Direct electricity production from green onion's roots

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Abstract

In recent years, extensive scientific efforts are conducted to develop clean bio-energy technologies that have the potential to compete with the contaminating fossil fuels. Electricity production was previously generated from photosynthetic organisms in bio-photo electrochemical cells (BPECs), and microbial fuel cells (MFCs). The main approach of such methods was to exploit the ability of the organisms to perform external electron transfer (EET) to reduce the anode of an electrochemical cell. By far, these methods were applied to microorganisms, seaweeds, and plant leaves. The ability of roots to perform EET was applied in solutions that consist of molecular electron acceptors, however, it was not used for electricity production yet. In this work, we integrate green onion roots in a bio-electrochemical cell and show that it can produce continuous bias-free electric current for more than 24 h. This current is enhanced upon irradiation of light on the onion's leaves. We apply 2D-fluorescence maps to show that NADH serves as a major electron mediator between the roots and the anode, while its concentration in the external root matrix is increased upon irradiation of the leaves. Direct electricity production from roots may be integrated with soil-based MFCs or plant leaves-based BPECs and pave the way towards the establishment of novel renewable energy technologies.

Introduction

In recent years, many efforts are being done to replace fossil fuels with green energy solutions. The utilization of microbial fuel cells (MFCs) for electricity generation was extensively studied. [1–9] In this method, bacteria is utilized as an electron donor at the anode [10] or acceptor at the cathode. [10,11] The reduction of the anode can be done by mediated electron transfer (MET) [6,7,12–15] or direct electron transfer (DET). [1,16,17] In the case of MET, redox-active metabolites such as derivatives of flavines and phenazines are secreted from the cells to reduce the anode. The MET can be enhanced by the addition of an exogenous electron mediator such as potassium ferricyanide. [18–23] DET is performed by conductive *pili* [24–27] or complexes such as MTR complexes. [28,29] Among the species that perform a high degree of DET are *Shewanella onedis* and *geobacter*. [24–29]

Another approach that was evolved from MFCs is the utilization of live photosynthetic unicellular organisms such as cyanobacteria and microalgae. [30–35]. In this case, the electron source is photosynthesis, while the electron mediation is mostly conducted by the redox couple $NADP^+$/NADPH that can cycle between the photosystems inside the organisms and the external anode. [30] The photocurrent can be enhanced and prolonged by the addition of exogenous NAD^+, $NADP^+$, or vitamin b1[30,31] that can enter the cells and accept an electron from photosystem I. Exogenous artificial electron mediators such as Ferricyanide [36] can also enhance the photocurrent by accepting electrons at the external surface of the cells. Recently, it was discovered that bio-photo electrochemical cells are not limited to unicellular organisms and can be directly produced from macroalgae (seaweeds)[37] and green tissues of terrestrial plants such as leaves and stems. [38] The photocurrent production of such systems is about 1000 times greater than for microorganism-based BPECs.

Rather than using live organisms, photoelectricity generation can be done by isolating photosynthetic components at different degrees of purification, such as Chloroplasts, Thylakoid membranes, and photosystems. [39–46] In contrast with live organisms in which the addition of an external mediator is optional, isolated compounds are incapable to perform EET by themselves, and require the addition of an exogenous electron mediator. [47]

An additional interesting method is to integrate soil MFCs with plants farming. In this method, plants conduct photosynthesis and secrete through their roots different compounds including organic carbon sources that are being uptaken by the bacteria and enable its cultivation in the soil. [48–50]

Rather than secreting components, roots can sense and electrochemically interact with their environment. Such a phenotype can be observed in electro-tropism responses of the roots. Upon application of an electric field, roots can decompose the starch granules that direct them toward gravitropism (elongation along the gravitational direction) and re-orient their growth direction. [51,52]

The electro-tropic mechanism involves biomolecules that are capable of charge transfer. Integration of these electro-tropic additives in organic solar cells may increase its power efficiency. [53] It was previously reported that roots can reduce the molecular electron acceptors FeCN and 2,6-dichlorophenol indophenol (DCPIP) [54]. In this work, we follow the ability of green onion roots to reduce Cytochrome C by absorption measurement. We show for the first time that electricity can be harvested directly from green onion roots by attaching them to the anode in an electrochemical cell. This electricity generation is enhanced upon the illumination of the onions' leaves. Finally, we use 2D fluorescence maps to detect the electron mediators NADH and NADPH (NAD(P)H) in the external matrix of the roots suggesting that like in photosynthetic micro and macro-organisms based BPECs, NADH performs MET between the roots and the anode.

