Roots fuel cell produces and stores clean energy.

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Abstract

In recent years, extensive scientific efforts have been conducted to develop clean bio-energy technologies. A promising approach that has been under development for more than a hundred years is the microbial fuel cell (MFC) which utilizes exo-electrogenic bacteria as an electron source in a bio-electrochemical cell. The viability of bacteria in soil MFCs can be maintained by integrating plant roots which release organic materials that feed the bacteria. In this work, we show that rather than organic compounds, roots also release redox species that can produce electricity in a bio-fuel cell. We first study the reduction of the electron acceptor Cytochrome C by green onion roots. We integrate green onion roots into a bio-fuel cell to produce a continuous bias-free electric current for more than 24 h in the dark. This current is enhanced upon irradiation of light on the onion's leaves. We apply cyclic voltammetry and 2D-fluorescence measurements to show that NADH and NADPH act as major electron mediators between the roots and the anode, while their concentration in the external root matrix is increased upon irradiation of the leaves. Finally, we show that roots can contribute to energy storage by charging a supercapacitor.

1. Introduction

In recent years, many efforts have been made to replace fossil fuels with green energy technologies. The utilization of MFCs for electricity generation has been extensively studied for more than a hundred years.(Bond and Lovley, 2003; Menicucci et al., 2006; Min et al., 2005; Rabaey et al., 2005; Ramu et al., 2020; Rhoads et al., 2005; Ringeisen et al., 2006; Slate et al., 2019; Wei et al., 2016) In this method, bacteria acts as an electron donor at the anode (Fang et al., 2020) or an electron acceptor at the cathode (Bergel et al., 2005; Fang et al., 2020). The electron transfer between the exo-electrogenic bacteria and the anode may be conducted by either of two mechanisms: mediated electron transfer (MET) (Bond et al., 2002; Bond and Lovley, 2003; Chaudhuri and Lovley, 2003; Liu et al., 2006; Min et al., 2005; Pham et al., 2003) or direct electron transfer (DET) (Sekar et al., 2016; Thirumurthy et al., 2020; Wei et al., 2016). In the case of MET, redox-active metabolites such as derivatives of flavins, phenazines, or NADH (Shlosberg et al., 2023a) 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 (Huang et al., 2015; Lan et al., 2013; Laohavisit et al., 2015; McCormick, 2013, 2011; Ochiai et al., 1983). DET is performed by conductive *pili* (Heidary et al., 2020; Lovley, 2012; Nevin et al., 2008; Yi et al., 2009) or metal respiratory complexes (Hartshorne et al., 2007; Shi et al., 2012). Among the species that perform a high degree of DET are *Shewanella onedis* and *Geobacter* (Hartshorne et al., 2007; Heidary et al., 2020; Lovley, 2012; Nevin et al., 2008; Shi et al., 2012; Yi et al., 2009). The power generation of MFCs can be enhanced by implementing them into the soil, while environments with higher organic carbon content and lower pH enhance the power generation (Jiang et al., 2016).

Another approach that evolved from MFCs is the utilization of live photosynthetic unicellular organisms such as cyanobacteria or microalgae (Grattieri, 2020; Grattieri et al., 2019a, 2019b; Shlosberg et al., 2021b, 2020; Tschörtner et al., 2019). In this case, the electron source is photosynthesis where the electron mediation is mainly conducted by the redox couple $NADP^+$/NADPH that can cycle between the photosystems inside the organisms and the external anode (Shlosberg et al., 2020). The photocurrent can be enhanced and prolonged by the addition of exogenous NAD^+, $NADP^+$, or vitamin B1 (Shlosberg et al., 2022f, 2021b, 2020) that can enter the cells and accept an electron from photosystem I. Exogenous artificial electron mediators such as potassium ferricyanide (Lee et al., 2020) 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) (Shlosberg et al., 2022b) and green tissues of terrestrial plants such as leaves and stems (Shlosberg et al., 2022c). The photocurrent production of such systems is about 2-3 orders of magnitude greater than for microorganism-based bio-photo electrochemical cells (BPECs) (Shlosberg et al., 2022d). Interestingly, photocurrent may also be harvested from non-photosynthetic cells and organisms such as non-photosynthetic bacteria, (Shlosberg et al., 2023a) and fruits (Shlosberg et al., 2023b).

Rather than using live organisms, photoelectricity generation can be accomplished by isolating photosynthetic components at different degrees of purification, such as chloroplasts, Thylakoid membranes, and photosystems (Efrati, 2016; Efrati et al., 2013; Hartmann et al., 2020; Hasan et al., 2017; Li, 2016; Yehezkeli et al., 2012; Zhao et al., 2014) or by catalysis of isolated enzymes (Herkendell, 2021; Herkendell et al., 2017). In contrast with live organisms in which the addition of an external mediator is optional, isolated compounds are incapable of performing EET by themselves, and require the addition of an exogenous electron mediator (Pinhassi et al., 2016). Rather than making a specific isolation of a component of interest, a different approach utilizes yeast extracts. Similar to bacteria, live yeast may also conduct exo-electrogenic activity in MFCs (Bahartan et al., 2012). Yet, most of the reducing molecules are located inside the cell cytoplasm. Luria-Bertani media which is mostly based on yeast extract consists of a rich content of components which can produce photocurrent in biofuel cells (Shlosberg et al., 2022e).

An additional interesting method is plant-microbial fuel cells which integrate soil MFCs with plant farming. In this approach, plants release through their roots organic materials that are consumed by the bacteria enabling their cultivation in the soil (Moqsud et al., 2017; Strik et al., 2008).

Rather than secreting components, roots can sense and electrochemically interact with their environment. Such a phenotype can be observed in the 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 (Cassab, 2007; Volkov et al., 2013). The electro-tropic mechanism involves biomolecules that are capable of charge transfer. Integration of these electro-tropic additives in organic solar cells may increase their power efficiency (Karak et al., 2015). It was

previously reported that roots can reduce the molecular electron acceptors FeCN and 2,6-dichlorophenol indophenol (DCPIP) (Pardha-Saradhi et al., 2014).

In this work, we follow the ability of green onion roots to reduce Cytochrome C by absorption measurements. We show for the first time that electricity can be harvested directly from green onion roots by associating them with the anode in an electrochemical cell. This electricity generation is enhanced upon the illumination of the onions' leaves. We apply cyclic voltammetry and 2D fluorescence to detect the electron mediators NADH and NADPH (NAD(P)H) in the external matrix of the roots, suggesting that like in photosynthetic organisms based BPECs, NAD(P)H perform MET between the roots and the anode. Finally, we design a supercapacitor by laser ablation of graphene and show that it can be charged directly charged by the green onion's roots.

2. 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 an NADH calibration curve based on increasing concentrations measured at (λ_{Ex} = 350 nm, λ_{Em} = 450 nm).

