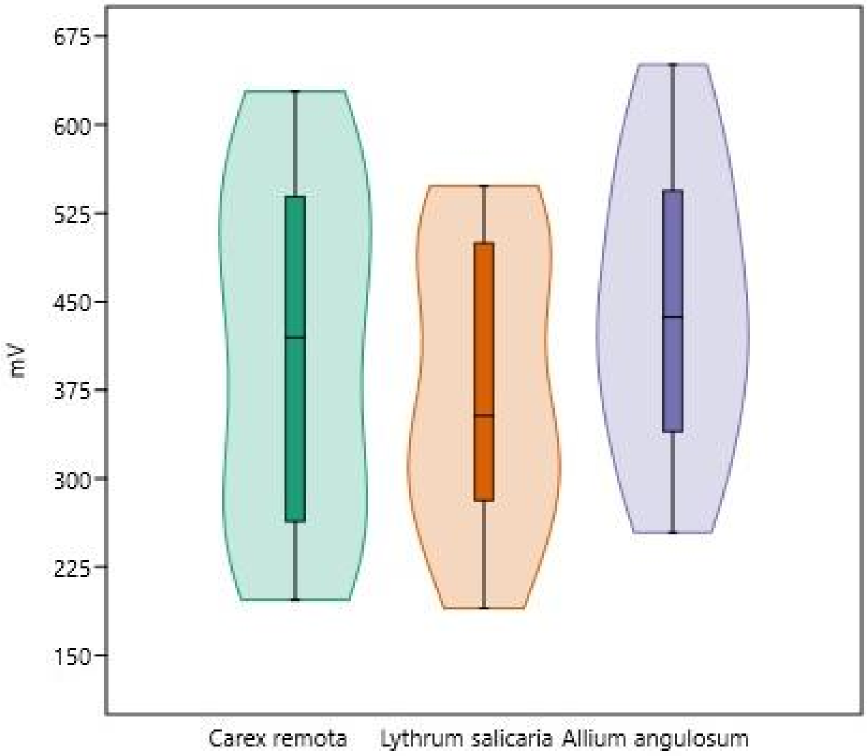
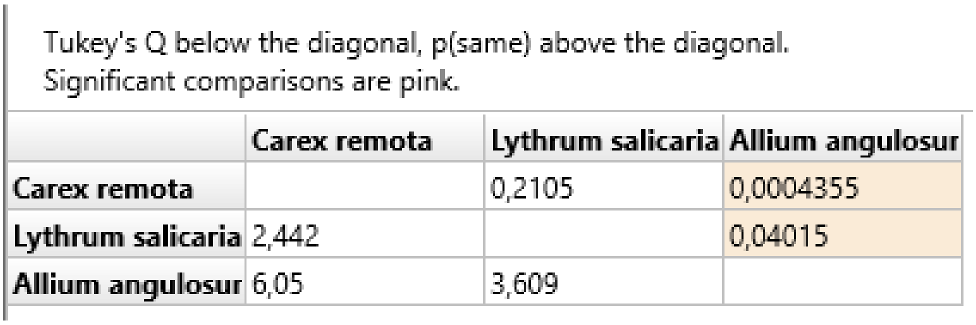


Figure 30: Violin and box plots for the outdoor voltage parameters

For the peak current parameter the distributions of mean values were normal. ANOVA indicates significant differences between sample means [*p* (same) = 0,00096]. Tuckey's pairwise test shows *Allium angulosum* set being significantly different from the other two species sets (**Figure 31**).



Tukey's Q below the diagonal, p(same) above the diagonal.
Significant comparisons are pink.

	Carex remota	Lythrum salicaria	Allium angulosur
Carex remota		0,2105	0,0004355
Lythrum salicaria	2,442		0,04015
Allium angulosur	6,05	3,609	

Figure 31: Tuckey's pairwise test results for the outdoor peak current parameter

The violin and box plots show the distributions of the mean peak current values (mA) for each group (**Figure 32**).

