

**Lab Book of Group 5 for InnoBioDiv POL 2022/23**

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21.11.22 – 13.02.23

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## 1. Introduction of concept Anna, Anita, Suvendu

Due to a faster growing world, increasing energy demand can be seen over the last few decades. It is expected that this rising demand will continue and might even go faster than it was before. More than half of all electricity is currently generated from the combustion of fossil solid and liquid fuels in particular coal, oil or natural gas. The share of fossil fuels in total electricity generation has declined over the past 50 years and is expected to continue to decline over the coming decades. The usage fossil fuel entails some main threats for the world:

- Easily accessible fossil fuels are being depleted
- They are unevenly distributed over the world, leading to dependence of several countries on sometimes politically unstable regions
- The combustion of fossil fuels is accompanied by adverse environmental impacts due to the emissions of sulfur dioxide, carbon dioxide and carbon monoxide

To meet future electricity demand and decrease negative effects on the environment, alternative electricity generating technologies are necessarily. A new alternative electricity generation technology is the Plant Microbial Fuel Cell (PMFC).

In this paper the plant *Pisum Sativum* was used to evaluate the voltage generation in an electrochemical device adapted at the root system of the plant. For this, *Pisum Sativum* was planted either with or without compost and varying the adding of rhizobia.

**Relevance:** Research on technologies to innovate and improve sustainable Energy Sources.

**Topic:** The impact of bacterial symbiosis and compost on the electricity flow between soil and the root of *Pisum sativum*.

**Subject:** In this research project we will aim at understanding some of the underlying mechanisms that are suspected to hinder or be of significant importance to the enhancement of the plant microbial fuel cell /To research how different intakes of nutrition and bacterial symbiosis influence the plant-microbial-interactions and hence electricity generation.

**Scientific question:** Does bacterial symbiosis and added nutrients (compost) improve the voltage that is generated from microbial-root interactions?

**Hypothesis:** The bacterial symbiosis and added nutrients improve the growth of the plant, which in turn increases the voltage that can be measured.

**Visualization:** We are going to germinate the seeds of *Pisum sativum* plants with the mycorrhizal fungus *Rhizophagus irregularis* (RI). The plants are going to be divided into 4 groups: all of the groups will have microbes in their soil to enable the electricity flow between root and soil. The control group which is the first group will have six pots, three with soil and plant while other three with only soil. Second group will get compost mixed more nutrient soil whereas the third group has the same soil type as the first one but with RI added. The last group will get the compost mixed RI added soil. The seeds will be pre-germinated and inoculated

right after their planting. The seed germination part is expected to start from 13.01, followed by observation of growth period until 08.02 which is the harvesting point. The measurement will be done by a multimeter. Possible use of Farmbot for watering, measuring and photographing the plants.

## **2. Idea forming process** Suvendu, Anna, Anita

**Weeks 21.11.22 - 14.12.22**

- We want to address a potential development, that's currently relevant

### **Idea discussion:**

- Rates of irrigation for chosen plant?
- Aspects of climate change (e.g., soil components, droughts, floods) that influence plants during growth
- Example: In 2021, some parts of Germany suffered from bad floods and 2022 is considered to be the driest year in recent times, so research on effect of this transition on the plants or how the plant can work against the stress using the help of microorganisms.
- Choosing plants from *Lotus japonicus*, *Pisum sativum*, *Arabidopsis thaliana*, *Zea mays*.
- Letting the plants dry out and then watering those a lot to observe how the change takes place with bacteria as a second factor and measure the current with electrodes in the ground. We want to find out if we can generate electricity with mycorrhizal plants.

### **Implications:**

- Influence of the plant-microbial interactions due to different intakes of nutrition and mycorrhiza symbiosis
  - How can different environmental factors and root-microbial interactions impact the electron flow in a plant?
  - Mycorrhizal symbiosis and added nutrients improve the voltage that is generated from microbial-root interactions
  - To what degree can root-microbial/root-Mycorrhiza interactions generate flow of electrons? Can this energy be used/harvested sustainably?
- Possibilities:
- Can the root microbial interactions generate enough electricity to light up a bulb?
  - How this concept may be introduced into everyday households as waste repurposing tool

[1] S. Tyagi, R. Singh, and S. Javeria, "Effect of Climate Change on Plant-Microbe Interaction: An Overview," *Eur. J. Mol. Biotechnol.*, vol. 5, 2014, doi: 10.13187/ejmb.2014.5.149.

[2] E. Fosso-Kankeu, S. Marx, F. Waanders, and V. Jacobs, "Impact of soil type on electricity generation from a Microbial Fuel Cell," 2015.

[3] C. Sophia Ayyappan, V. M. Bhalambaal, and S. Kumar, “Effect of biochar on bio-electrochemical dye degradation and energy production,” *Bioresour. Technol.*, vol. 251, pp. 165–170, 2018, doi: <https://doi.org/10.1016/j.biortech.2017.12.043>.

