The Impact of Bacterial Symbiosis and Compost on Electricity Generation in the roots of *Pisum sativum*

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1. Introduction 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 project, 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 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.

Implications: 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

2. Materials and Methods Anita, Suvendu, Anna

2.1 Concept development Anna, Anita

The Plant Microbial Fuel Cell (PMFC) is a novel technology in which makes use of naturally occurring processes around the roots of plants to directly generate electricity[1]. The PMFC is a fuel cell that utilizes organic matter as dead root material, lysates, mucilage and exudates that is available in soils[2]. Organic matter is converted into electricity by living bacteria around the roots in the soil[3]. Potential applications could include electricity production, methane reduction, and nature conservation[4].

But this concept, however, is still in its infancy due to commercially unprofitable because of low practical efficiency (typically around 0.022%)[4]. From this follows that this technology needs a lot of improvements to enhance performance of generating electricity.

Pisum sativum was finally considered for the concept of the experiment due to their ability to engage in a smooth symbiosis with rhizobia (Rhizophagus irregularis)[5]. Also, the plant does not grow that fast in height in contrast to other possible crops for this project (Lotus japonicus, Arabidopsis thaliana, Zea mays) and wouldn’t interfere with the watering done by the Farmbot[6][7].

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 brought.

2.2 Battery (PMFC) design and experimental setup Anna, Anita

The first experiment was performed to evaluate the effect of the addition of compost and rhizobia on voltage generation.

Pisum Sativum were planted in pots. 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[8]. 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.

Compost was collected from outside the greenhouse

In all experiments graphite rods were used as anode and cathode.

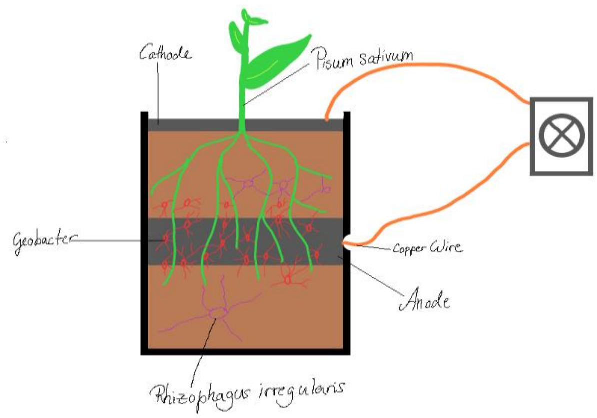


Fig. 1(a) Planned experimental setup, this Drawing shows how the PMFC was originally designed. The anode is buried in the pot and is in direct contact with the plant roots, the bacterial mix and Rhizobia. The Electrodes are connected to the Multimeter with a wire. This setup was not possible due to the 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).

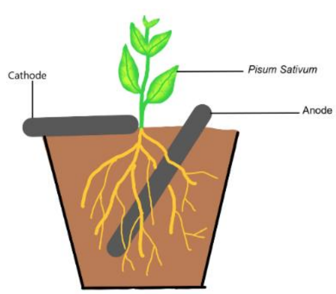


Fig. 1(b) Experimental setup, used because the electrodes could not be placed in the pots as planned, the Anode was put in vertically and isolated the top part, which was sticking out, from Oxygen. This can create another problem (which I (Anita) did not know about until taking the measurements); When taking the measurements, the electrodes only measure in between the parts that are closest to each other (view Fig. 1(b)). By placing the electrodes far away from each other this can be avoided but was also difficult in this setup.

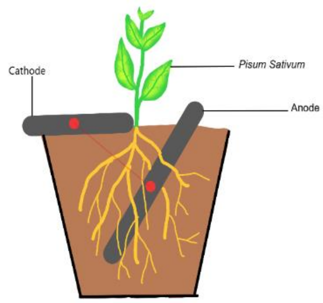


Fig. 1(c) Experimental setup, used with measuring points, the red dots outline the space that the electrodes probably measure in. it is a lot less space than could be measured and could also explain why the results were very low.