Materials and Methods

Materials

All chemicals were purchased from Merck unless mentioned otherwise.

Absorption measurements

O.D measurements of the cells were done at 750 nm (Nanodrop 2000 UV-Vis spectrophotometer, Thermo Fisher Scientific).

2D – FM measurements

The 2D-FM was conducted using a Fluorolog 3 fluorimeter (Horiba) with excitation and emission slits bands of 4 nm. Evaluation of NAD(P)H concentrations was calculated by the construction of the NADH calibration curve based on increasing concentrations measured at (λ_{Ex} = 350 nm, λ_{Em} = 450 nm).

Chronoamperometry (CA) measurements

The system was designed based on the description in the previous work of Shlosberg et al.[37] CA measurements were performed in 4.5 cm^3 rectangular transparent glass vessels. Illumination was performed using 2 LEDs that were placed vertically to illuminate the onion's leaves. The distance between the LEDs and the leaves was ~ 10 cm. All measurements were performed in a two-electrodes mode without the application of electrical potential on the WE. A stainless-steel clip was applied as the WE and a Pt wire as the CE. In all measurements, the current density was calculated based on the surface area between the WE the was totally covered by roots of 0.08 cm^2.

Results and Discussion

Onion roots reduce the electron acceptor Cytochrome C.

We first wished to quantitate the ability of green onion roots to perform external electron transport (EET). To do that, we incubated about 8-12 roots of green onion in a solution of 30 µM Cytochrome C (Cyt) for 10 min in dark, while the absorption spectra of the solution (without the roots) was measured right before the beginning of the incubation, and every 1 min. The obtained spectra showed an increase in the peak with the maxima at λ = 550 nm that correlated with the increasing incubation time (Fig. 1a), while the increase of this peak derives from the reduction of Cyt. However, the increase in the incubation time has also elevated the intensity of the whole measured spectrum λ = 475 – 550 nm, because of the secretion of molecules that also absorb in the wavelength range. Therefore, to make a more accurate quantification, the Cyt reduction was calculated by subtracting the intensity at the maxima of the peaks (λ = 550 nm) by their minima (λ = 540 nm) (Fig. 1b). This analysis showed that a maximal reduction of Cyt (that represent EET of 0.15 µmol electrons) was obtained after ~8 min.