- Trying to find answers to generate electricity in a renewable manner -- The concept of PMFC (Plant-Microbial Fuel Cell) to store electricity generated by the interactions between plant root microbes.
- Focus of PMFC design: Would be interesting to look into house plants as that could be used in big cities

[4] M. Benghernit, M. Kameche, F. Zerhouni, F. Krim, T. Sahraoui, and C. Innocent, “The study of the performance of a microbial fuel cell: a progress towards the improvement of low electrical bioenergy output by using an amplification system,” *Biotechnol. Lett.*, vol. 44, 2022, doi: 10.1007/s10529-022-03304-4.

[5] R. Shaikh *et al.*, “Bioelectricity production using plant-microbial fuel cell: Present state of art,” *South African J. Bot.*, vol. 140, pp. 393–408, 2021, doi: 10.1016/j.sajb.2020.09.025.

[6] M. Helder, *Design Criteria for the Plant-Microbial Fuel Cell: Electricity Generation with Living Plants : from Lab Tot Application*. 2012.

### **Challenges:**

There are various technological and feasibility challenges like low voltage generation, lack of steady voltage production, short lifespan of the plant species used in the PMFC and insufficient data to analyse and standardize area specific MFC design and configuration which restricts the PMFC from the commercial application.

### **Opportunities:**

It is essential to explore solely on power generation potential and development of advanced design and configuration of PMFC. In addition to this, more plant species with high biomass generation capacity, longer lifespan and high salt tolerance should be explored for power generation.

[7] S. Maddalwar, K. Kumar Nayak, M. Kumar, and L. Singh, “Plant microbial fuel cell: Opportunities, challenges, and prospects,” *Bioresour. Technol.*, vol. 341, p. 125772, 2021, doi: <https://doi.org/10.1016/j.biortech.2021.125772>.

[8] S. Aftab, A. Shah, J. Nisar, M. N. Ashiq, M. S. Akhter, and A. H. Shah, “Marketability Prospects of Microbial Fuel Cells for Sustainable Energy Generation,” *Energy & Fuels*, vol. 34, no. 8, pp. 9108–9136, Aug. 2020, doi: 10.1021/acs.energyfuels.0c01766.

[9] D. Nath, I. Chakraborty, and M. M. Ghangrekar, “Integrating microbial electrochemical technologies for methane-to-bioelectricity and water-splitting to impart

self-sustainability to wastewater treatment plants,” Bioresour. Technol. Reports, vol. 13, no. December 2020, p. 100644, 2021, doi: 10.1016/j.biteb.2021.100644.

### **Bacteria**

*Geobacter* produces current densities in microbial fuel cells that are higher than any known organism. They harvest electricity from aquatic sediments and organic waste by colonizing the electrodes. Their high efficiency in electron transfer is attributed to the *pili* along the length of their body that work as “microbial nanowires”. This property along with the ability of biofilms to work as super capacitors can contribute to bioelectronics.

[10] M. Morita *et al.*, “Potential for Direct Interspecies Electron Transfer in Methanogenic Wastewater Digester Aggregates,” *MBio*, vol. 2, no. 4, pp. e00159-11, 2011, doi: 10.1128/mBio.00159-11.

*Geobacter anodireducens* would have been the most suitable for our project but finally we decided to use microbial mix for growing microorganisms naturally in soils also it requires less time (2 weeks) as compared to mycorrhiza (4-6 weeks). Also sterilizing the soil is needed to use the type of bacteria.

[11] D. R. Lovley, “Powering microbes with electricity: Direct electron transfer from electrodes to microbes,” *Environ. Microbiol. Rep.*, vol. 3, no. 1, pp. 27–35, 2011, doi: 10.1111/j.1758-2229.2010.00211.x.

### **Electrodes (anode and cathode) materials**

The most frequently used materials in plant microbial fuel cells include carbon (fiber, cupboard, mesh, mat, felt, brush, granular activated carbon, glassy carbon). Carbon is also a high-conductive and non-inhibitive. Looking at different anodes (using carbine granules in one and carbon felt at another). Because one of the studies shows that carbon felt obtained higher current but granules are more suitable for the plant roots.

[12] C. Sánchez, P. Dessì, M. Duffy, and P. N. L. Lens, “Microbial electrochemical technologies: Electronic circuitry and characterization tools,” *Biosens. Bioelectron.*, vol. 150, p. 111884, 2020, doi: 10.1016/j.bios.2019.111884.

[13] R. Nitisoravut, T. Cao, and R. Regmi, “Microbial Fuel Cells: Advances in Electrode Modifications for Improvement of System Performance,” *Int. J. Green Energy*, vol. 14, 2017, doi: 10.1080/15435075.2017.1326049.

### **Plant selection:**

It has to be a plant that is able to form a symbiosis with mycorrhiza (*Rhizophagus irregularis*). We want to work with a common crop, like *P. sativum* that is used in agriculture and form the symbiosis smoothly.

### **Contradiction:**

If we can reach a pH-level of around 5.0-5.5 in the soil, that would support the flow of electrons. But the model organisms we can choose from mostly react badly to a pH-level

like that. Maybe we can use a different model organism? Organisms work better with lower pH levels but with mature composts pH level goes higher, also more organisms tend to raise the flow of electrons. So finally we decided not to take pH level measurement into consideration.