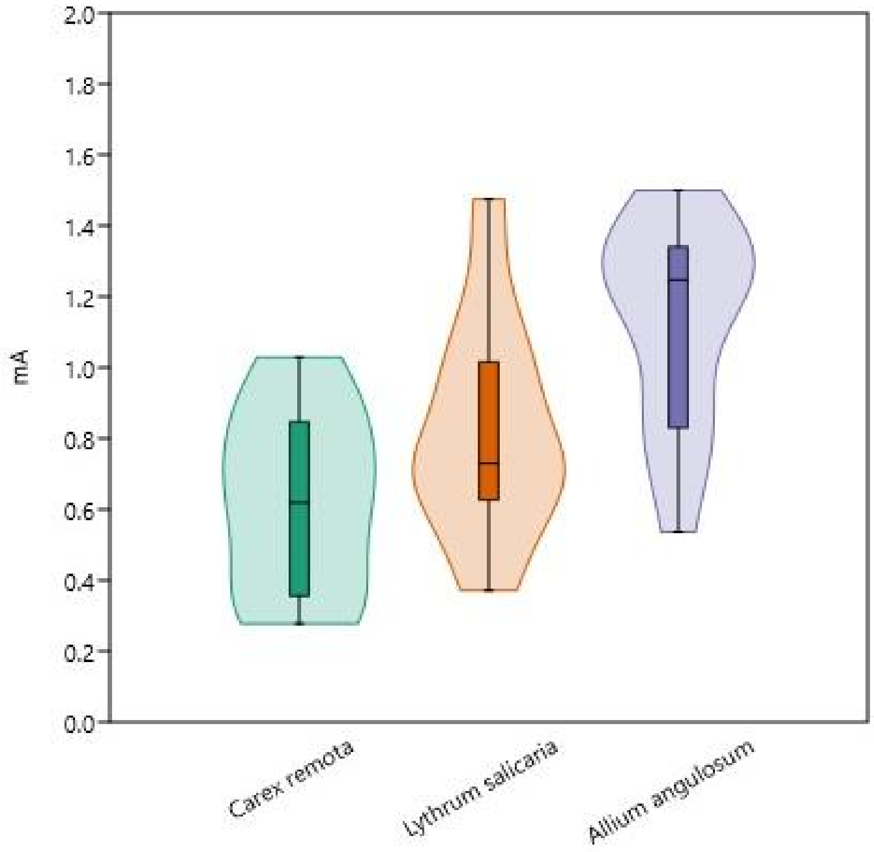
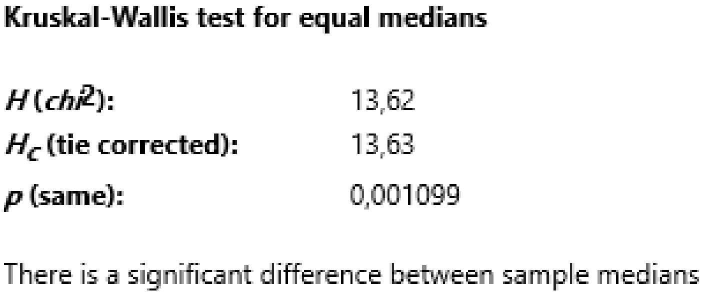


Figure 32: Violin and box plots for the outdoor peak current parameter

For the current after five seconds parameter the distribution of mean values of MFCs wetted with percolate of *Allium angulosum* were not normal. Kruskal-Wallis test indicates significant differences between sample medians (**Figure 33**). Dunn’s post hoc shows *Allium angulosum* set being significantly different from *Carex remota* set (**Figure 34**).

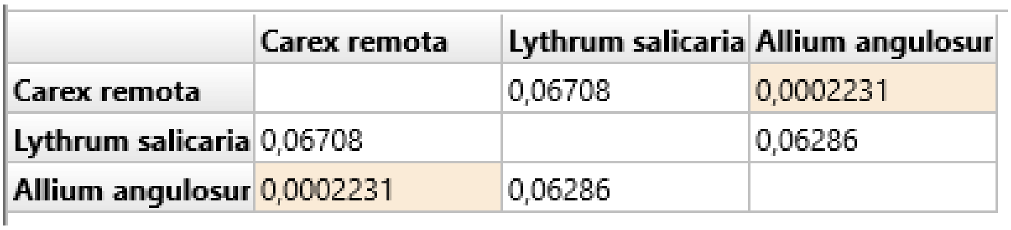


Kruskal-Wallis test for equal medians

H (*chi2*): 13,62
H_c (tie corrected): 13,63
p (same): 0,001099

There is a significant difference between sample medians

Figure 33: Kruskal-Wallis test results for the outdoor current after five seconds parameter



	Carex remota	Lythrum salicaria	Allium angulosur
Carex remota		0,06708	0,0002231
Lythrum salicaria	0,06708		0,06286
Allium angulosur	0,0002231	0,06286	

Figure 34: Tuckey’s pairwise test results for the outdoor current after five seconds parameter

The violin and box plots show the distributions of the mean current after 5 second values (mA) for each group (**Figure 35**).