Chronoamperometry measurements

CA measurements were conducted using a system designed based on the description in the previous work of Shlosberg et al.(Shlosberg et al., 2022b) 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-electrode mode without the application of electrical potential on the working electrode (WE). A stainless-steel clip was applied as the WE and a platinum wire as the counter electrode (CE). In all

measurements, the current density was calculated based on the surface area of the WE that was totally covered by roots of 0.08 cm^2.

Chronopotentiometry measurements

Chronopotentiometry was carried out using a Palmsens4 potentiostat. The potentiostat was connected to the supercapacitor in parallel with a connection to a roots fuel cell. The measurements were conducted under real sunlight whose intensity was measured by a light meter at the maximal height of the green onion leaves, measuring a sunlight intensity of about 150 W/m^2.

Cyclic voltammetry measurements

Cyclic voltammetry measurements were conducted using a palmsens4 potentiostat connected to screen-printed electrodes (Basi), with a graphite working electrode (1 mm in diameter), a graphite counter electrode, and a silver coated silver chloride reference electrode. Green onion roots were incubated in 0.1 M NaCl for 10 min in the dark or under white light illumination. Right after the incubation, a drop of 60 µL NaCl solution was placed on the screen-printed electrodes, and the CV was measured in the range of -1 – 1 V, with a scan rate of 0.1 V/s.

Supercapacitor fabrication

Supercapacitors were fabricated using a previously published method.(El-Kady and Kaner, 2013) Briefly, graphite oxide (GO) was synthesized using a modified Hummer's method and was made into an aqueous dispersion of 2.7 mg mL^{-1}. A sheet of polyethylene terephthalate (PET) plastic film was cut and glued onto a LightScribe disc. 16 mL of the stock GO dispersion were then drop-cast on the PET film. After drying overnight under ambient conditions, the prepared GO on PET film was laser-scribed with the desired interdigitated pattern using a LightScribe DVD burner. Subsequently, the edges of the supercapacitor were painted with conductive silver paint, and copper tape was applied on top to act as a current collector. Finally, polyimide Kapton was used to cover the supercapacitor leaving only the interdigitated electrodes exposed.

3. Results and Discussion

3.1. Onion roots can 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 10 roots of green onion in a solution of 30

μM Cytochrome C (Cyt) for 10 min. The absorption spectra of the pure Cyt solution and the roots were measured at 1 min time intervals. The obtained spectra showed an increase in the peak with a maxima at λ = 550 nm that correlated with the increasing incubation time (**Fig. 1a**). This intensity peak derives from the reduction of Cyt.(Larom et al., 2015) However, the increase in the incubation time has also elevated the baseline intensity of the whole measured spectrum λ = 475 – 550 nm, probably because of the secretion of molecules that also absorb in this 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) from their minima (λ = 540 nm) (**Fig. 1b**). This analysis showed that a maximal reduction of Cyt (that representing EET of 0.15 μmol electrons) was obtained after 8 min.

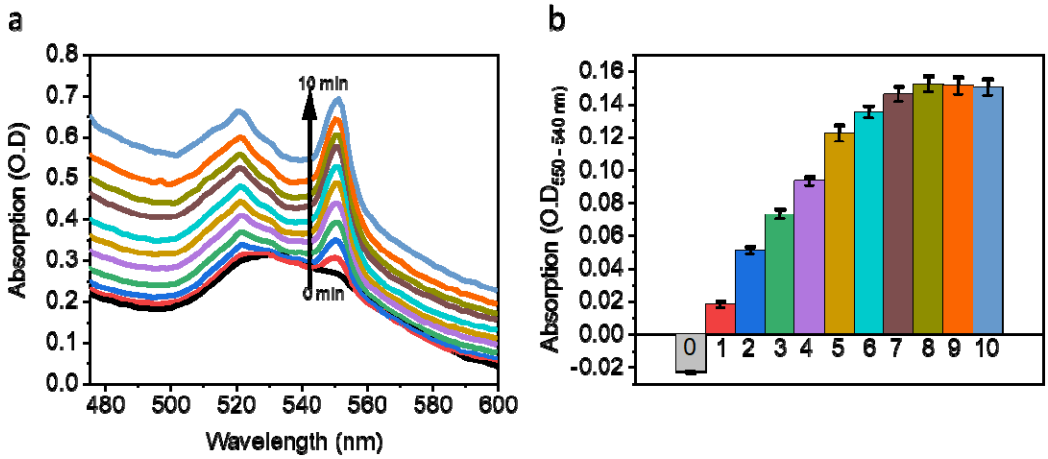

Fig. 1 – Onion roots reduce the electron acceptor Cytochrome C.

Ten green onion roots were incubated for 10 min with Cyt. The absorption spectra were measured before the incubation and then every 1 min. **a** Absorption spectra of the Cyt solution as a function of incubation time. 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 dependent on the absorption of additional components in the solution. The error bars represent the standard deviation over 3 independent repetitions.

3.2. NAD P H is released from roots

Previous studies about fuel cells based on various cells and organisms such as photosynthetic organisms,(Shlosberg et al., 2022d) marine animals, (Shlosberg et al., 2022a) showed that part of the electrical current production derives from the secretion of the redox-active molecules NADH or NADPH (NAD(P)H). In the case of plants, 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 (Shlosberg et al., 2022c). To elucidate this, green onion roots were incubated in 0.1 M NaCl solution for 10 min in the dark and in high light. The plant (with the roots) was 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 (Shlosberg et al., 2021a, 2020). Interestingly, as previously reported for photosynthetic organisms, (Shlosberg et al., 2022d) 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 that release it to donate electrons at 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) with increasing NADH concentrations. The NAD(P)H concentrations were determined to be ~0.15 and 0.27 µM in dark and light, respectively (**Fig. 2c**) (that is equivalent to 15 and 27 nmol). Since unknown reducing molecules rather than NAD(P)H may be secreted from the roots, we wished to estimate the contribution of NAD(P)H to the total reduction of Cyt reduction (**Fig. 1**). To evaluate this, the number of secreted NAD(P)H molecules in the 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 shows that NAD(P)H can reduce about 10% of the Cyt molecules. Based on these results, we suggest that the roots secrete additional reducing molecules rather than just NAD(P)H. However, it is possible that more than 10% of Cyt derives from NAD(P)H reduction if some of the $NAD(P)^+$ or NAD(P)H molecules are being up taken up by the roots. Such a cyclic electron transport mechanism was previously reported for various cyanobacterial species(Shlosberg et al., 2020) and microalgae(Shlosberg et al., 2021b) in which NAD(P)H was the major electron mediator. Roots secrete many kinds of components such as minerals, proteins, carbohydrates, and vitamins (Greisenegger, J. K.; Vorbuchner, 1920; Gvamichava, 1966). We postulated that some of the reported vitamins that are capable of charge transfer such as ascorbic acid, thiamine, and nicotinic acid, may also play a role in the reduction of Cyt. To further study the released redox active species, we applied cyclic voltammetry measurements on an 0.1 M NaCl solution after incubation for 10 min with the roots in the dark and in the light (**Fig. 2d**). A small peak was obtained around 0.5 V in the dark, which may originate from the presence of ascorbic acid (Shlosberg et al., 2023b). After illumination, a bigger peak appeared around 0.6 V (that may have masked the

small peak at 0.5 V) which may correspond to NADPH (Wang et al., 2021). The diagonal trend of the obtained voltammogram indicates the presence of non-conductive organic materials in the measured solution whose release from the roots is in agreement with previous studies (Moqsud et al., 2017; Strik et al., 2008).