#### **Soil selection:**

We prioritized soil type with high organic carbon as it plays an important role in increasing microbial activity to generate higher peak voltage. Compost plays role as fuel to generate power. Another research shows that using clay soils that are enriched with compost in different rates also are good to use in our research to generate electricity but problem is sensors don't work with it. Black soil is possessed with different levels of nutrition and this type of soil could be taken into consideration list. Additionally, balanced carbon to nitrogen (C/N) ratio is recommended and should be 24:1 because the generated electricity will decelerate if it is higher. For major part of research this parameter of soil is essential. Finally settled with sand soil mix with compost in some of the pots (1:4) as extra nutrient.

[14] M. Acosta-Coll, A. Ospino-Castro, S. Carbonell-Navarro, J. Escobar-Duque, R. Peña-Gallardo, and R. Zamora-Musa, "Substrate treatment for the increment of electric power potential from plants microbial fuel cells," *Int. J. Electr. Comput. Eng.*, vol. 11, no. 3, pp. 1933–1941, 2021, doi: 10.11591/ijece.v11i3.pp1933-1941.

[15] A. C. Sophia and S. Sreeja, "Green energy generation from plant microbial fuel cells (PMFC) using compost and a novel clay separator," *Sustain. Energy Technol. Assessments*, vol. 21, pp. 59–66, 2017, doi: 10.1016/j.seta.2017.05.001.

#### **Watering the plant:**

Measuring water field capacity is crucial and therefore when the value of water field capacity is under 70%, it's time to water. But it's complicated, and maybe water the pots approximately around 2.5 cm per week or more frequently if the temperature is right. Also, dried out soil is harmful for this type of plant, so proper monitoring of soil condition and drainage is important.

#### **Compost making:**

Possible start of compost processing in the ongoing month as it will require some extra time if we do it ourselves, adding organic destructor to accelerate the process.

[16] Y. Bin Jiang, W. H. Zhong, C. Han, and H. Deng, "Characterization of electricity generated by soil in microbial fuel cells and the isolation of soil source exoelectrogenic bacteria," *Front. Microbiol.*, vol. 7, no. NOV, pp. 1–10, 2016, doi: 10.3389/fmicb.2016.01776.

[17] R. A. Timmers and P. dr. ir. C. J. N. ; H. Buisman Dr. ir. H.V.M. ; Strik, Dr. ir. D.P.B.T.B., Electricity generation by living plants in a plant microbial fuel cell, vol. PhD. 2012.

#### **Week 15.12.22 – 22.12.22**

- Presentation
- Concretion of setup
- Topic idea: Impact of mycorrhizal symbiosis on electricity generation from microbial-root interactions – Problem: Low electrical bioenergy output is very difficult to measure – Possible solution: Use of voltage amplifier recommended by Prof. Dettmar
- Raising questions: what size of pots will we use? Therefore we will know how much soil we will use, ratio of soil:compost, size of the electrodes and the distance between of those
- Finalizing the list of components and were ordered subsequently.

#### **Weeks 04.01.23 – 15.01.23**

- Preparing the plants
- Finalizing the setup

#### **16.01.22: Start of Experiment**

### **3. Hardware procurement** Suvendu

#### **Materials for project:**

- Plants/ microorganisms: *Pisum sativum* (24 seeds), soil microbiota mix and rhizobia
- Soil: soil and sand mix and compost
- Equipment/ device: graphite rod electrodes, copper wire, resistors, multimeter

The next step after selecting the plants and the experimental procedure to analyze the plant growth, we needed equipment to precisely measure the voltage differences generated from root-microbial



interactions. So, for that, we need a very accurate and precise low-voltage applications supported digital multimeter to detect the voltage nuances and hence VOLTcraft VC-655 BT multimeter was ordered.

1. **VC-655 BT Digital Bench multimeter:** Digital multimeters are essential for detecting, analyzing and correcting faults in electronic circuits. The Voltcraft-655 BT is used in cat I 1000 V and cat II 600 V, so it is suitable for low-voltage applications as well as for troubleshooting devices connected to low-voltage systems (e.g., household appliances). In addition to measuring functions such as True RMS measurement of AC voltage and current or continuity test with acoustic signal, this multi-talent also supports temperature sensors of ITS90 K type and PT100. The optimal measuring ranges are conveniently set automatically. The Voltcraft digital desktop multimeter can carry out up to 64 measurements per second and SCPI is supported for control and programming.



Technical data:

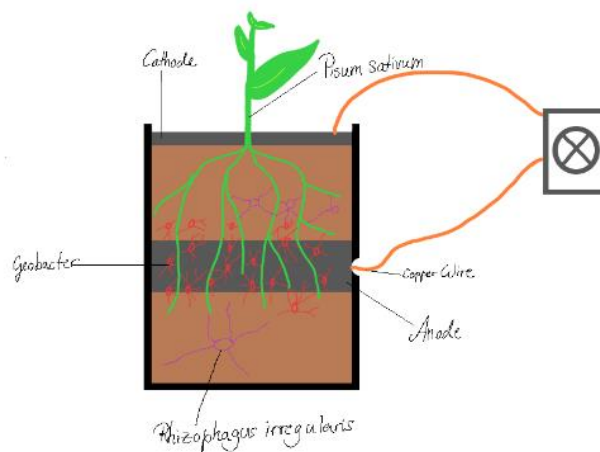
V reading range DC (min.)	0.001 mV
V reading range DC (max.)	1000 V
V reading range AC (min.)	0.001 mV
V reading range AC (max.)	750 V
A reading range DC (min.)	0.01 $\mu$ A
A reading range DC (max.)	10 A
A reading range AC (min.)	0.01 $\mu$ A
A reading range AC (max.)	10 A

2. **Electrodes:** We need electrodes (anode and cathode) and connect it to a multimeter via copper wire to measure the flow of electrodes. We chose graphite rods as the electrode material because of the super conductivity that it provides.



