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Photo-induced charge transfer between *Lemna minor* and anode of photosynthesizing plant fuel cell

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The photosynthesizing plant fuel cells (PPFC) are bioelectrochemical devices, in which aquatic plants are grown as biocatalysts in fuel cell. Recently, it was proved that higher electrical current is generated by duckweed-PPFC during daytime. In this study a new experimental set-up is developed, so that the *Lemna minor* duckweeds grown in the PPFC can be irradiated with polarized monochromatic light with precise wavelength, 650 nm (red) and 450 nm (blue-violet), while the electrical current generated is monitored. The higher current values during photoperiod show that photo-induced charge transfer between duckweed and fuel cell anode takes place. The results from both chronopotentiometric and electrochemical impedance spectroscopy analyses reveal the contribution of duckweed` light absorbing photosystems (PS I and PS II) and ETC in the thylakoid membranes to a direct electron transfer to the anode.

Keywords: Charge transfer; Duckweeds; Electricity generation; Monochromatic light; Photosynthesizing plant fuel cell.

INTRODUCTION

Microbial fuel cells (MFC) are bioelectrochemical devices operating on the principles of the galvanic cells. They convert chemical energy into electrical one. Instead of chemical catalysts, however, their specific feature is the utilization of living cells as biocatalysts. During the last decade, a huge diversity of MFCs has been developed. Depending on the used biocatalysts, the MFCs are referred to as bacterial fuel cells, yeast-based biofuel cells, sediment microbial fuel cells, plant fuel cells, etc. shortly biofuel cells, famous as X-MFC [1]. Among the advantages of the technology is that the biofuel cells have the potential for different applications – wastewater treatment, autonomous electricity generation, bioremediation and even biosynthesis. Bringing the MFC-technology to higher technological readiness level (TRL) is possible by improving the devices toward higher electrical outputs, finding new exoelectrogenic organisms and better understanding the intra- and extracellular processes contributing to enhanced extracellular electron transfer, having in mind that the electrical current is proportional to the cellular electrons reached the anode [2].

One of the newest types of X-MFCs - the plant microbial fuel cell (P-MFC) has been developed recently by using *Oryza sativa*, *Spartina angelica*, *Arundinella anomala* and *Glyceria maxima* as producers of organic matter by photosynthesis [3-8]. The P-MFCs operate based on the mutualism between plants and the soil microorganisms in the rhizosphere that feed on rhizodeposition and the proteins and sugars released by roots [9]. By positioning of an anode in the rhizosphere [10], a part of the chemical energy of plant-derived organics, oxidized by bacteria and fungi, is transformed in electrical current, which could even supply low-power consumers [11].

In our previous studies [12, 13], we reported that higher aquatic plants - *Lemna minuta* and *Lemna valdiviana* duckweeds, can be used as biocatalysts in a Direct Photosynthesizing Plant Fuel Cell (DPPFC) without the participation of electrogenic bacteria. High power density (380±19 mW/m^2), corresponding to 119.83±5.99 GJ/ ha year [8, 14], were achieved during DPPFC operation under natural sunlight illumination. It was established that abiotic factors as temperature, humidity and light intensity, as well as the day/night cycle influence the generation of the current. At permanently connected resistance higher values of electrical parameters were achieved during daytime than through the nights, indicating the contribution of light-dependent photosynthetic processes. The twice higher duckweeds` biomass and the increased content of monosaccharides (44 %), proteins (47 %) together with the decreased inorganic phosphates reveal that the metabolic processes in the duckweed are intensified when grown under fuel cell polarization. We proved that the quantity of the reserve carbohydrate in form of starch was increased with ca. 30 % at these conditions, while the amylase activity was slightly decreased. Enhanced oxidative

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phosphorylation processes have been suggested due to the doubly increased phytase activity delivering phosphate groups by phytate hydrolysis in the lack of inorganic phosphate in the medium. The polarization also up-regulates a secondary metabolic pathway and the secretion of electrochemically active metabolite, supposed to play a role of a plant endogenous mediator, shuttling electrons extracellularly to the anode.