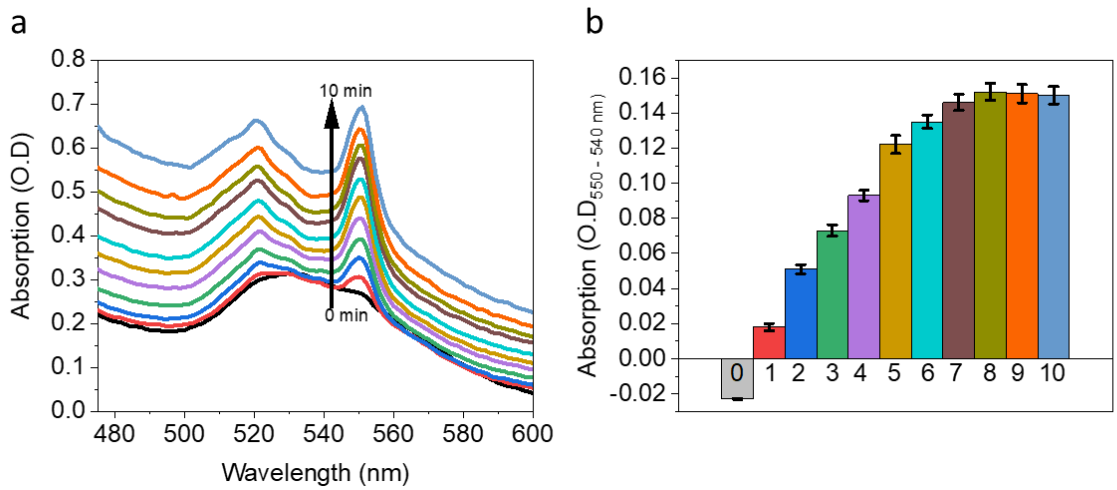

Fig.1 – Onion roots reduce the electron acceptor Cytochrome C. Green onion roots were incubated for 10 min with Cyt. The absorption spectra were measured before the incubation and every 1 min. **a** Absorption spectra of the Cyt solution. The black arrow represents the time increase that correlates with the intensity increase at λ = 550 nm. **b** The intensities at λ = 550 nm were subtracted by the intensities at λ = 540 to estimate the net absorption increase that derives from Cyt C reduction and is not dependant on the absorption of additional components in the solution. The error bars represent the standard deviation over 3 repetitions.

NAD(P)H is secreted from roots

Previous studies about BECs based on cyanobacteria[30], microalgae[31], seaweeds[37], and plant leaves[38], showed that part of the electrical current production derives from the secretion of the redox-active molecules NADH or NADPH (NAD(P)H), while the secretion of NADPH was influenced by its formation in the photosynthesis pathway, and was therefore enhanced upon illumination. We

postulated that the same molecules may be secreted from roots to reduce external acceptors [38]. To elucidate this, green onion roots were incubated in 0.1 M NaCl solution for 10 min in dark and high light. The plant (with the roots) were removed, and 2D-fluorescence maps (2D-FM) λ_{Ex} = 250 - 500 nm, λ_{Em}= 280 – 800 nm were measured (Fig. 2a,b). The spectral fingerprints of NAD(P)H ($\lambda_{Ex\ max}$ = ~350 nm, $\lambda_{Em\ max}$= ~450 nm), and the amino acids tryptophan and tyrosine ($\lambda_{Ex\ max}$ = ~280 nm, $\lambda_{Em\ max}$ = ~350 nm) were identified based on previous studies [30,55]. Interestingly, as previously reported for cyanobacterial cell[1], the intensity of the peaks of NAD(P)H and the amino acids were higher after the illumination of the onion's leaves. We suggest that NADPH that is formed by photosynthesis may be transported from the leaves to the roots and exist to reduce the anode. To evaluate the NAD(P)H concentrations in the external solution, a calibration curve was prepared by measuring the fluorescence intensity (λ_{Ex} = 350 nm, λ_{Em} = 450 nm) of increasing NADH concentrations. The NAD(P)H concentrations were determined to be ~ 0.15 and 0.27 µM in dark and light respectively, (that is equivalent to 15 and 27 nmol). Since unknown reducing molecules rather than NAD(P)H may be secreted from roots, We wished to estimate the contribution of NAD(P)H to the total reduction of Cyt reduction. To evaluate this, the number of secreted NAD(P)H molecules in dark was divided by the number of reduced Cyt molecules over 10 min (each NAD(P)H or Cyt molecule can donate/accept 1 electron). The calculation showed that NAD(P)H reduces 10 % of the Cyt molecules. Based on these results, we suggest that the roots secrete additional reducing molecules rather than NAD(P)H. However, it is possible that more than 10 % of Cyt derives from NAD(P)H reduction, assuming that some of the $NAD(P)^+$ or NAD(P)H molecules are being up taken by the roots. Such a cyclic electron transport mechanism was previously reported for various cyanobacterial species[30] and microalgae[31] in which NAD(P)H was the major electron mediator. Roots secrete many kinds of components such as minerals, proteins, Carbohydrates, and Vitamins[56,57]. We postulate that some of the reported vitamins that are capable of charge transfer such as Ascorbic acid, Thiamine, Nicotinic acid, may also play a role in the reduction of Cyt.