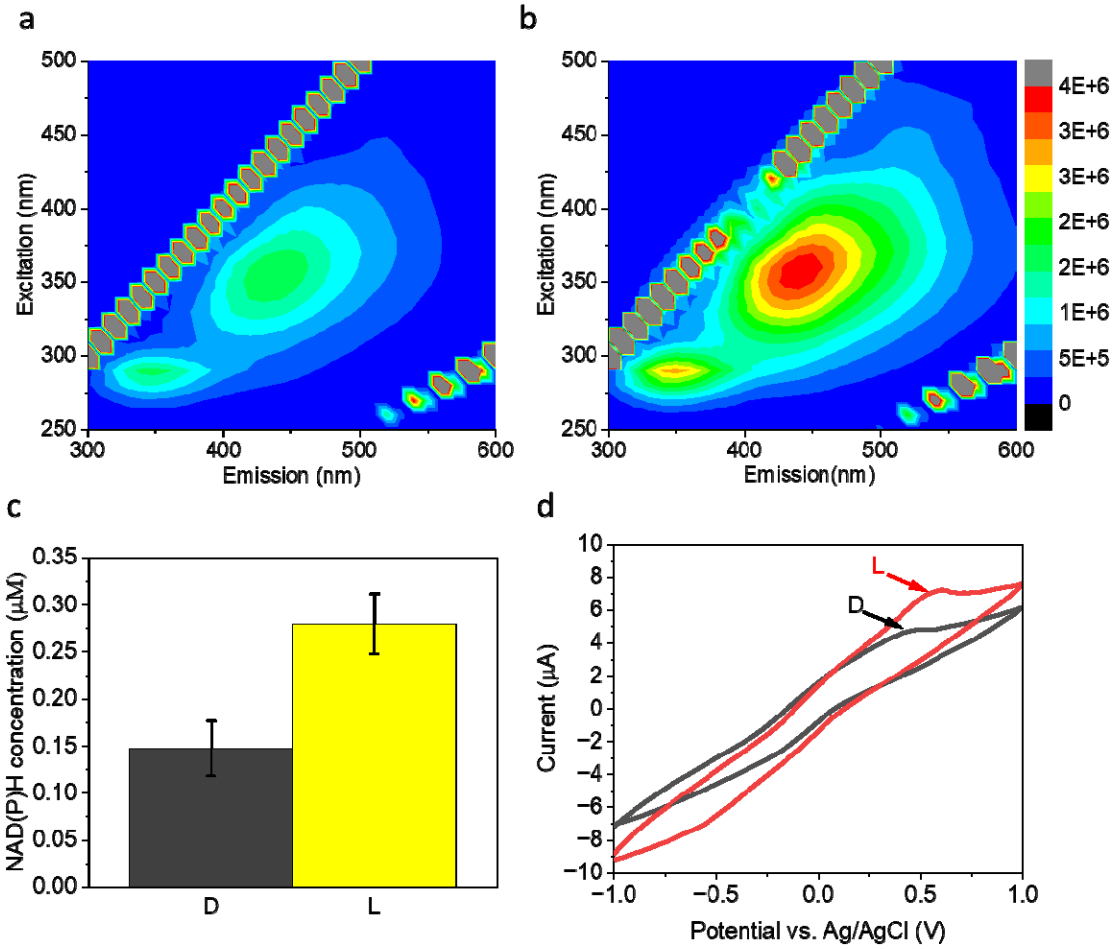

Fig. 2 – NAD(P)H is secreted from roots. Ten green onion roots were incubated in 0.1 M NaCl solution for 10 min with or without the illumination of the onion's leaves. Following the incubation, the 2D-FM and cyclic voltammetry of the solutions were measured. **a** 2D-FM after incubation in the dark.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

3.3. Harvesting bio-electricity from roots

Based on our results (**Fig. 1**), and previous works that reported the ability of roots to reduce electron acceptors in solution, (Pardha-Saradhi et al., 2014) we thought that the roots could produce an electric current in a bio-fuel cell. For this purpose, a new setup was designed (**Fig.**

3a-c). The setup consists of a rectangular glass vessel with 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 roots were attached to 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 LEDs that were placed on a stand ~10 cm from the leaves. A similar setup that utilizes a stainless steel clip as an anode was previously reported for seaweeds and plant leaves based BPECs (Shlosberg et al., 2022b, 2022c). To optimize the NaCl concentration, Chronoamperometry (CA) of increasing NaCl concentrations (100 – 500 mM) was measured using a 2-electrode mode without the application of an electrical potential bias (bias-free) for 24 h in the dark. The maximal current density that was obtained for the NaCl concentrations: 0.1, 0.2, 0.3, 0.4, and 0.5 M were ~4.5, 8, 20, 35, and 40 $\mu A/cm^2$, respectively (**Fig. S1**). This electrolyte concentration of 500 mM was also previously determined to be optimal in seaweed and plant leave-based BPECs (Shlosberg et al., 2022b, 2022c).

Next, 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 in this range did not have any significant influence on the current production (**Fig. S2**). Previous studies on BPECs that were based on photosynthetic micro, (Shlosberg et al., 2021b, 2020) and macro- organisms (Shlosberg et al., 2022b, 2022c) suggested that although the mechanism of the electron transfer as conducted by diffusive 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 must 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 the dark, in the light and without a direct association of the roots with 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 the dark and in the light were ~40 and 140 $\mu A/cm^2$, respectively (**Fig. 3d,e**). 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 donate electrons at the anode.