The aim of the present study is to verify whether the plant photosystems, absorbing light and transferring energy and electrons in the chloroplasts, contribute to the higher current density of the PPFCs. For this purpose, a special experimental set-up for growing the duckweed in fuel cells under controlled light access and constant temperature was developed. Monochromatic red and blue-violet polarized light were applied for periodical irradiation and the electrical parameters were compared with those obtained when visible light was used.

MATERIALS AND METHODS

Plant material and construction of the PPFC

Duckweed (*Lemna minor*), collected from river Maritza, near the town of Plovdiv, Bulgaria, was used in the experiments. Each plant was washed twice with clean water and grown under autotrophic conditions (in potable water without additives) prior to experiments. 1 g of wet biomass *Lemna minor*, equal to ca. 100 plants, was put onto the anode surface of each P-MFC. The used Photosynthesizing Plant Fuel Cell (PPFC) construction is identical with the DPPFC previously described [12]. The anode (carbon felt) is fixed in such a way that the plant roots are covered by water, while the fronds are on the water surface. Potable water in a volume of 40 ml plays a role of anolyte. Five identical fuel cells differing in irradiation conditions (Table 1) were operated simultaneously. After two days of acclimatization period toward polarization by load resistor (1 kΩ), the samples were explored on varying the irradiation.

Development of the experimental set-up

A lamp with illuminance of 60 kLx was used as a coherent light source. Light was transmitted through 12 optical fibers of 60 cm length, 3 of which illuminate a single PPFC. A filter for polychromatic or monochromatic light (wavelengths 450 nm and 650 nm) was put at the end of the fiber after a polaroid (PPFC1, 2, 3). The light filter was fastened so that the light illuminated the entire surface of the anode covered with plants. PPFC4 was a control irradiated by light, which is emitted in all directions (isotropically). The fuel cells bodies were wrapped with black non-transparent foil. The imitation of night conditions was achieved by using a black, light-tight lid.

Electrochemical analyses

The voltage of the fuel cells was monitored continuously by using a multiplexer connected to PalmSens 3 potentiostat in chronopotentiometric mode. The current was calculated by the Ohm`s law. In the graphs, it is presented as a current density in respect to the anodic geometric area. The open circuit voltage (OCV) was measured at the beginning and the end of the experimental window after recovery of the PPFCs.

At the 10^{th} day after the start-up, electrochemical impedance spectroscopy (EIS) was applied during the irradiation. The EIS was carried out in a three electrode mode, in the frequency range from 50 kHz to 7 mHz with an applied ac signal with an amplitude 10 mV and E_{dc} equal to -0.1V. The bioanode was connected as a working electrode, Pt-wire – as a counter, and Ag/AgCl (3 M KCl) - as a reference electrode. Cyclic voltammetry (CV) in the potential range from +0.6 to -0.6 V (*vs*. Ag/AgCl) with a scan rate of 10 mV/s were also performed by PalmSens 3 handheld potentiostat.

RESULTS AND DISCUSSION

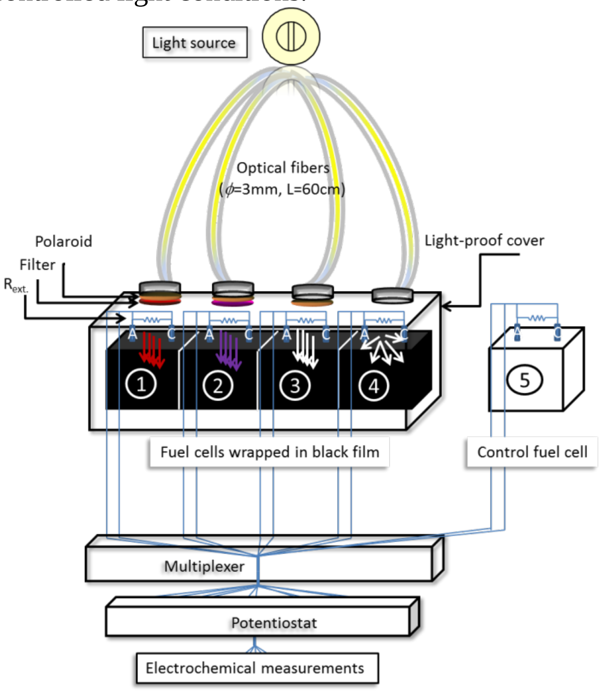
Representatives of Lemna duckweeds – *L. minuta* and *L. valdiviana,* have been shown to be capable of extracellular electron transferring (EET) when cultivated in a fuel cell [12, 13].