2.3 Final experiment Anita

The final setup we chose consists of the following components:

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

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 were divided into 4 groups. All the groups had Microbes in their soil to enable the electricity flow between root and soil. The first group had three pots with plant and soil and three pots without a plant (just soil). This was our control group. The second group had more nutrients in the soil, provided by compost mixed into the soil. Group three had the same soil as group one, but the rhizobia will be added as well. The last group had the compost-mix soil and rhizobia added.

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

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**

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

- The Voltage [VDC] and Ampere [ADC/ µADC] was measured on every plant
- 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, te 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)

The experiment ran for two weeks from 16.01.23 to 30.01.23 in the greenhouse of the University of Cologne.

2.4 **Implementing the Farmbot** Suvendu

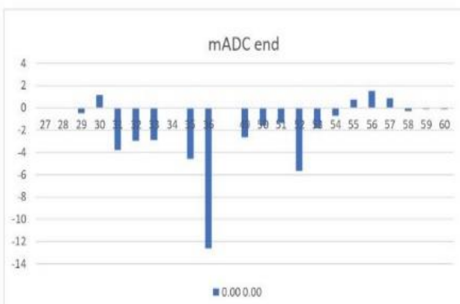
We initially thought about programming the Farmbot but due to some personal circumstances I (Suvendu Barai) couldn't complete it. The preliminary discussions involved

- 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)

3. **Results** Anita

Voltage and current measurements from 07.02.23 and 13.02.23 were entered in an Excel sheet (link:https://1drv.ms/x/s!AhMXDLX5w4YeglChEFiqjTXfyhHa?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



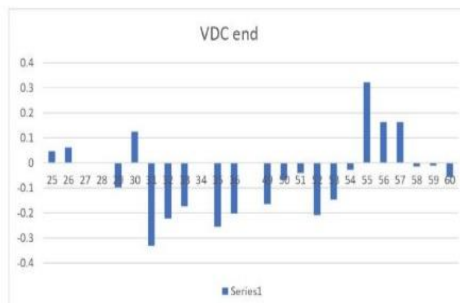


Table 1: Measurement of voltage and current (collected data from 7th and 13th of February), the different colors show the groups: Blue: Group 1 (compost), Red: Group 2 (control), Green: Group 3 (Rhizobia), Yellow: Group 4 (Rhizobia + compost). The columns (from left to right) show (1) the pot number, (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 taken on 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[5][9][10]. 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 (view Image 3). 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.