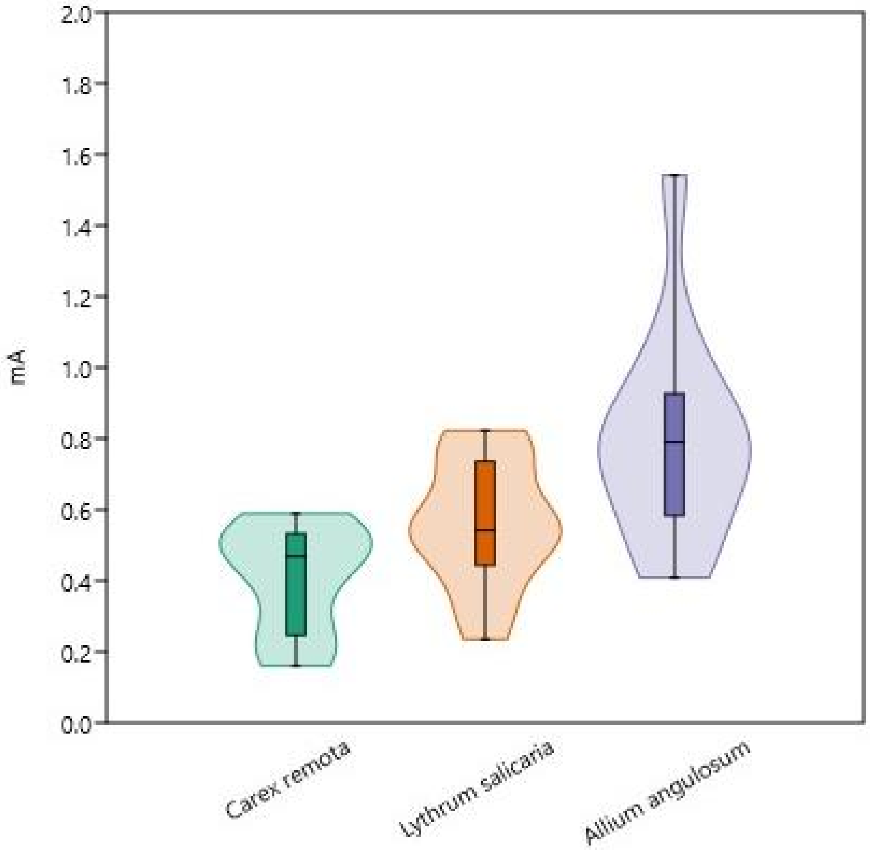
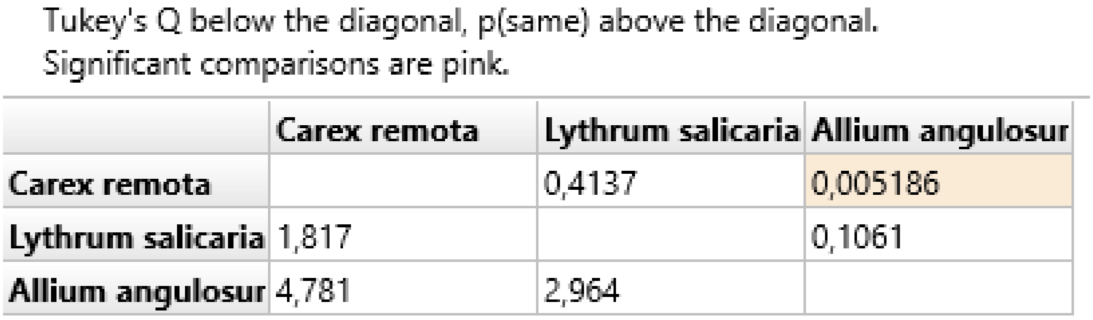


Figure 35: Violin and box plots for the outdoor current after five seconds parameter

For the power parameter the distribution of mean values were normal. ANOVA indicates significant differences between sample means [*p* (same) = 0,00832]. Tuckey's pairwise test shows *Allium angulosum* set being significantly different from the *Carex remota* set (**Figure 36**).