Table 1. Photosynthesizing Plant Fuel Cells irradiated with monochromatic polarized light: red (PPFC1), blue-violet (PPFC2); polarized visible light (PPFC3); non-polarized visible light (PPFC4). The duckweeds in the PPFC5 were grown as a control in laboratory conditions. The light irradiation characteristics of each PPFC are presented.

PPFC	Irradiation with	Measured illuminance on PPFC / Lx	Irradiance / (W/m^2)	Characteristics: wavelength, frequency, photon energy
1	Monochromatic red light	40±5	0.059±0.007	650 nm, 400THz, 1.65eV
2	Monochromatic blue-violet light	500±100	0.732±0.146	450 nm, 668 THz, 2.75eV
3	Polarized visible light	280±60	0.410±0.088	390 to 700 nm
4	Non-polarized visible light	1050±300	1.537±0.439	Random mixture of waves
5	Light in the lab	1870±200	2.738±0.293	Mixed - natural and artificial

Depending on the illumination intensity and the period of cultivation, their response to fuel cell polarization has been related to the membrane potential of the fronds/roots, as well as to the secretion of plant endogenous mediator. The higher generated current during daytime showed a possible contribution of the light-phase of photosynthesis to the anodic reaction. Until now, however, no report has been published about the capabilities of direct electron transfer between a photosynthesizing plant biocatalyst and the anode of a PPFC.

In the present study, we used *L. minor* as a biocatalyst in a PPFC and developed a new experimental set-up (Scheme 1), by which it is possible to measure important electrochemical parameters, while the duckweeds are grown under controlled light conditions.



Scheme 1. Schematic presentation of the developed experimental set-up for controlled irradiation of photosynthesizing plant fuel cells.

To assess the influence of different light wavelengths on the electrical current generated by the PPFC, filters for polychromatic or monochromatic light with wavelength 450 nm and 650 nm were assembled with a polarizing filter (PPFC1, 2, 3) and used for temporary irradiation of the plants. Right after the irradiation of the plants in the fuel cell with polarized red or blue-violet light, the generated current increases and with small oscillations is stable up to 3 h (Fig. 1a). The periodic irradiation with red light leads to the highest values of the current densities. The wavelengths of 650 and 450 nm are near to those reported for the characteristic absorption peaks of chlorophyll a and b, which are the most essential pigments in the light-harvesting system of green plants.