#### 4. Battery (PMFC) design Anna, Anita

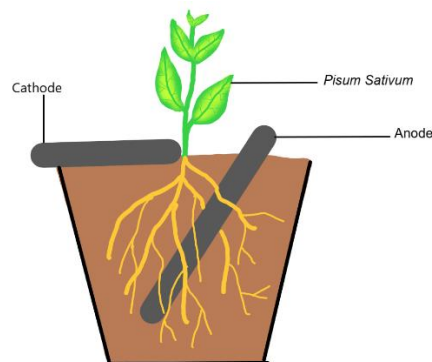
The first experiment was performed to evaluate the effect of the addition of compost and rhizobia on voltage generation in the pots *Pisum Sativum* was planted. Electrodes were placed in parallel to each other. The anode was set into the soil near to the roots to limit the contact with oxygen and to increase the contact the microorganisms. The cathode was placed on the surface of the soil to ensure access to oxygen. To measure the electricity that can flow between plant-root and microorganism, some form of device that can measure or show the electricity flow, must be connected. To do that, electrodes need to be connected to the source of electricity. Both electrodes will be connected to a multimeter via a copper wire (a). Compost was collected from outside the greenhouse. In all experiments graphite was used as anode and cathode.



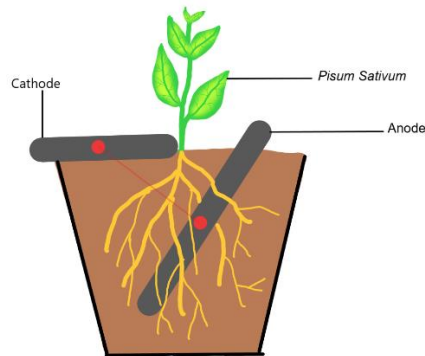
**Fig. 1(a) Sketch of the planned setup,**

this Drawing shows how the PMFC was originally designed. The anode is buried in the pot and is in direct contact with the plant roots, the bacterial mix and Rhizobia. The Electrodes are connected to the Multimeter with a wire. This setup was not possible due to the placement of the pots in the watering robot. (view Fig. 1(c)). The pots are too close to each other and the electrodes were sticking out a little bit.

During the set up, it was discovered that the Anode could not be placed horizontally into the pots like planned, due to the placement in the FarmBot (b). Because part of the Anode was sticking out of the pot, it was difficult to take the pots out of it, which was needed to be done to connect the pot to the Multimeter. The decision was made to put the Anodes placed diagonally (c).



**Fig. 1(b) Problem with placing the anode horizontally as planned,** because the electrodes could not be placed in the pots as planned, the Anode was put in vertically and isolated the top part, which was sticking out, from Oxygen.



**Fig. 1(c) Putting the anode diagonally**, the red dots outline the space that the electrodes probably measure in. it is a lot less space than could be measured and could also explain why the results were very low.

## 5. Experimental setup Anita

Plant species: *Pisum Sativum*

Abiotic/biotic factors: Soil Microbial mix, nutrient deficiency, rhizobia

Soil: Soil and sand mix in ratio 1:1, compost and soil mix in 1:1 ratio and then this mixture was mixed in 1:1 ratio with sand

Groups/treatments (6 replicates per group): The plants are going to be divided into 4 groups: all the groups will have Microbia in their soil to enable the electricity flow between root and soil. The first group will have three pots with plant and soil and three pots without a plant (just soil). This will be our control group. The second group has more nutrients in the soil, provided by compost mixed into the soil. Group three has the same soil as group one, but the rhizobia will be added as well. The last group will have the compost-mix soil and rhizobia added.

- Tray with 12 plants: group one and two
- Tray with 12 plants: group three and four

Control group: Three pots with plant and soil and three pots without a plant (just soil)

Data to be collected: Measure voltage, resistance and current with a multimeter and stem length

## **6. Implementing the Farmbot** Suvendu

We initially thought about programming the Farmbot but due to some personal circumstances I (Suvendu Barai) couldn't complete it. The preliminary discussions involved

- The amount of water for the plant to grow
- How frequent the Farmbot is going to water the plant
- Measure the temperature level of water, as many researches stated that warm water (30 – 35 degree C) accelerates power generation
- Take photographs of the plant
- Monitor the growth rate
- Measure the soil moisture (possible with Genesis kits)