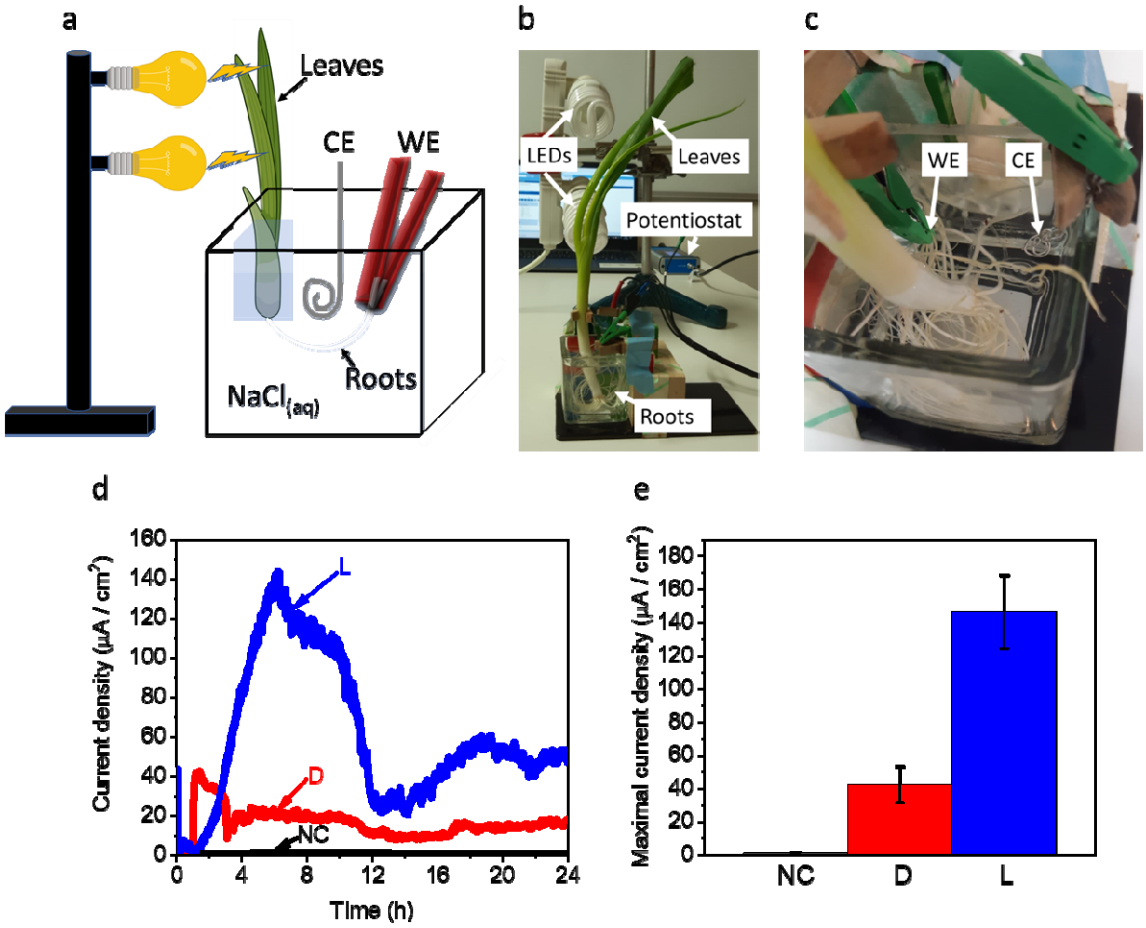

Fig. 3 - Harvesting bioelectricity from roots.

Bias-free CA measurements of green onion roots in a bio-fuel cell in the dark and in light. **a** A schematic illustration of the system: the bottom of a green onion was placed inside the NaCl solution. The edge of the roots was attached to stainless-steel clips (red) that were used as the working electrode (WE), and a platinum wire (gray) was used as a counter electrode (CE). The leaves of the onion were illuminated by white LEDs (100 W) that were 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. **d** CA measurements of green onion roots were conducted over 24 h in the dark (D), in the light (L), and with roots that were not connected to the anode in the light (NC). **e** Maximal current over 24 h. The error bars represent the standard deviation over 3 independent measurements.

3.4. Charging a supercapacitor by a roots fuel cell

Electricity production may be used by connecting the biofuel cell directly to the electric grid. However, in many cases, the production is conducted far from the grid or generates more electricity than is needed at a specific time point. To improve energy management, the energy may be stored and used in a time of need. To demonstrate the ability to store energy that is

produced by roots bio-fuel cells, we wished to charge a supercapacitor. We fabricated a supercapacitor that consists of graphene/graphene oxide (**Fig. 4a**) by laser ablation as reported in our previous work.(Shlosberg et al., n.d.) We designed a system that consists of 2 beakers with a 50 mL solution of 0.5 M NaCl. The first beaker consisted of green onion stainless steel anode clips and a platinum wire as a cathode. Five roots were connected to the anode. Connective leads were wired from the anode and cathode to the graphene/graphene oxide supercapacitor that was placed inside 0.5 M NaCl solution in the second beaker. A potentiostat was connected to the supercapacitor in parallel with the bio-fuel cell (**Fig. 4b**). Chronopotentiometry (CP) was measured under real sunlight illumination (150 W/m^2) for 10 min. A NaCl solution without the roots was applied as a control experiment. A potential increase was obtained only with the onion roots reaching a maximal potential of 4.5 V after 10 min (**Fig. 4c,d**).

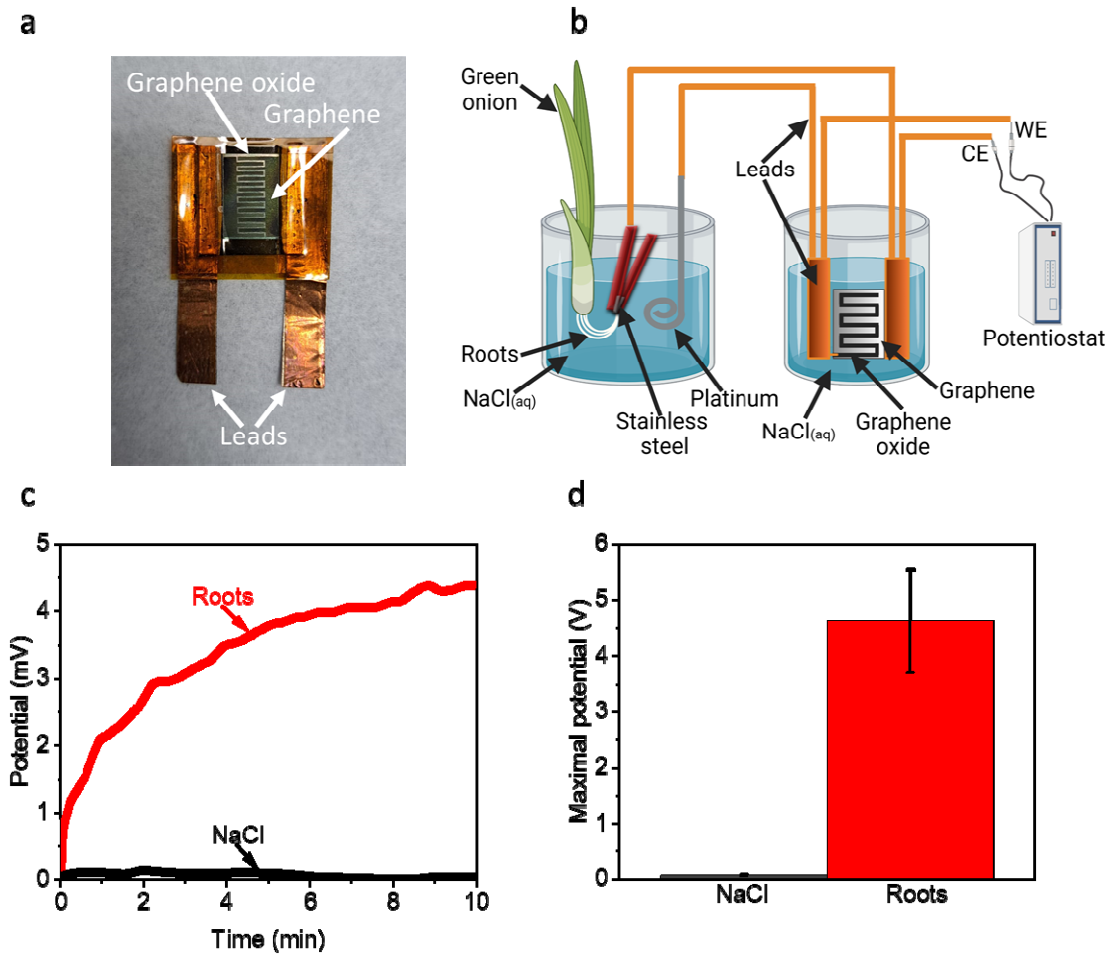

Fig. 4 - Charging a supercapacitor by a roots fuel cell. A biofuel cell based on green onion roots was applied to charge a graphite oxide/graphene supercapacitor. **a** A photo of the supercapacitor. **b** A schematic description