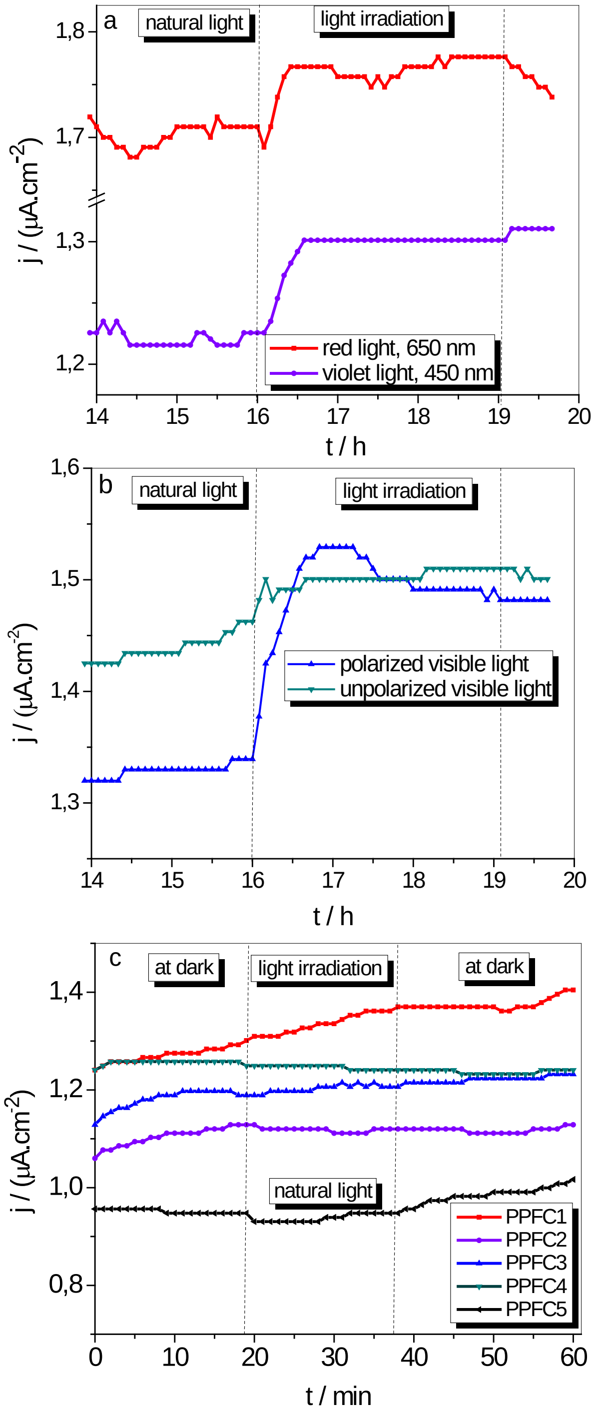


Fig. 1. Current density measured at the time of irradiation with: a) Red and blue-violet light introduced after the PPFCs have been incubated at natural lab light: b) polarized visible and non-polarized visible light; c) the irradiation is applied after dark incubation of the samples.

In this way, the contribution of chlorophyll a and chlorophyll b to the current generation by PPFC is supposed. The differences in current generation by PPFC3 and PPFC4 overlapped fast after applying irradiation, showing that the duckweed is capable of absorbing polarized visible light (Fig. 1b).

The participation of photosensitive complexes in the current generation could be deduced also by the results from the experiment, in which the duckweeds were incubated at dark and after that irradiated (Fig. 1c). In this case none of the PPFCs except PPFC1 responded to the irradiation, which indicates that the energy of the absorbed red light by duckweeds contributes not only to the photosynthetic processes, but also to an enhanced EET. The lowest current was achieved with the control fuel cell (PPFC5), where the irradiation was carried out naturally. After removal of the artificial light sources and exposure to the lab light illumination, the current of all fuel cells tends to equalize (Fig. 2), which is an additional evidence for the sensitivity of the duckweeds' response to the different sources of irradiation.

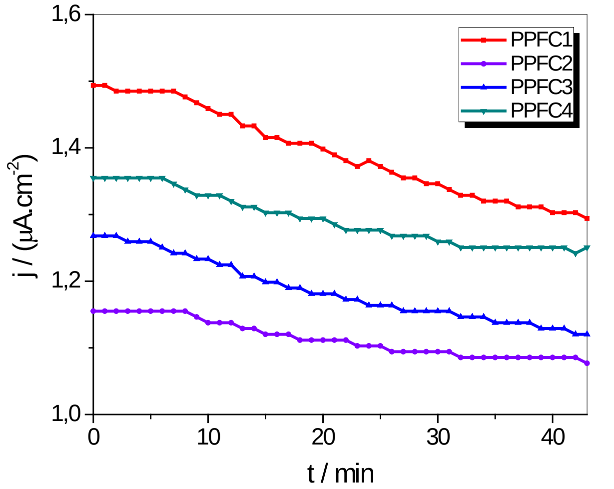


Fig. 2. PPFC current generation after removal of artificial light sources and incubation at lab light.