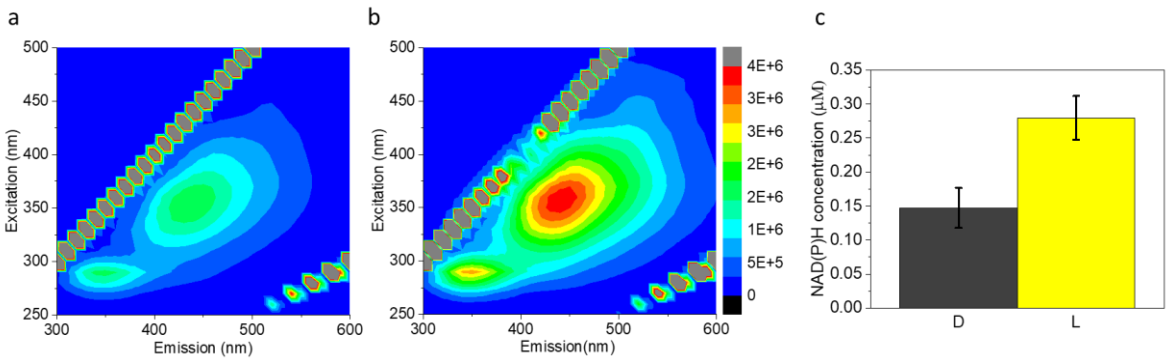

Fig.2 – NAD(P)H is secreted from roots. Roots of green onion were incubated in 0.1 M NaCl solution for 10 min with or without the illumination of the onion's leaves. Following the incubation, the 2D-FM of the solutions were measured. **a** 2D-FM after incubation in dark. **b** 2D-FM after incubation in light. **c** NAD(P)H concentrations were calculated for the solutions after incubation in dark (D) and light (L). The error bars represent the standard deviation over 3 independent repetitions.

Designing a bio-electrochemical setup for harvesting electricity from roots

Based on our results (Fig.1), and previous works that reported the ability of roots to reduce electron acceptors in a solution, we sought that the roots could produce an electric current in a bio-electrochemical cell (BEC). For this purpose, a new BEC setup was designed (Fig. 3). The setup consisted of a rectangular glass vessel with a width, length, and height of 4.5 cm. 50 mL NaCl solutions with increasing concentrations of 0.1 – 0.5 M were used as electrolytes. The bottom of the green onion and its roots were placed in the solution. 0.2 cm of the edges of ~5 – 6 the roots were grabbed by the stainless-steel anode clips, to cover the entire anodic area of 0.04 cm^2. A platinum wire was used as a cathode. The illumination of the leaves was conducted by 2 white light LEDs that were placed on a stand with ~10 cm from leaves. A similar setup that utilizes a stainless steel clip as an anode was previously reported for seaweeds and plants' leaves based BPECs.[37,38]