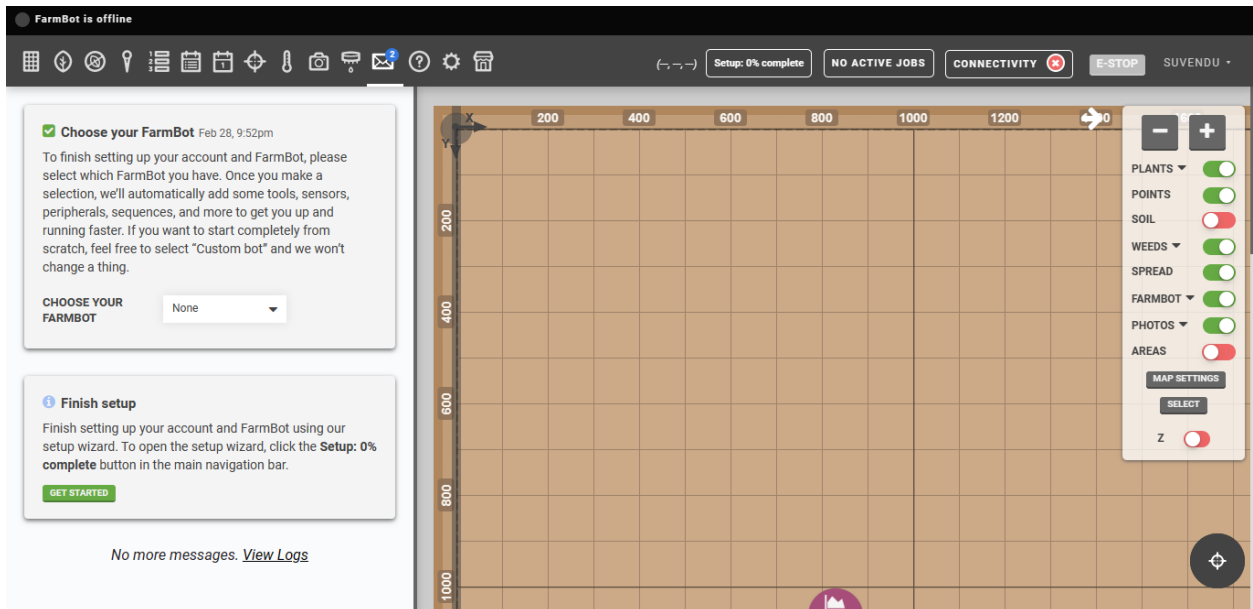
### **Development:**

**The Firmware:** The firmware that is flashed onto the Farmduino (The Farmduino is the central component of the Farmbot from an electronic systems perspective) microcontroller is responsible for physically operating Farmbot's motors, tools, sensors, and other electronics. It receives G and F codes from Farmbot OS, and then moves the motors and reads and writes pins accordingly. It also sends collected data from the rotary encoders and pin reads back to the Raspberry Pi.

**The Raspberry Pi Controller:** The Raspberry Pi is the communications link to the Farmbot Web App. The Raspberry Pi communicates directly with the Farmduino board.

**The Farmbot web app:** The web app allows easily control and configure the FarmBot from a web browser using laptop, tablet, or smartphone. The application features real-time manual controls and logging, a sequence builder for creating custom routines for Farmot to execute.

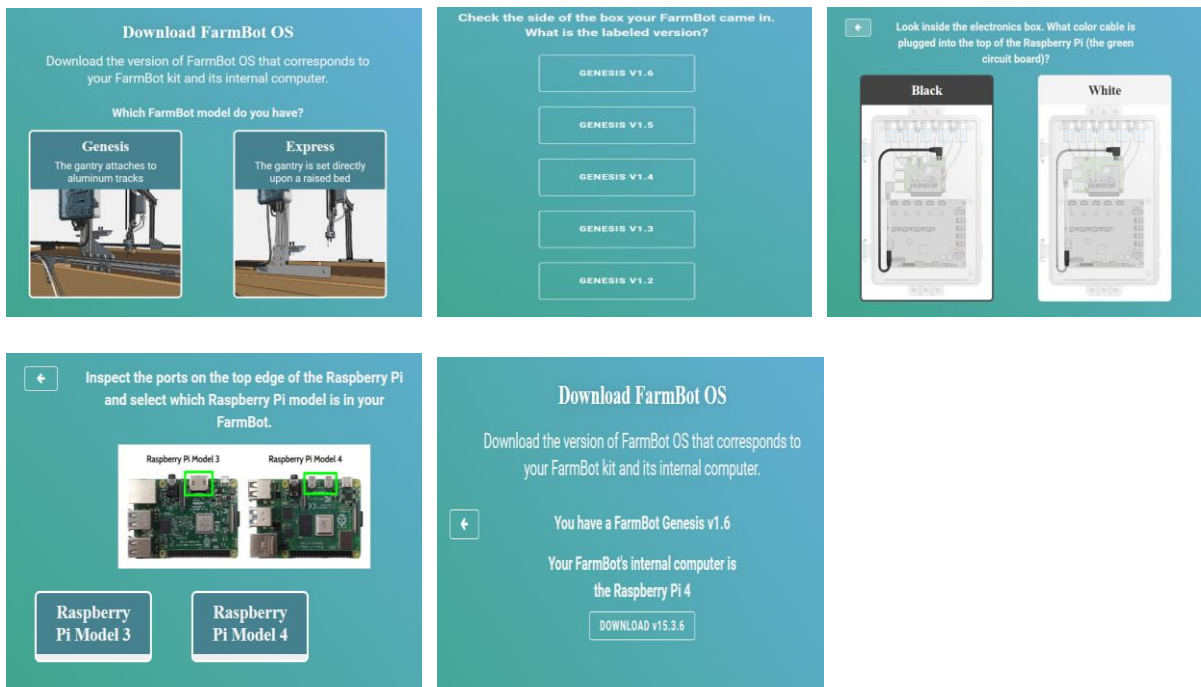
**The Farmbot OS:** Farmbot's Raspberry Pi runs a custom operating system named Farmbot OS to maintain a connection and synchronize with the web application via the message broker. This allows Farmbot to download and execute scheduled events, be controlled in real-time, and upload logs and sensor data. The OS communicates with the Farmduino/Arduino over a USB cable or serial connection to send G and F code commands, and also receive collected data from sensors and rotary encoders.