For all samples except PPFC3 the open circuit voltage (OCV) values decreased with several tenths of millivolts after 6 days of polarization with external load (Fig. 3).

The smallest OCV, comparable with that of the control, is registered for the PPFC2, which directs to possible damages of reaction centres of the photosystems by the blue-violet light irradiation, possessing the highest photon energy (Table 1). It was assumed that the reaction centres consisting of enzymes that use light to reduce molecules providing them with electrons or their surrounding by light-harvesting complexes could contribute to the membrane potential changes of the cells and therefore the fuel cell performance. The maximal OCV was observed for PPFC3.

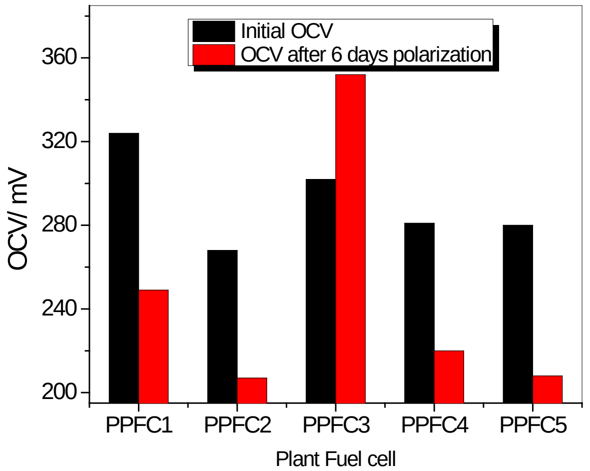


Fig. 3. The change of the open circuit voltage after 6 days of fuel cell polarization.

On the 10th day of the fuel cell polarization, electrochemical impedance spectroscopy analyses were implemented for establishment of the charge transfer mechanism during irradiation. The obtained impedance spectra, presented as Niquist and Bode plots on Fig. 4, show that the system responds in a different way depending on the applied irradiation. While the spectra of PPFC1 (monochromatic red light) and PPFC4 (non-polarized visible light) are well-fitted to a simplified one-time constant Randels model (Fig. 4a), those of PPFC2 (blue-violet light) and PPFC3 (polarized visible light) are consistent with an equivalent circuit model consisting of two successive RC time constants (Fig. 4b)

The values extracted from the fitted spectra of the resistances and capacitances, included in the corresponding equivalent circuit models, are summarized in Table 2.

Following the approach proposed by Jung [15] it may be assumed that the two-time constant model represents the impedance of the intracellular (second bigger semi-arc) and extracellular (smaller semi-arc) charge transfer processes. Considering the use of photosynthetic plants as a biocatalyst in our system, the intracellular charge transfer processes in this case should be assigned to the transport of photo excited electrons and protons across the thylakoid membranes.

Table 2. Values of resistances and capacitances, derived from the fitted EIS.

Parameter/FC	PPFC1	PPFC2	PPFC3	PPFC4
R_s /(kΩ.cm^2)	0.8	-	0.8	0.8
R_{ex} / (kΩ.cm^2)	12.2	1.3	2.0	2.0
R_{in} / (kΩ.cm^2)	-	21.5	70.0	-
C_{ex} (µF.cm^2)	1.9	14.7	14.6	3.5
C_{in} (mF.cm^2)	-	54.5	48.0	-

The intracellular charge transfer resistances (R_{in}) of PPFC2 and PPFC3 are respectively 16.5 and 35 times larger than the corresponding extracellular charge transfer resistances (R_{ex}), indicating that the intracellular charge transfer processes are the rate-limiting step when the system is irradiated by blue-violet or polarized visible light.

At the same time, the 3-fold higher intracellular capacitance (C_{in}) in both cases shows that almost all electrical charges generated through the photosynthetic processes reside in the intracellular reaction step [15].