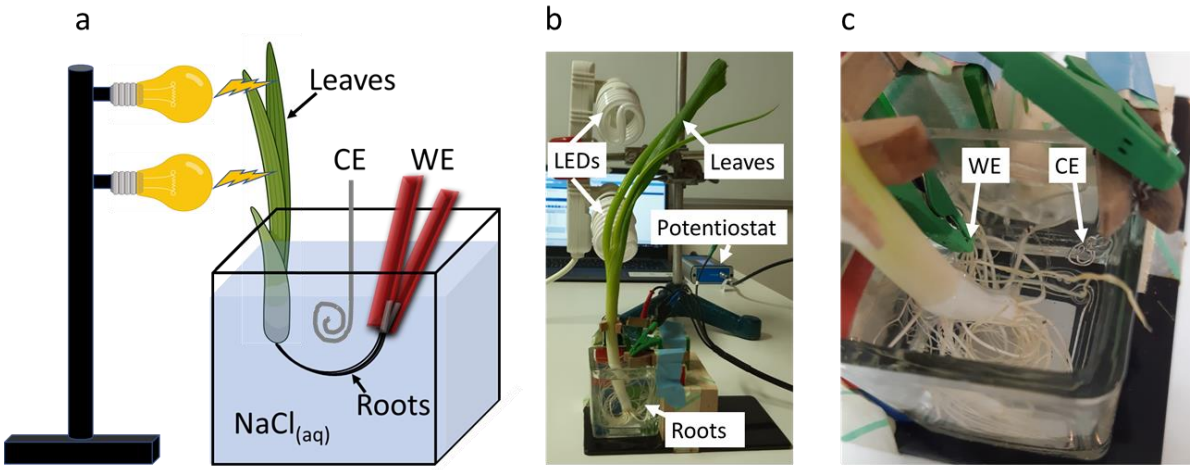

Fig.3 - Designing a BEC system for harvesting electricity from roots. A schematic description and photos of the system for electricity generation from green onion roots. **a** A schematic illustration of the system. the bottom of a green onion is placed inside the NaCl solution. The edge of the roots is grabbed by the stainless-steel clips (red shape) that apply as working electrode (WE), a platinum wire (Grayline) is used as a counter electrode (CE). The leaves of the onion can be illuminated by white LEDs that are placed on a stand at a distance of ~10 cm from the leaves. **b** A picture of the measurement system. white labels with arrows mark the roots, leaves, potentiostat, and LEDs. **c** An enlargement of the BEC system. white labels with arrows mark the WE, and CE.

Direct Bias-free current production from green onion roots in dark and light.

To optimize the NaCl concentration, Chronoamperometry (CA) of increasing NaCl concentrations (100 – 500 mM) were measured using a 2-electrode mode without application of an external potential bias for 12 h in dark. The maximal current density that was obtained for the NaCl concentrations: 100, 200, 300, 400, and 500 M were about 4.5, 8, 20, 35, and 40 µA / cm^2 respectively (Fig. S1). No destruction of the onion was observed at the end of this measurement. The electrolyte concentration of

500 mM was also previously determined to be optimal in seaweeds and plant leaves-based BPECs.[37,38] In addition, we wished to assess whether different pH values affect the current production. For this purpose, the CA of roots was measured using 500 mM NaCl dissolved in MES buffer with pH values of 5 - 8. The change in the pH did not have any significant influence on the current production (Fig. S2).

Previous studies about BECs that were based on photosynthetic micro[30,31], and macro- organisms[37,38] suggested that although the mechanism of the electron transfer was conducted by diffusible electron mediators, the organism must be in a close approximation with the anode to supply a sufficient concentration of the mediator. We wished to explore if roots also have to be attached to the anode to produce current and whether illumination of the leaves can influence the current production of the roots. Bias-free CA measurements of the onion roots were conducted in 0.5 M NaCl for 24 h in dark, light and without a direct association of the roots to the anode (the roots were inside the electrolyte solution but not touching the anode). No significant current was obtained for the roots without a direct association to the anode. The maximal current densities of the associated roots in dark and light were ~40 and 140 µA / cm^2 respectively (Fig.4). We suggest that the dark current derives from the secretion of NADH, and vitamins such as Ascorbic acid, Thiamine, Nicotinic acid, while the current enhancement in light derives from the trafficking of NADPH molecules that are formed in photosynthesis towards the roots, and outside to reduce the anode.