Tukey's Q below the diagonal, p(same) above the diagonal.
Significant comparisons are pink.

	Carex remota	Lythrum salicaria	Allium angulosur
Carex remota		0,4137	0,005186
Lythrum salicaria	1,817		0,1061
Allium angulosur	4,781	2,964	

Figure 36: Tuckey's pairwise test results for the outdoor power parameter

The violin and box plots show the distributions of the mean power values (mW) for each group (**Figure 37**).

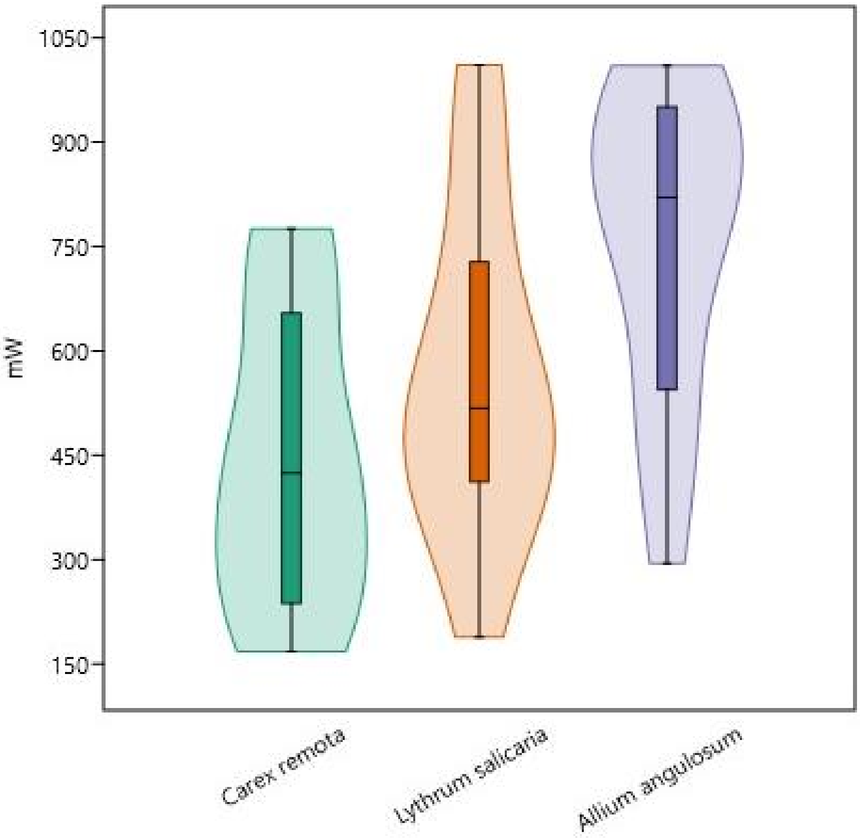


Figure 37: Violin and box plots for the outdoor power parameter

		Maximum	Minimum	Median
Voltage (mV)	*Carex remota*	628	197	420
	Lythrum salicaria	548	190	353
	Allium angulosum	651	254	437
Peak Current (mA)	*Carex remota*	1.029	0.278	0.619
	Lythrum salicaria	1.475	0.371	0.729
	Allium angulosum	1.500	0.536	1.247
Current after 5s (mA)	*Carex remota*	0.589	0.160	0.468
	Lythrum salicaria	0.823	0.235	0.542
	Allium angulosum	1.542	0.408	0.790
Power	*Carex remota*	775.223	167.797	424.228
	Lythrum salicaria	1010.401	188.661	517.446
	Allium angulosum	1009.756	294.213	820.507

Table 3: Data of maximum, minimum and median collected for each set

In **Table 3** there are the data of maximum, minimum and median measured for each set.

VOLTAGE: For the voltage parameter, the PMFCs with *Allium angulosum* show higher values compared to the other two plants. *Carex remota* and *Allium angulosum* have similar values even if *Allium angulosum* is a little bit higher as regards the maximum and the median while for the minimum they have the same value.