Farmbot OS has a built-in utility named configurator that allows to easily enter WiFi and web app credentials from a WiFi enabled device (such as a laptop or smartphone). This is used for initial setup in order to get the Farmbot connected to the home WiFi and web app account.

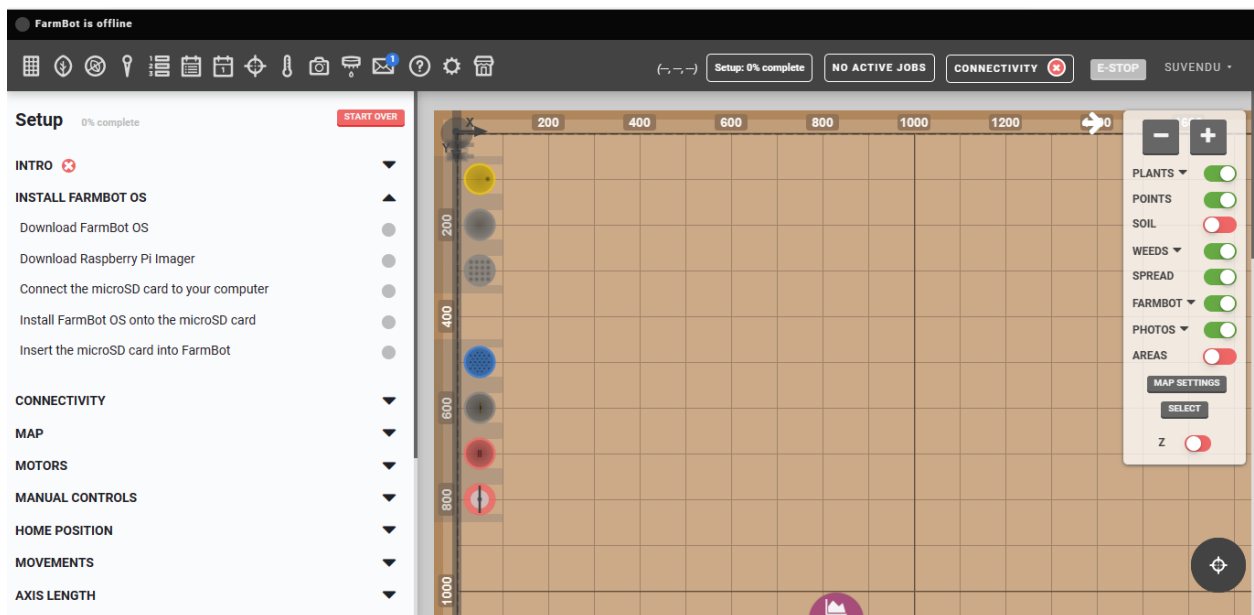
### The summarized configuration:

Depending on the type of Farmbot kit (Genesis/ Genesis XL/ Express), download the .img file of the Farmbot OS [The FarmBot Web App](#)





- Download and install Etcher <https://www.balena.io/etcher/>
- Insert an SD card into the PC
- Open Etcher. Select the downloaded .img file and burn it into the SD card
- Plug SD card into the Raspberry Pi and power it up to get started



## Implementation:

### Measure Soil Moisture:

- Add the soil moisture sensor
- Build watering sequences
- Measure the soil moisture
- Interpret the soil moisture reading
- Use an IF statement to decide how much to water

### Perform Actions on Many Plants:

- Create a group



- Perform an action on one plant
- Execute the action on every plant in the group

#### Scan The Garden (Photographing)

- Create a point group
- Group the points
- Create a sequence

### 7. Experimental procedure Anita, Uliana, Anna

#### 7.1 Preparation 09.01.23

- Autoclaved soil and sand were mixed in 1:1 ratio and 12 pots were filled with 770g
- Autoclaved soil and compost were mixed in 1:1 ratio and this mixture was then mixed in 1:1 ratio with autoclaved sand. This mixture was filled into 12 pots, 660g each
- Pots were placed on the tray

#### Tray 1

#### Tray 2

25	28		31	34		49	52		55	58
26	29		32	35		50	53		56	59
27	30		33	36		51	54		57	60

**Compost**

**Control group**

**Rhizobia**

**Rhizobia + Compost**



**24 pots with seeds on trays**

### **7.2 Germination and planting 13.01.23**

Pisum Sativum seeds were placed in a sterile petri dish on 13.01.23 and then kept in the dark for 2-3 days

### **7.3 Planting the seeds 17.01.2023**

The seeds were planted on 17.01.23 and watered per 80 ml in each pot

### **7.4 Addition of rhizobia 18.01.23**

Added 1 ml of 0.01% Rhizobia solution to compost-microbes group. The bacteria were pre-grown by Ana Moraes, and we were not sure if they are already alive, so we have repeated this.