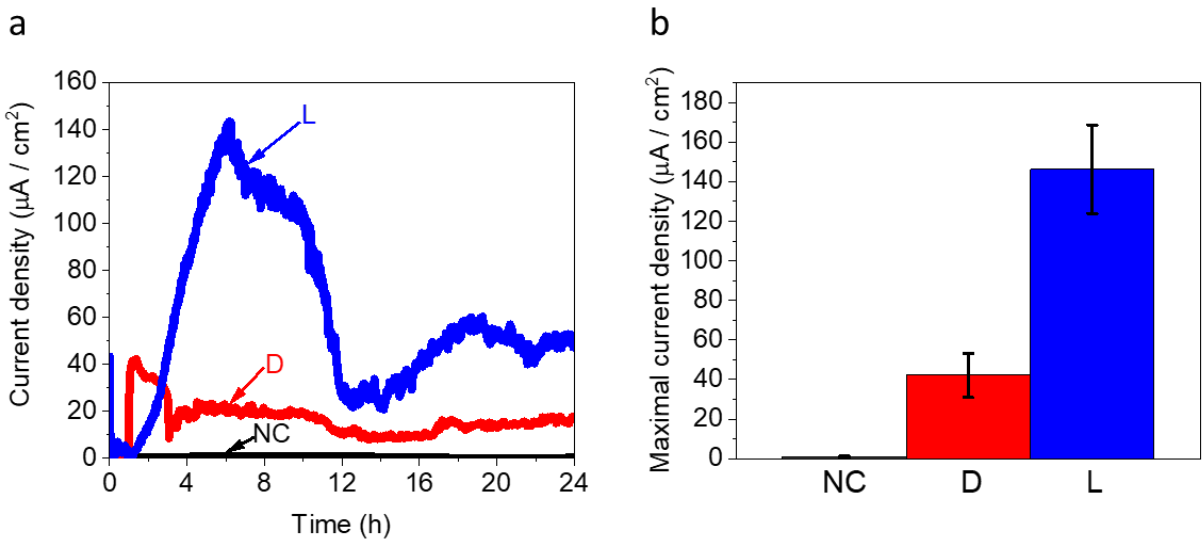

Fig.4 - Direct Bias-free current production from green onion roots in dark and light. CA measurements of green onion roots were conducted in dark (D), light (L), and with roots that are not connected to the anode on light (NC). **a** bias-free CA measurement over 24 h. **b** Maximal current over 24 h. The error bars represent the standard deviation over 3 independent measurements.

A model for electrical current production in roots-based BECs.

Previous studies showed that the metabolic pathways of photosynthesis and respiration are connected, while the NADH molecules that are formed in the TCA cycle can reduce the plastoquinone molecules that proceed into multistep redox reactions that end by the formation of NADPH[58]. In our work, we showed that NAD(P)H is being secreted from the roots, while its secretion is enhanced upon illumination. Based on the previous and our work, we suggest a model for the electron transfer mechanism, in which NADH, NADPH, and other unknown redox-active molecules can move between the leaves and the roots and be secreted outside to reduce the anode (Fig.5).

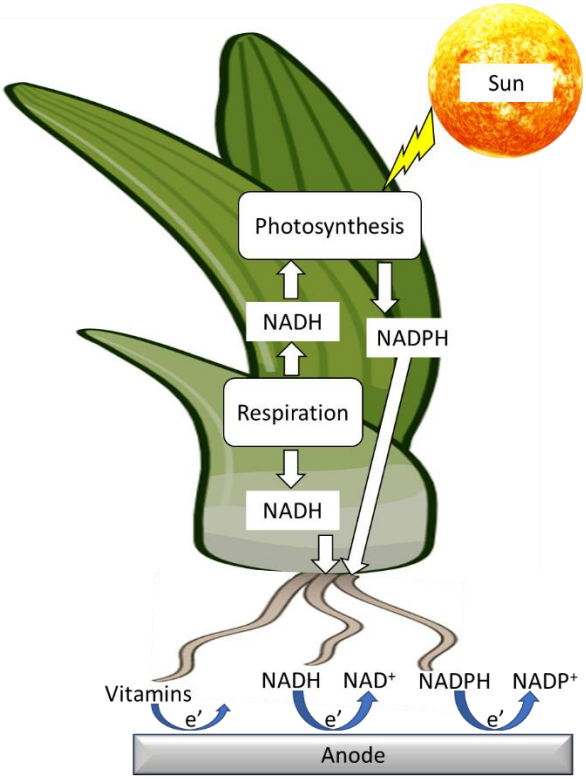

Fig.5 - A model for electrical current production in roots-based BECs. A schematic illustration of the suggested electron transport mechanism. NADH molecules that are formed in the respiration pathway can migrate towards the roots or continue to the photosynthesis pathway that produces NADPH, which can also migrate toward the roots. NADH, NADPH, and other unknown redox-active molecules can exit the roots to reduce the anode of the BEC and produce current. A Sharp yellow shape represents the illumination of the sun. White arrows represent the trafficking of NADH and NADPH between the metabolic pathways of Respiration and photosynthesis and their physical movement toward the roots. Round blue arrows represent the reduction of the anode by NADH, NADPH, and additional unknown redox-active molecules.