PEAK CURRENT: For the peak current parameter, the PMFCs with *Allium angulosum* show higher values. In this case *Allium angulosum* and *Lythrum salicaria* have similar maximum values while the lowest values are from *Carex remota.*

CURRENT AFTER 5s: For the current after 5s parameter, the PMFCs with *Allium angulosum* show higher values. In this case the values are not similar to the other plants in fact the values of *Allium angulosum* are significantly higher than the others.

POWER: For the power parameter, the PMFCs with *Lythrum salicaria* show a higher maximum value, even if it is very similar to the one from *Allium angulosum.* For the median the highest value is reached by *Allium angulosum. Carex remota* shows the lowest values.

4.2.2 - TEST AMONG CELLS WITH GOLD AGAINST CLASSICAL COPPER CATHODE CABLES

For the voltage parameter the distributions of mean values were normal. T-test indicates significant evidence for unequal means [*p* (same) = 0,0393].

The violin and box plots show the distributions of the mean voltage values (mV) for each group (**Figure 38**).

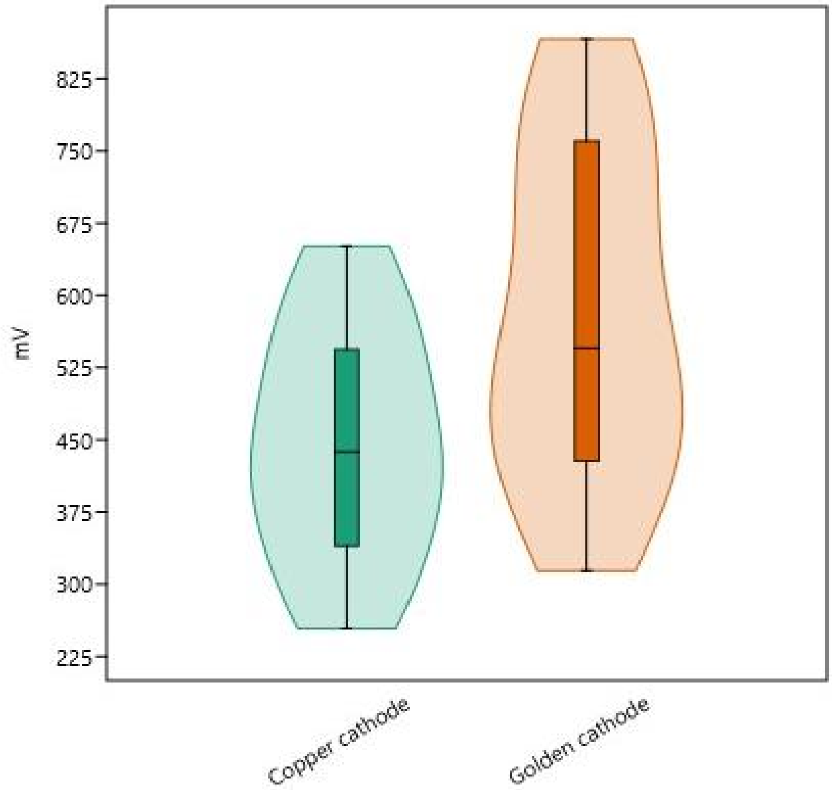


Figure 38: Violin and box plots for the cathode voltage parameter

For the peak current parameter the distributions of means values were normal. T-test indicates significant evidence for unequal means [*p* (same) = 0,0194].

The violin and box plots show the distributions of the mean peak current values (mA) for each group (**Figure 39**).