of the system. The system consists of 2 beakers with 0.5 M NaCl solution. The first beaker is the bio-fuel cell which consists of the bottom of a green onion with 5 roots connected to a stainless-steel anode and a platinum cathode. The anode and the cathode were wired to a graphene/graphene oxide-based supercapacitor placed inside the second beaker. The supercapacitor was also connected in parallel to the working electrode (WE) and counter electrode (CE) leads to a potentiostat to measure its charging. The experiment was conducted under real sunlight conditions (~150 W/m^2). **c** Chronopotentiometry measurements with and without the green onion roots. **d** Maximal potential with and without roots over 10 min. The error bars represent the standard deviation over 3 independent repetitions.

3.5. Proposing a mechanism for the electrical current production in root fuel cells

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 in the formation of NADPH (Saper et al., 2018). In our work, we showed that NADH and NADPH are being released from the roots, while their excretion is enhanced upon illumination. Based on the previous work and ours, we propose a mechanism for electron transfer (**Fig. 5**). NADH and other unknown redox-active biomolecules such as vitamins can be released from the roots to donate electrons at the anode. Upon solar illumination, NADPH is formed in the photosynthetic pathway while some of it moves from the leaves to the roots and is released to donate electrons at the anode. The oxidized forms NAD^+ and $NADP^+$ may be taken up by the roots and be reduced inside the onion.

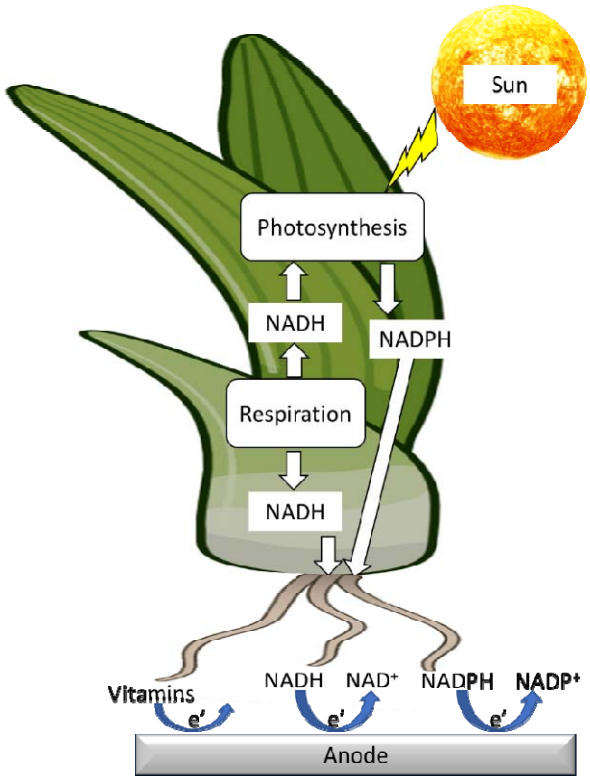

Fig. 5 - Proposed mechanism for the electrical current production in root fuel cells. A schematic illustration of the suggested electron transport mechanism. NADH molecules that are formed in the respiration pathway can migrate toward the roots or continue to the photosynthesis pathway that produces NADPH, which can also migrate toward the roots. NADH, NADPH, and other unknown redox-active molecules can exit the roots to donate electrons at the anode to produce current. The yellow lightening bolt represents illumination from the sun. White arrows represent the movement of NADH and NADPH between the metabolic pathways of respiration and photosynthesis and their physical movement toward the roots. The round blue arrows represent the reduction of the anode by NADH, NADPH, and additional unknown redox-active molecules.

4. Conclusions

In this work, we show that the roots of green onions can release molecules that can reduce the electron acceptor Cyt. We apply 2D-fluorescence and cyclic voltammetry measurements to identify the secretion of the electron mediators NADH and NADPH from the roots. We show for the first time a bio-fuel cell that can produce bias-free electricity directly from intact roots for more than 24 h. This current production is significantly enhanced by the illumination of the onion's leaves. Finally, we show the ability of the roots fuel cell to store energy by charging a supercapacitor. The ability to produce electrical current from roots especially in hydroponic cultures may pave the way toward the establishment of novel renewable energy technologies that supply the energy demand of green houses facilities. Such technologies can

be based on a circular economy model in which agricultural crops will be used for both food and energy generation and storage.

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Author contributions

Y.S. conceived the idea. Y.S. and A.H. designed the experiments. Y.S. and A.H. performed the main experiments. Y.S., A.H, T.N.T., and R.B.K. wrote the paper. T.N.T and R.B.K. supervised the entire research project.

Conflicts of interest

There are no conflicts to declare.

References

Bahartan, K., Amir, L., Israel, A., Lichtenstein, R., Alfonta, L., 2012. In stu fuel processing in a microbial fuel cell. ChemSusChem 5, 1820–1825. https://doi.org/10.1002/cssc.201200063

Bergel, A., Féron, D., Mollica, A., 2005. Catalysis of oxygen reduction in PEM fuel cell by seawater biofilm. Electrochem. commun. 7, 900–904. https://doi.org/10.1016/j.elecom.2005.06.006

Bond, D.R., Holmes, D.E., Tender, L.M., Lovley, D.R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments.