### **7.5 Watering I 18.01.23**

All pots were watered per 80 ml.

### **7.6 Watering II 20.01.23**

We checked out the state of seed germination and watered all pots per 80 ml.



**Pots with electrodes and sensors**





**Sprouting**



***Pisum sativum* plants aged 12 days**

### **7.7 Watering III 22.01.23**

All pots were watered per 80 ml.

Then, the farm bot has been doing watering relating to the sensors.

### **7.8 Addition of new rhizobia solution 23.01.23**

Prepared new solution with fresh Rhizobia and added the 1 ml of 0.01% solution to the compost-microbes group.

### **7.9 Measurements I 07.02.23**

- Ricarda came to the University as well and showed me how to connect the wires to the multimeter and the plant
- The Voltage [VDC] and Ampere [ADC/  $\mu$ ADC] was measured on every plant
- I wasn't sure, how the others had measured before, so I waited until the value on the multimeter was stable and wrote down that value

### **7.10 Measurements II 13.02.23**

- The Voltage and Ampere was measured again
- For a better overview it was decided that all measurements will be held for 2.5 minutes each, and the start and end value is written down
- The height of the plants was measured as well
- After the measuring is done, the plants were taken out of the pot, to look at how the roots had grown around the electrode
- The roots have grown around the electrode, but they haven't connected to it (the electrode just fell out, as soon as the soil was removed)



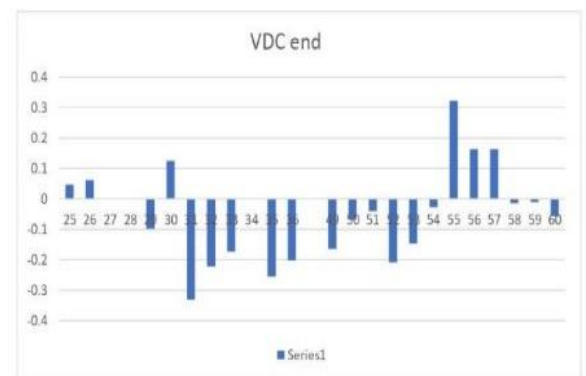
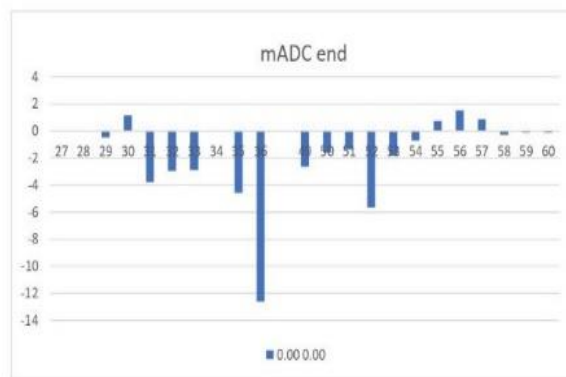


**Plant root with bacteria**

#### **8. Evaluation, discussion and analyzing of data Anita**

- Voltage and current measurement for 07.02.23 and 13.02.23 was entered in an Excel sheet (link: <https://1drv.ms/x/s!AhMXDLX5w4YegIChEFiqjTXfyhHa?e=qEJXMt>)

Pot Nr.:	Ampere (S) Increase (= Decrease ( End			Volt [VDC], Increase (= Decrease ( End			Height [cm]		Verlust VD	Verlust mADC
25	0,00		0,00	0,065	x	0,047	26,4	All measurements were taken for 2.5 min.	0,018	#VALUE!
26	0,00		0,00	0,0688	x	0,0626	25,20		0,0062	
27	No Elektrode						27,60			
28	No Elektrode						20,20			
29	-1,24	x	-0,48	-0,115	X	-0,0981	20,70		-0,0169	-0,76
30	2,10	x	1,14	0,0636	x	0,1251	23,90		-0,0615	0,96
31	-6,44	x	-3,76	-0,3433		-0,331	31,00	Was bouncing a lot between -0.340 and -0.451	-0,0123	-2,68
32	-9,00	x	-2,94	-0,0989	X	-0,2215	21,30		0,1226	-6,06
33	-3,18	x	-2,88	-0,175		-0,1737	29,50	Bounced a lot	-0,0013	-0,30
34	No Elektrode						15,40			
35	-10,21		-4,58	-0,2234	X	-0,2553	No plant (control group)		0,0319	-5,63
36	-30,40	x	-12,61	-0,01785	X	-0,2021	26,00		0,18425	-17,79
49	-17,62	X	-2,63	-0,0859	X	-0,1638	19,10		0,0779	-14,99
50	-2,92	X	-1,58	-0,028	X	-0,0682	32,80		0,0402	-1,34
51	-6,38	X	-1,36	-0,0159	X	-0,0401	27,60		0,0242	-5,02
52	-23,95	x	-5,64	-0,0869	X	-0,2084	29,50		0,1215	-18,31
53	-13,25	x	-1,81	-0,1446	X	-0,1464	31,80		0,0018	-11,44
54	3,80	x	-0,71	-0,004	X	-0,0274	1,00		0,0234	4,51
55	2,27	x	0,76	0,3833	x	0,3227	19,50		0,0606	1,51
56	7,54	x	1,52	0,0772	X	0,1638	27,40		-0,0866	6,02
57	2,29	x	0,87	0,1692	x	0,1642	24,70		0,005	1,42
58	-6,21		-0,27	-0,0072	X	-0,0147	32,00		0,0075	-5,94
59	-0,25		-0,11	0,0014	x	-0,0105	14,10		0,0119	-0,14
60	4,87	X	-0,13	-0,0148	X	-0,0568	19,50		0,042	5,00