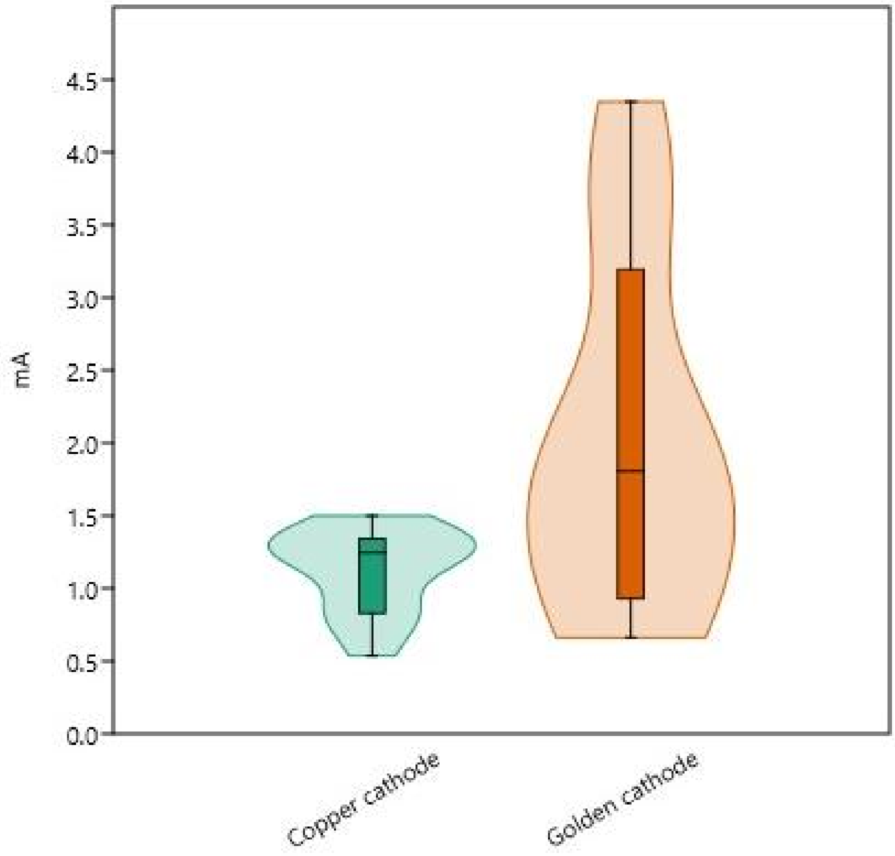


Figure 39: Violin and box plots for the cathode peak current parameter

For the current after five seconds parameter the distribution of mean values of copper cathode MFCs was not normal. Mann-Whitney test indicates no significant difference between sample medians [*p* (same) = 0,1503].

The violin and box plots show the distributions of the mean current after 5 second values (mA) for each group (**Figure 40**).

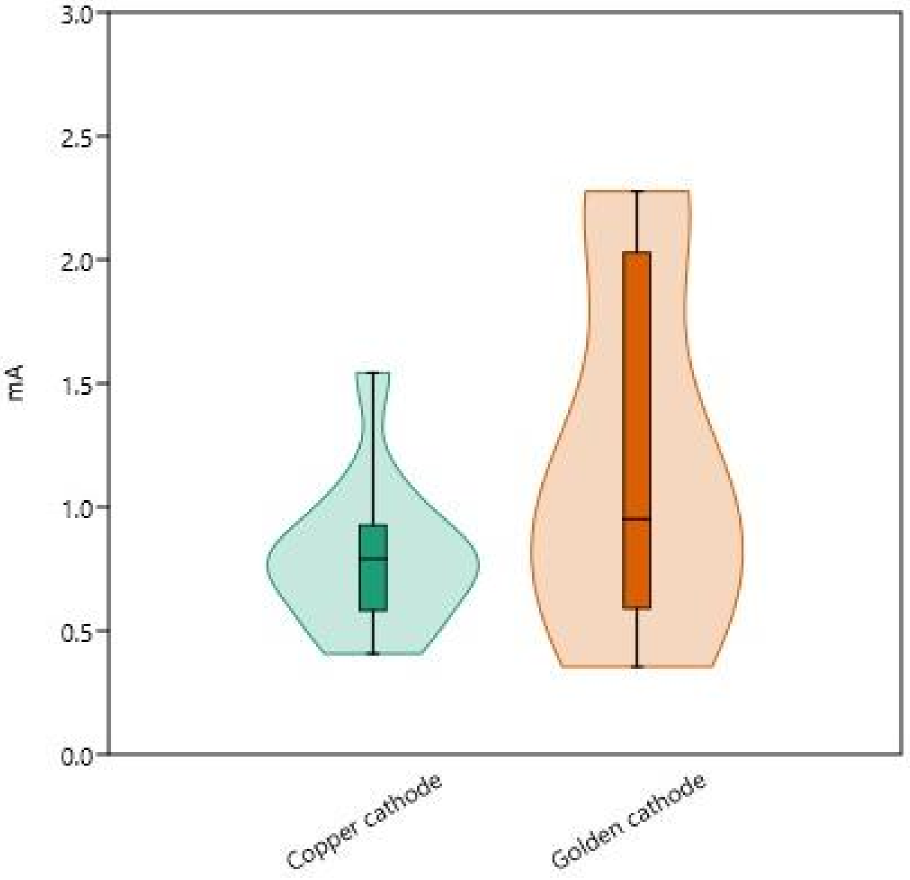


Figure 40: Violin and box plots for the cathode current after five seconds parameter

For the power parameter the distribution of mean values of the “Golden cathode” set was not normal. Mann-Whitney test indicates a significant difference between sample medians [*p* (same)=0,0481].