Conclusions

In this work, we show that the roots of green onion can secrete molecules that can reduce the electron acceptor Cyt. We identify secretion of the electron mediator NAD(P)H from the roots by 2D-fluorescence measurements. We present for the first time a BEC system that can produce electricity directly from intact roots for more than 24 h. We show that the current production is significantly

enhanced by illumination of the leaves of the onion, while no current is produced if the root is not directly attached to the anode. The ability to produce electrical current from roots may pave the way towards the establishment of novel renewable technologies for bioelectricity production.

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Conflicts of interest

There are no conflicts to declare.

Author contributions

YS and LS conceived the idea. YS and LS designed the experiments. YS performed the main experiments. YS and LS wrote the paper. LS supervised the entire research project.

Supplementary Information

Fig. S1

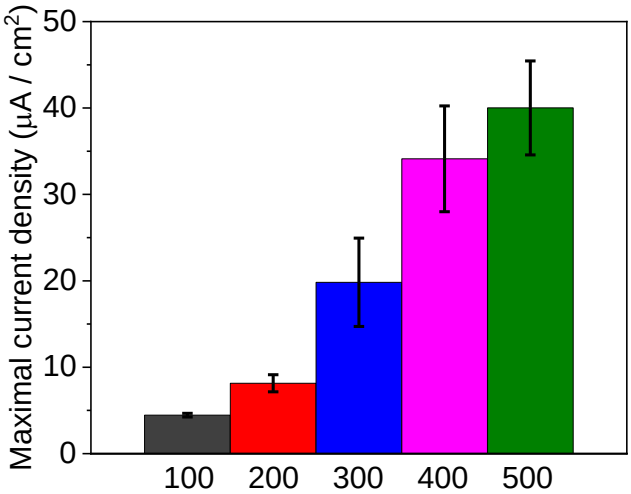

Fig. S1 – Electric current production from roots at increasing electrolyte salinity. CA of Onion's roots was measured in water solutions with various increasing salinities of 100, 200, 300, 400, and 500 mM NaCl. The error bars represent the standard deviation over 3 independent repetitions.

Fig. S2

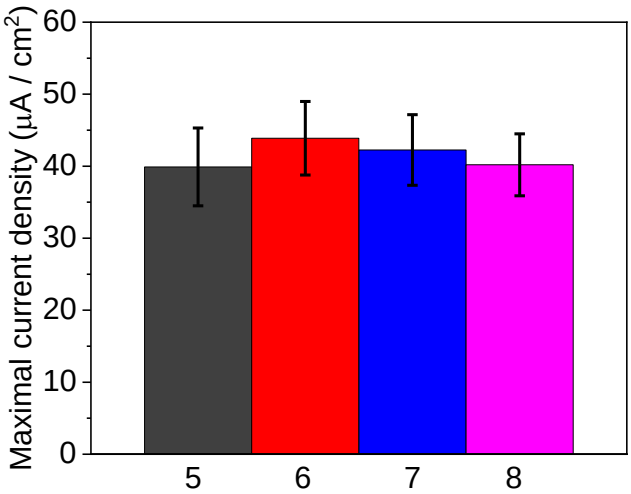

Fig. S2 – Electric current production from roots at increasing pH values. CA of Onion's roots was measured in a solution of 500 mM NaCl + MES buffer with increasing pH values of 5, 6, 7, and 8. The error bars represent the standard deviation over 3 independent repetitions.