Bond, D.R., Lovley, D.R., 2003. Electricity Production by Geobacter sulfurreducens Attached to Electrodes. Appl. Environ. Microbiol. 69, 1548–1555. https://doi.org/10.1128/AEM.69.3.1548-1555.2003

Cassab, G.I., 2007. Other Tropisms and their Relationship to Gravitropism, in: Plant Tropisms. John Wiley & Sons, Ltd, pp. 123–139.

https://doi.org/https://doi.org/10.1002/9780470388297.ch6

Chaudhuri, S.K., Lovley, D.R., 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat. Biotechnol. 21, 1229–1232. https://doi.org/10.1038/nbt867

Efrati, A., 2016. Assembly of photo-bioelectrochemical cells using photosystem I-functionalized electrodes. Nat. Energy 1. https://doi.org/10.1038/nenergy.2015.21

Efrati, A., Tel-Vered, R., Michaeli, D., Nechushtai, R., Willner, I., 2013. Cytochrome c-coupled photosystem I and photosystem II (PSI/PSII) photo-bioelectrochemical cells. Energy Environ. Sci. 6, 2950–2956. https://doi.org/10.1039/c3ee41568f

El-Kady, M.F., Kaner, R.B., 2013. Scalable fabrication of high-power graphene micro-supercapacitors for flexible and on-chip energy storage. Nat. Commun. 4, 1475. https://doi.org/10.1038/ncomms2446

Fang, X., Kalathil, S., Reisner, E., 2020. Semi-biological approaches to solar-to-chemical conversion. Chem. Soc. Rev. 49, 4926–4952. https://doi.org/10.1039/c9cs00496c

Grattieri, M., 2020. Purple bacteria photo-bioelectrochemistry: enthralling challenges and opportunities. Photochem. Photobiol. Sci. 19, 424–435. https://doi.org/10.1039/C9PP00470J

Grattieri, M., Beaver, K., Gaffney, E.M., Minteer, S.D., 2019a. Tuning purple bacteria salt-tolerance for photobioelectrochemical systems in saline environments. Faraday Discuss. 215, 15–25. https://doi.org/10.1039/C8FD00160J

Grattieri, M., Rhodes, Z., Hickey, D.P., Beaver, K., Minteer, S.D., 2019b. Understanding Biophotocurrent Generation in Photosynthetic Purple Bacteria. ACS Catal. 9, 867–873. https://doi.org/10.1021/acscatal.8b04464

Greisenegger, J. K.; Vorbuchner, K., 1920. Root excretion. Oster.-ungar. Z. Zuckerind. und Landw. 47, 82–91.

Gvamichava, N.E., 1966. Vitamin secretion by roots. Fiziol. Drev. Rastenii 2, 5–16.

Hartmann, V., Harris, D., Bobrowski, T., Ruff, A., Frank, A., Günther Pomorski, T., Rögner, M., Schuhmann, W., Adir, N., Nowaczyk, M.M., 2020. Improved quantum efficiency in an engineered light harvesting/photosystem II super-complex for high current density

biophotoanodes. J. Mater. Chem. A. https://doi.org/10.1039/D0TA03444D

Hartshorne, R.S., Jepson, B.N., Clarke, T.A., Field, S.J., Fredrickson, J., Zachara, J., Shi, L., Butt, J.N., Richardson, D.J., 2007. Characterization of Shewanella oneidensis MtrC: a cell-surface decaheme cytochrome involved in respiratory electron transport to extracellular electron acceptors. JBIC J. Biol. Inorg. Chem. 12, 1083–1094. https://doi.org/10.1007/s00775-007-0278-y

Hasan, K., Milton, R.D., Grattieri, M., Wang, T., Stephanz, M., Minteer, S.D., 2017. Photobioelectrocatalysis of Intact Chloroplasts for Solar Energy Conversion. ACS Catal. 7, 2257–2265. https://doi.org/10.1021/acscatal.7b00039

Heidary, N., Kornienko, N., Kalathil, S., Fang, X., Ly, K.H., Greer, H.F., Reisner, E., 2020. Disparity of Cytochrome Utilization in Anodic and Cathodic Extracellular Electron Transfer Pathways of Geobacter sulfurreducens Biofilms. J. Am. Chem. Soc. 142, 5194–5203. https://doi.org/10.1021/jacs.9b13077

Herkendell, K., 2021. Status update on bioelectrochemical systems: prospects for carbon electrode design and scale-up. Catalysts 11. https://doi.org/10.3390/catal11020278

Herkendell, K., Tel-Vered, R., Stemmer, A., 2017. Switchable aerobic/anaerobic multi-substrate biofuel cell operating on anodic and cathodic enzymatic cascade assemblies. Nanoscale 9, 14118–14126. https://doi.org/10.1039/C7NR06233H

Huang, L.F., Lin, J.Y., Pan, K.Y., Huang, C.K., Chu, Y.K., 2015. Overexpressing ferredoxins in Chlamydomonas reinhardtii increase starch and oil yields and enhance electric power production in a photo microbial fuel cell. Int. J. Mol. Sci. 16, 19308–19325. https://doi.org/10.3390/ijms160819308

Jiang, Y.-B., Zhong, W.-H., Han, C., Deng, H., 2016. Characterization of electricity generated by soil in microbial fuel cells and the isolation of soil source exoelectrogenic bacteria. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01776

Karak, S., Page, Z.A., Tinkham, J.S., Lahti, P.M., Emrick, T., Duzhko, V. V, 2015. Raising efficiency of organic solar cells with electrotropic additives. Appl. Phys. Lett. 106, 103303. https://doi.org/10.1063/1.4914847

Lan, J.C.W., Raman, K., Huang, C.M., Chang, C.M., 2013. The impact of monochromatic blue and red LED light upon performance of photo microbial fuel cells (PMFCs) using

Chlamydomonas reinhardtii transformation F5 as biocatalyst. Biochem. Eng. J. 78, 39–43. https://doi.org/https://doi.org/10.1016/j.bej.2013.02.007

Laohavisit, A., Anderson, A., Bombelli, P., Jacobs, M., Howe, C.J., Davies, J.M., Smith, A.G., 2015. Enhancing plasma membrane NADPH oxidase activity increases current output by diatoms in biophotovoltaic devices. Algal Res. 12, 91–98. https://doi.org/10.1016/j.algal.2015.08.009

Larom, S., Kallmann, D., Saper, G., Pinhassi, R., Rothschild, A., Dotan, H., Ankonina, G., Schuster, G., Adir, N., 2015. The Photosystem II D1-K238E mutation enhances electrical current production using cyanobacterial thylakoid membranes in a bio-photoelectrochemical cell. Photosynth. Res. 126, 161–169. https://doi.org/10.1007/s11120-015-0075-3

Lee, J., Cho, H., Kim, S., 2020. Enhanced Photocurrent Generation From a Single-Mediated Photo-Bioelectrochemical Cell Using Wild-Type Anabaena Variabilis Dispersed in Solution. ChemElectroChem 7, 4075–4083. https://doi.org/https://doi.org/10.1002/celc.202001026

Li, J., 2016. Integrating photosystem II into a porous TiO 2 nanotube network toward highly efficient photo-bioelectrochemical cells. J. Mater. Chem. A 4. https://doi.org/10.1039/C6TA04964H

Liu, Z.-D., Lian, J., Du, Z.-W., Li, H.-R., 2006. Construction of sugar-based microbial fuel cells by dissimilatory metal reduction bacteria. Chin. J. Biotechnol. 22, 131–137. https://doi.org/https://doi.org/10.1016/S1872-2075(06)60010-1

Lovley, D.R., 2012. Electromicrobiology. Annu. Rev. Microbiol. 66, 391–409. https://doi.org/10.1146/annurev-micro-092611-150104

McCormick, A.J., 2013. Hydrogen production through oxygenic photosynthesis using the cyanobacterium Synechocystis sp. PCC 6803 in a bio-photoelectrolysis cell (BPE) system. Energy Environ. Sci. 6. https://doi.org/10.1039/c3ee40491a

McCormick, A.J., 2011. Photosynthetic biofilms in pure culture harness solar energy in a mediatorless bio-photovoltaic cell (BPV) system. Energy Environ. Sci. 4. https://doi.org/10.1039/c1ee01965a

Menicucci, J., Beyenal, H., Marsili, E., Veluchamy, Demir, G., Lewandowski, Z., 2006.