The violin and box plots show the distributions of the mean power values (mW) for each group (**Figure 41**).

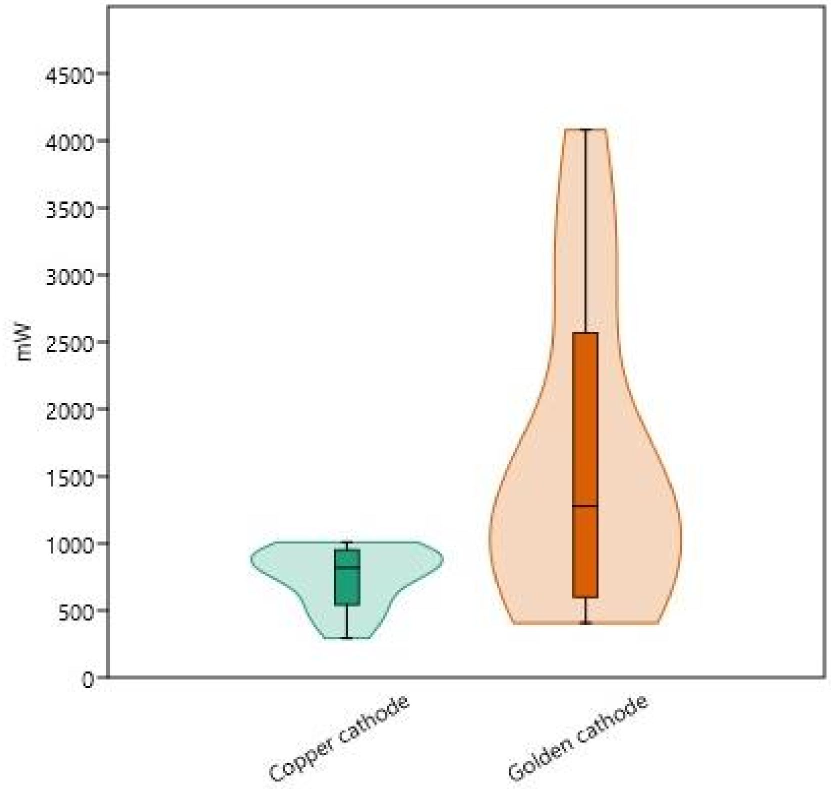


Figure 41: Violin and box plots for the cathode power parameter

		Maximum	Minimum	Median
Voltage (mv)	Copper cathode	651	254	437
	Golden cathode	866	314	545
Peak Current (mA)	Copper cathode	1.500	0.536	1.247
	Golden cathode	4.349	0.661	1.807
Current after 5s (mA)	Copper cathode	1.542	0.408	0.790
	Golden cathode	2.276	0.354	0.952
Power	Copper cathode	1009.756	294.213	820.507
	Golden cathode	4082.036	406.835	1279.119

Table 4: Data of maximum, minimum and median collected for each set

In **Table 4** there are the data of maximum, minimum and median measured for each set.

For all the parameters the highest values are reached by the golden cathode that shows to have a trend of higher efficiency.

5. DISCUSSIONS

Indoor experimentations demonstrated that electrical performances of PMFCs can be affected by different configurations.

Significant differences in voltage among the Soil Control set, *Trichoderma* Plant Soil set and *Trichoderma* Plant Membrane set seem to suggest that the interaction between *Trichoderma* and the plant could promote greater electricity production (despite the ones where *Trichoderma* is in the cells). Moreover, *Trichoderma* Plant Membrane set seems to have a more stable performance (due to its highest median value if compared with the other sets).

Significant differences in current after 5 second parameter among Soil Control, *Trichoderma* Cell Soil and *Trichoderma* Plant Membrane sets, seem to confirm the above mentioned consideration. *Trichoderma* seems to enhance the performance of the cells, when inoculated both in the plant and in the cell.