**Table 1: Measurement of voltage and current (collected data from 7 and 13<sup>th</sup> of February),** the different colors show the groups: Blue: Group 1 (compost), Red: Group 2 (control), Green: Group 3 (Rhizobia), Yellow: Group 4 (Rhizobia + compost). The columns (from left to right) show (1) the pot number, (2) uADC at the start of the measurement, (3) uADC increase?, (4) uADC decrease?, (5) uADC at the end of the measurement, (6) VDC at the start of the measurement, (7) VDC increase?, (8) VDC decrease?, (9) VDC at the end of the measurement, (10) Height of the plant in cm, (11) comments, (12) VDC difference of value between beginning and end of the measurement, (13) uADC difference of value between beginning and end of the measurement. Underneath the Table: on the left the visualization of the uADC measurements (end) and on the right the visualization of the VDC measurement (end).

Now, looking at the measurements, the Voltage that was generated was not very high. But if we compare the measurements from the 07.02 and 13.02, we can see that the values have increased.

The plants were about 4 weeks old at this point and if given more time to grow and develop a symbiosis with the Rhizobia they probably could have generated more energy.

To compare the voltage obtained from PMFC with or without compost (control group)) (view table 1) we can see that the results do not differ from each other so much. Actually, we were expecting that pots with added compost would show higher current flow and voltage value. One of our explanations of this fact is that the presence of organic matter in soil and their degradation by microorganisms take more time into low molecular weight compounds and electrons, which were captured by the anode to produce voltage. Therefore, if we had had extra time for the experiment, we would have obtained different data in particular higher amount of voltage in order to added compost.

What immediately falls out of place is that some measurements are positive, while most of them are negative. That is because the electricity flow went in the other direction. That means that the Anode became the Cathode and the other way around. This can happen when the Anode was not isolated properly. Another reason could be that, because we had to put the Anode in vertically, the top parts of the electrodes are closer to each other than the lower parts. If that happens the top part of the pot is measured but not the lower half and the measurement can be out of place. We tried avoiding this by placing the Electrodes as far away from each other as possible.

## Discussion:



**Fig. 2 VDC (end of measurement) with trendlines**, this shows the different Groups and how the Voltage which was measured is different from Group to Group. Group 1 (Blue), with



compost. Group 2 (Orange), control. Group 3 (Grey), Rhizobia. Group 4 (yellow), Rhizobia with compost.

In Fig. 2, the Voltage at the end of measuring is shown, the different colors represent the Groups 1-4. The control Group (Group 2, orange) is the one with the most negative result. While the Groups 1 and 4 (both with compost) are the ones where most measurements turned out positive. Group 3, where only Rhizobia was added, also had negative results, but these were closer to zero. This shows that the symbiosis with the Rhizobia, *Bacteria* and *Pisum sativum* influence the Voltage which could be measured. These results can be increased by adding compost to the soil. In fact, adding the compost resulted in 75% of Group 1 and 50% of Group 4 showing positive Voltage measurements. This could point to two things. Either the PMFC was destabilized and the Cathode and Anode switched places. This could happen because the electrodes were not isolated properly or the compost, which could not be analyzed, impacted the PMFC system negatively. Or, because a positive Voltage can also mean that the battery has electrons available to discharge, this could show that the Voltage that is produced can be increased by adding a symbiosis partner, like Rhizobia, for the plant and compost to the soil. Comparing this to Image 4, shows that in most measurements the Voltage or Ampere decreases over the 2.5 min. This points to the PMFC discharging somehow. But connecting a Multimeter should just redirect the electricity, not take it out. This could be explained by assuming the multimeter just increased the size of the circuit, which is why the measurements decreased. But while measuring, the Voltage and Ampere started with very high figures which started to slowly and steadily decrease after about one minute. Which means there's a stabilization, which can be seen, in the PMFC, which points to reliable results. If there was a discharge here, it happened very slowly. It also points to the few measurements where the results increased being unstable. It could be that in these cases the Anode was not isolated properly.