4. Discussion Anita

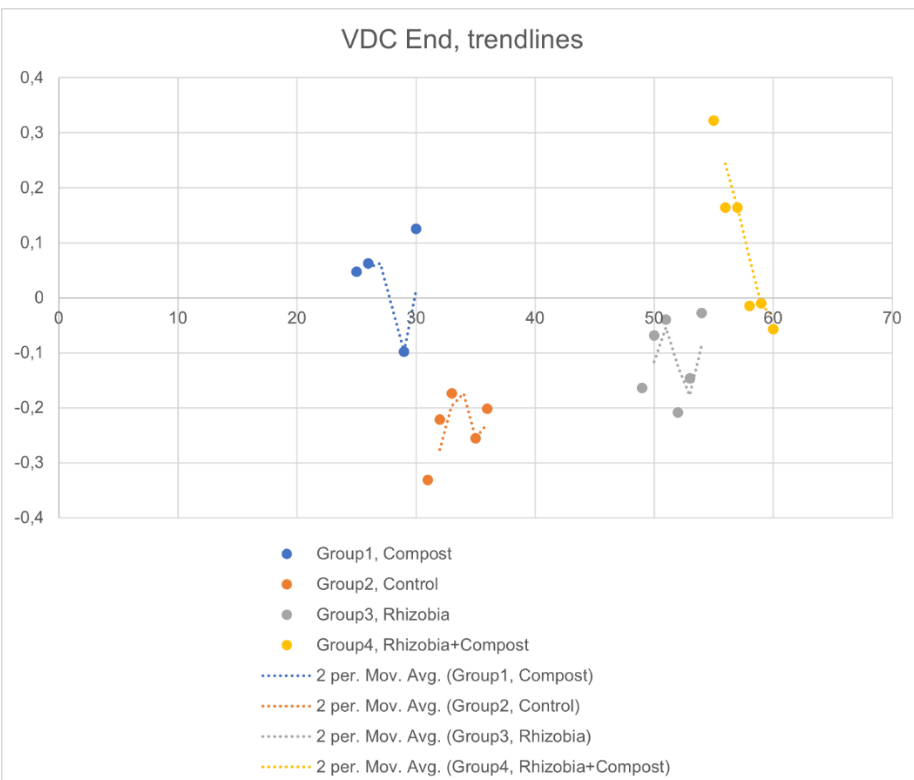


Fig. 2 VDC (end of measurement) with trendlines, this shows the different Groups and how the Voltage which was measured is different from Group to Group. Group 1 (Blue), with compost. Group 2 (Orange), control. Group 3 (Grey), Rhizobia. Group 4 (yellow), Rhizobia with compost.

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 were 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 were the results increased being unstable. It could be that in these cases the Anode was not isolated properly.

5. Improvements for future experiments Anita, Suvendu

5.1 Project related:

During the experiment there were a few things that could be improved in future projects to further increase the relevance and amount of data.

- Geobacter produces current densities in microbial fuel cells that are higher than any known organism[6][9]. They harvest electricity from aquatic sediments and organic waste by colonizing the electrodes. *Geobacter anodireducens* would have been the most suitable for the project. Hypothetically, it would have given more electricity generation, because this type of bacteria serves as conductor for better transfer of electrons to anode.

- If we had more time to develop the root system we might get higher voltage and current.

5.2 Relevant researches-based suggestions:

Comparing project results with and without compost: According to data from one article, the maximum voltage generated without compost was at the start of experiment, around 6 - 14 days, that's decreasing. In contrast, with compost the highest voltage was generated after 31 days. It could be associated to the presence of organic matter is soils and their more slowly degradation by microorganisms into low molecular weight compounds and electrons which were captured by anode[11][12].

Effects of biochar addition on the performance of MFC: A low-cost additive which was explored for increasing the voltage generation in PMFC research is coconut-based biochar. 1 g and 0.5 g each of biochar were introduced in the MFC for dye degradation and removal of COD. In this process, the maximum current reported in MFC was 1.07 mA and the voltage was 722 mV from 1 g of biochar addition as compared to the control. Hence, these results concluded the possibility for the application of coconut-based biochar as an additive component in MFC to enhance power production after standardization[13].

5.3 Challenges of PMFC:

There are various technological and technical and economic challenges, such as low voltage generation, lack of stable voltage production, short lifespan of plant species used, and lack of data on of the plant species used in the PMFC, and insufficient data for analysis and insufficient data to analyze and standardize the design and configuration of site-specific PMFCs, which limits PMFCs from being commercialized[1][14]. In order to address these issues, additional future research is needed to clarify each possible aspect in turn or simultaneously. simultaneously.

High electricity production and shorter payback time are two important parameters that need to be carefully investigated to compare PMFCs with existing renewable energy sources such as solar panels and wind turbines and wind turbines[15]. Although the operation of PMFCs is a complex process and involves numerous factors that are responsible for overall performance, in this report we will look at some of these factors.

5.4 Standardization of design for PMFC:

In order to commercialize the PMFC technology, it is required to standardize certain aspects like electrode material, electrode position and spacing, oxygen diffusion, cation diffusion composition of catholyte and anolyte and identification of high-power generating microbes[16]. It is also essential to standardize the carrier or media containing substrate for microbial activity at appropriate parameters like pH and porosity for required internal resistance. Plant species with more rhizodeposition potential should be screened and used for PMFC application[17][14].

5.5 Optimization of the functionality:

Basically, optimization aspects should vary from design and configurations as per geographic locations, PAR (APAR) wavelength, concentration of carbon dioxide at that place, atmospheric temperature, rootzone temperature and type of microbial community used in anode as well as cathode[18]. The ultimate aim of this optimization should be generation of steady, sustainable, and long-term power via PMFC system.

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