Procedure for determining maximum sustainable power generated by microbial fuel cells. Environ. Sci. Technol. 40, 1062–1068. https://doi.org/10.1021/es051180l

Min, B., Cheng, S., Logan, B.E., 2005. Electricity generation using membrane and salt bridge microbial fuel cells. Water Res. 39, 1675–1686. https://doi.org/https://doi.org/10.1016/j.watres.2005.02.002

Moqsud, M.A., Gazali, T.A., Omine, K., Nakata, Y., 2017. Green electricity by water plants in organic soil and marine sediment through microbial fuel cell. Energy Sources, Part A Recover. Util. Environ. Eff. 39, 160–165. https://doi.org/10.1080/15567036.2016.1159263

Nevin, K.P., Richter, H., Covalla, S.F., Johnson, J.P., Woodard, T.L., Orloff, A.L., Jia, H., Zhang, M., Lovley, D.R., 2008. Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells. Environ. Microbiol. 10, 2505–2514. https://doi.org/10.1111/j.1462-2920.2008.01675.x

Ochiai, H., Shibata, H., Sawa, Y., Shoga, M., Ohta, S., 1983. Properties of semiconductor electrodes coated with living films of cyanobacteria. Appl. Biochem. Biotechnol. 8, 289–303. https://doi.org/10.1007/BF02779496

Pardha-Saradhi, P., Yamal, G., Peddisetty, T., Sharmila, P., Nagar, S., Singh, J., Nagarajan, R., Rao, K.S., 2014. Reducing Strength Prevailing at Root Surface of Plants Promotes Reduction of Ag+ and Generation of Ag0/Ag2O Nanoparticles Exogenously in Aqueous Phase. PLoS One 9, 1–13. https://doi.org/10.1371/journal.pone.0106715

Pham, C.A., Jung, S.J., Phung, N.T., Lee, J., Chang, I.S., Kim, B.H., Yi, H., Chun, J., 2003. A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell. FEMS Microbiol. Lett. 223, 129–134. https://doi.org/10.1016/S0378-1097(03)00354-9

Pinhassi, R.I., Kallmann, D., Saper, G., Dotan, H., Linkov, A., Kay, A., Liveanu, V., Schuster, G., Adir, N., Rothschild, A., 2016. Hybrid bio-photo-electro-chemical cells for solar water splitting. Nat. Commun. 7, 1–10. https://doi.org/10.1038/ncomms12552

Rabaey, K., Boon, N., Höfte, M., Verstraete, W., 2005. Microbial Phenazine production enhances electron transfer in biofuel cells. Environ. Sci. Technol. 39, 3401–3408. https://doi.org/10.1021/es048563o

Ramu, S.M., Thulasinathan, B., Gujuluva Hari, D., Bora, A., Jayabalan, T., Mohammed, S.N., Doble, M., Arivalagan, P., Alagarsamy, A., 2020. Fermentative hydrogen production and bioelectricity generation from food based industrial waste: An integrative approach. Bioresour. Technol. 310, 123447. https://doi.org/https://doi.org/10.1016/j.biortech.2020.123447

Rhoads, A., Beyenal, H., Lewandowski, Z., 2005. Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant. Environ. Sci. Technol. 39, 4666–4671. https://doi.org/10.1021/es048386r

Ringeisen, B.R., Henderson, E., Wu, P.K., Pietron, J., Ray, R., Little, B., Biffinger, J.C., Jones-Meehan, J.M., 2006. High power density from a miniature microbial fuel cell using shewanella oneidensis DSP10. Environ. Sci. Technol. 40, 2629–2634. https://doi.org/10.1021/es052254w

Saper, G., Kallmann, D., Conzuelo, F., Zhao, F., Tóth, T.N., Liveanu, V., Meir, S., Szymanski, J., Aharoni, A., Schuhmann, W., Rothschild, A., Schuster, G., Adir, N., 2018. Live cyanobacteria produce photocurrent and hydrogen using both the respiratory and photosynthetic systems. Nat. Commun. 9, 1–9. https://doi.org/10.1038/s41467-018-04613-x

Sekar, N., Jain, R., Yan, Y., Ramasamy, R.P., 2016. Enhanced photo-bioelectrochemical energy conversion by genetically engineered cyanobacteria. Biotechnol. Bioeng. 113, 675–679. https://doi.org/10.1002/bit.25829

Shi, L., Rosso, K.M., Zachara, J.M., Fredrickson, J.K., 2012. Mtr extracellular electron-transfer pathways in Fe(III)-reducing or Fe(II)-oxidizing bacteria: A genomic perspective. Biochem. Soc. Trans. 40, 1261–1267. https://doi.org/10.1042/BST20120098

Shlosberg, Y., Brekhman, V., Lotan, T., Sepunaru, L., 2022a. Direct electricity production from Nematostella and Arthemia's Eggs in a Bio-Electrochemical Cell. Int. J. Mol. Sci. 23. https://doi.org/10.3390/ijms232315001

Shlosberg, Y., Eichenbaum, B., Tóth, T.N., Levin, G., Liveanu, V., Schuster, G., Adir, N., 2020. NADPH performs mediated electron transfer in cyanobacterial-driven bio-photoelectrochemical cells. iScience 24, 101892.

Shlosberg, Y., Farber, Y., Hasson, S., Bulatov, V., Schechter, I., 2021a. Fast label-free identification of bacteria by synchronous fluorescence of amino acids. Anal. Bioanal. Chem. 413. https://doi.org/10.1007/s00216-021-03642-8

Shlosberg, Y., Faynus, M.A., Huang, A., Carlini, A.S., Clegg, D.O., Kaner, R.B., n.d. Mammalian Fuel Cells Produce Clean Energy. Available SSRN 4388844.