Due to the low values of Soil Control set for all parameters, the realized experiments have proved that the presence of *Trichoderma* and the membrane affect positively the electrical activity of the cells.

However, when the Control sets, with and without membranes, are considered separately, no significant results were obtained, but higher values for the membrane set were observed. This shows how the presence of a membrane may influence the capacity of the cells.

Outdoor experimentations demonstrated that electrical performances of PMFCs can be affected by different species and used materials.

Regarding plant species, the results seem to indicate *Allium angulosum* as the best performing species (among those tested).

Regarding the cathode materials, the results seem to indicate the golden cathode having a trend of higher efficiency, if compared with the copper cathode.

When every single set is taken into consideration it can be observed how cells of the same set have different trends of values. In fact, discrepancies were found in the activity of cells in the same set, which may be due to several factors like differences among the cells.

6. CONCLUSIONS

During the thesis experiment, in the indoor setup we tested PMFCs with the addition of *Trichoderma* and with or without a membrane to verify whether these could affect the efficiency of the cells. For the outdoor setup we tested PMFCs with plants that have different traits, in particular root type and life form and the difference between classic PMFCs with copper cable cathodes and PMFCs with gold-part cable cathodes.

For the indoor setup the results showed that the use of *Trichoderma* can be a great advantage in increasing electrical activity so it can be used as an additive to improve performance.

Another interesting result is about the membrane. In fact, traditionally PMFCs are systems in which the membrane is not present as the soil represents the proton exchange substrate (Greenman et al., 2024; Regmi et al., 2018). The prototype developed in our study has a configuration in which the plant is detached from the cell, unlike in classic PMFCs. This allowed us to test the effectiveness or otherwise of the presence of the membrane within the cell stratification. They seem to indicate higher values for all parameters, although there is no statistical significance. Future studies on its influence are necessary to better evaluate its use.

As for outdoor performance, among the plants analyzed, *Allium angulosum* performed best. This may be due to its botanical characteristics described in the thesis, such as its root system and life form.
In fact, PMFCs performances appeared to be significantly influenced by both life forms and root architecture (Brugellis et al., 2024). The choice of species to use in PMFCs (especially for outdoor use) should be based also on the implications for biodiversity. In fact, biodiversity is a crucial component of sustainability and if we use invasive alien species in outdoor applications, their sustainability is compromised (Brugellis et al., 2024).

In terms of outdoor performance with different cables, cells with gold wiring performed better. This data is very interesting because, outdoors, rapid cathode oxidation is a problem and maintenance is difficult. Therefore, the use of different materials can help improve cell performance and activity.

For the discrepancies among the performances of cells (in a same set), several factors may have influenced the activity.
During the construction of the cells, we tried to maintain homogeneity in order to standardize every step. In fact, the same grams of soil and pellets were used in the construction method, and we tried to use cables of the same length. First and foremost, the capacity of the cells is influenced by the

biological variability between the soil samples and the domestic water used for the cells. In fact, some of this variability could be a result of the random distribution of electrogenic bacteria present in wastewater inoculum (Leicester et al., 2023). Maybe, some differences in bacterial proportions could be due to the use of universal potting mix as soil in the cells.
Another factor that may have influenced the results is electrode oxidation. Differences may have arisen during the construction of the cells due to the different lengths of the cables, variations in the size of the anode and cathode (a matter of millimeters), and during the cable stripping process, which was done manually. This means that the cables may have been exposed to varying degrees of oxidation.

All the data obtained represents an excellent future prospect for energy production using different PMFCs setups. However, further research is and will be necessary to fully understand the mechanisms of these systems that regulate these complex interactions, with the aim of making them usable with even greater awareness and precision.
In particular, their use in agriculture is very promising for the development of more efficient and sustainable bioelectrochemical systems powering biosensors and remote devices useful for field monitoring. This would allow farmers to have a self-sufficient service without having to rely on outer services. Several studies (particularly, (Rusyn et al., 2025)and Yadav et al., 2020) have demonstrated the usefulness of PMFCs and how they can be integrated into both traditional and modern agriculture (such as vertical farming).

Despites the challenges of integrating those systems at large scale production, more studies should be realized in order to limit those challenges and try to make the use of PMFCs sustainable in an economic and operational point of view.

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