Shlosberg, Y., Krupnik, N., Tóth, T.N., Eichenbaum, B., Meirovich, M.M., Meiri, D., Yehezkeli, O., Schuster, G., Israel, Á., Adir, N., 2022b. Bioelectricity generation from live marine photosynthetic macroalgae. Biosens. Bioelectron. 198, 113824. https://doi.org/https://doi.org/10.1016/j.bios.2021.113824

Shlosberg, Y., Limwongyut, J., Moreland, A.S., Bazan, G.C., 2023a. Non-photosynthetic bacteria produce photocurrent mediated by NADH. bioRxiv. https://doi.org/10.1101/2023.01.16.524302

Shlosberg, Y., Meirovich, M., Yehezkeli, O., Schuster, G., Adir, N., 2022c. Production of photocurrent and hydrogen gas from intact plant leaves. Biosens. Bioelectron. 114558. https://doi.org/https://doi.org/10.1016/j.bios.2022.114558

Shlosberg, Y., Rubino, K.C., Nasseri, N.S., Carlini, A.S., 2023b. Photocurrent Production from Cherries in a Bio-Electrochemical Cell. Electrochem 4, 47–55. https://doi.org/10.3390/electrochem4010005

Shlosberg, Y., Schuster, G., Adir, N., 2022d. Harnessing photosynthesis to produce electricity using cyanobacteria, green algae, seaweeds, and plants. Front. Plant Sci. https://doi.org/10.3389/fpls.2022.955843

Shlosberg, Y., Smith, M., Carlini, A., 2022e. Redox-active molecules in bacterial cultivation media produce photocurrent. bioRxiv. https://doi.org/10.1101/2022.12.03.518941

Shlosberg, Y., Spungin, D., Schuster, G., Frank, I.-B., Adir, N., 2022f. Trichodesmium Erythraeum produces a higher photocurrent than other cyanobacterial species in bio-photo electrochemical cells. Biochim. Biophys. Acta Bioenerg. https://doi.org/10.1101/2022.01.23.477402

Shlosberg, Y., Tóth, T.N., Eichenbaum, B., Keysar, L., Schuster, G., Adir, N., 2021b. Electron Mediation and Photocurrent Enhancement in Dunalliela salina Driven Bio-Photo Electrochemical Cells. Catal. 2021, Vol. 11, Page 1220 11, 1220.

https://doi.org/10.3390/CATAL11101220

Slate, A.J., Whitehead, K.A., Brownson, D.A.C., Banks, C.E., 2019. Microbial fuel cells: An overview of current technology. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2018.09.044

Strik, D.P.B.T.B., Hamelers (Bert), H.V.M., Snel, J.F.H., Buisman, C.J.N., 2008. Green electricity production with living plants and bacteria in a fuel cell. Int. J. Energy Res. 32, 870–876. https://doi.org/https://doi.org/10.1002/er.1397

Thirumurthy, M.A., Hitchcock, A., Cereda, A., Liu, J., Chavez, M.S., Doss, B.L., Ros, R., El-Naggar, M.Y., Heap, J.T., Bibby, T.S., Jones, A.K., 2020. Type IV Pili-Independent Photocurrent Production by the Cyanobacterium Synechocystis sp. PCC 6803. Front. Microbiol. 11, 1–11. https://doi.org/10.3389/fmicb.2020.01344

Tschörtner, J., Lai, B., Krömer, J.O., 2019. Biophotovoltaics: Green Power Generation From Sunlight and Water. Front. Microbiol. 10, 866. https://doi.org/10.3389/fmicb.2019.00866

Volkov, A.G., Volkova, M.I., Markin, V.S., 2013. Phytosensors and Phytoactuators. {ECS} Trans. 50, 3–11. https://doi.org/10.1149/05012.0003ecst

Wang, H.-W., Bringans, C., Hickey, A.J.R., Windsor, J.A., Kilmartin, P.A., Phillips, A.R.J., 2021. Cyclic Voltammetry in Biological Samples: A Systematic Review of Methods and Techniques Applicable to Clinical Settings. Signals 2, 138–158. https://doi.org/10.3390/signals2010012

Wei, X., Lee, H., Choi, S., 2016. Biopower generation in a microfluidic bio-solar panel 228, 151–155. https://doi.org/10.1016/j.snb.2015.12.103

Yehezkeli, O., Tel-Vered, R., Wasserman, J., Trifonov, A., Michaeli, D., Nechushtai, R., Willner, I., 2012. Integrated photosystem II-based photo-bioelectrochemical cells. Nat. Commun. 3, 1–7. https://doi.org/10.1038/ncomms1741

Yi, H., Nevin, K.P., Kim, B.-C., Franks, A.E., Klimes, A., Tender, L.M., Lovley, D.R., 2009. Selection of a variant of Geobacter sulfurreducens with enhanced capacity for current production in microbial fuel cells. Biosens. Bioelectron. 24, 3498–3503. https://doi.org/https://doi.org/10.1016/j.bios.2009.05.004

Zhao, F., Sliozberg, K., Rögner, M., Plumeré, N., Schuhmann, W., 2014. The role of

hydrophobicity of Os-complex-modified polymers for photosystem 1 based photocathodes. J. Electrochem. Soc. 161, H3035. https://doi.org/10.1149/2.0081413jes

Supporting Information for

Roots fuel cell produces and store clean energy.

This section includes Figs. S1-3.

Fig. S1

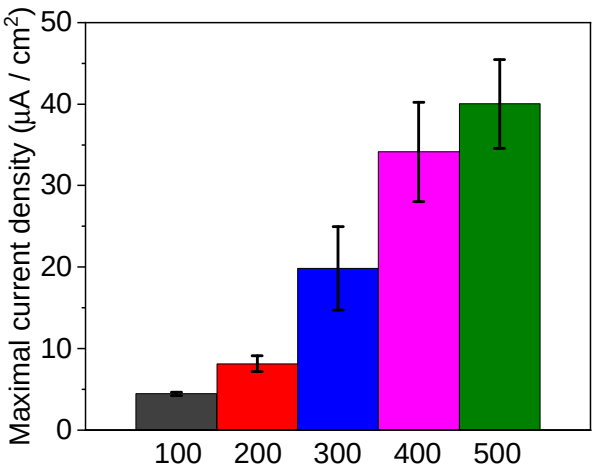

Fig. S1 – Electric current production from roots at increasing electrolyte salinity. CA of onion roots measured in water solutions with various increasing salinities of 100, 200, 300, 400, and 500 mM NaCl. The error bars represent the standard deviation over 3 independent repetitions.

Fig. S2

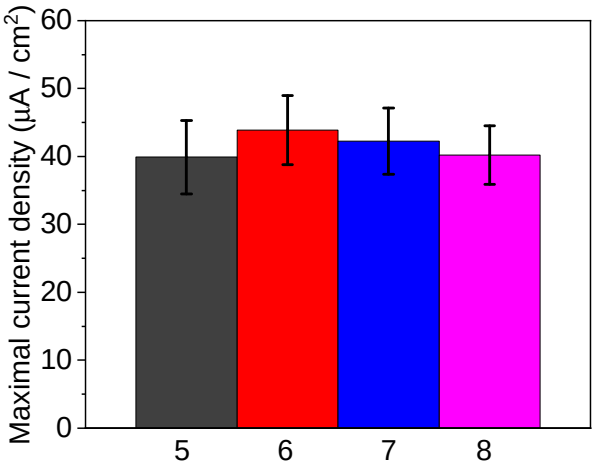

Fig. S2 – Electric current production from roots at increasing pH values. CA of onion roots measured in a solution of 500 mM NaCl + MES buffer with increasing pH values of 5, 6, 7, and 8. The error bars represent the standard deviation over 3 independent repetitions.