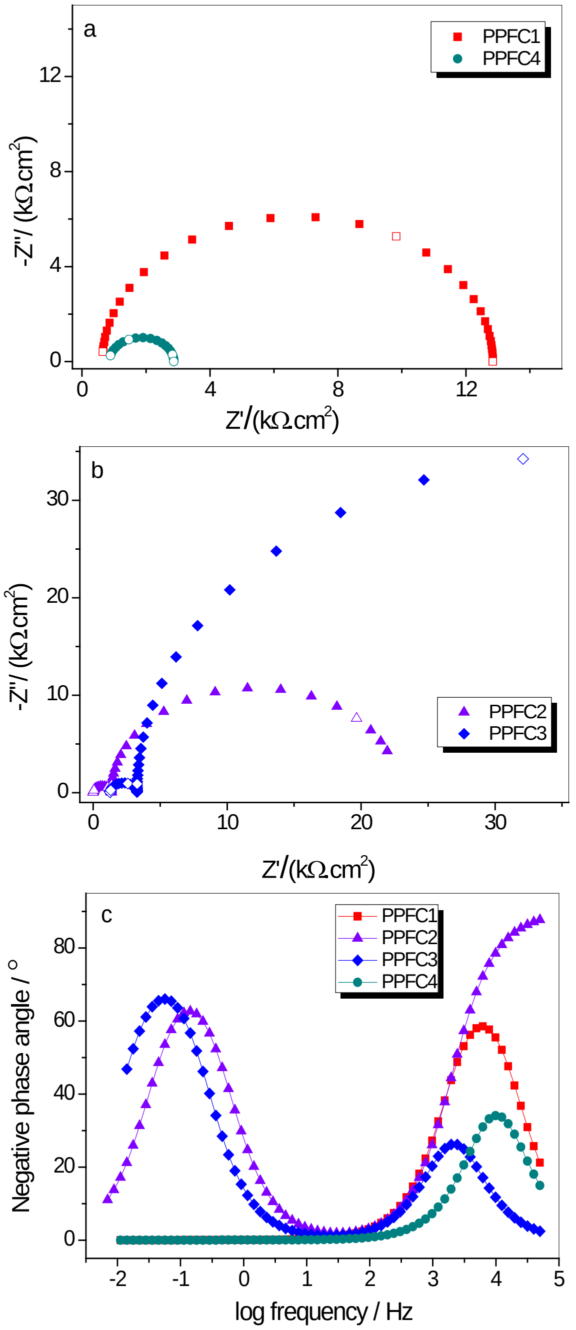


Fig. 4. EIS of bioanodes during irradiation, presented as: a) Niquist plot of PPFC1 (squares) and PPFC4 (circles); b) Niquist plot of PPFC2 (triangles) and PPFC3 (diamonds); c) Bode plots of all PPFCs.

On the other hand, the existence of only one, non-ideal semi-arc in the spectra (Fig. 4a) of the samples, irradiated by monochromatic red (PPFC1) and non-polarized visible light (PPFC4), can be explained supposing that the intracellular charge transfer processes are facilitated and there is no such huge accumulation of electric charges inside the plant cells in these cases. In other words, the values of the intracellular and extracellular resistances and capacitances are of the same order of magnitude and the total charge transfer process from the duckweeds to the anode is represented as a “pseudo” one-time constant reaction.

Based on the similarity of EIS obtained with PPFC1 and PPFC4, we speculate that complexes of the plants’ photosystems, capable of absorbing red light, could contribute to a direct electron transfer from duckweeds’ fronds/roots to the fuel cell anode. Although the plants’ chlorophyll *a* absorption maximum is at about 450 nm (blue-violet light), red light is more effective because both photosystems (PS I and PS II) absorb light of wavelengths in the red region. When PS II absorbs light, electrons in the chlorophyll reaction-center are excited to a higher energy level. Photo-excited electrons travel through the cytochrome b6f complex to photosystem I *via* an electron transport chain set in the thylakoid membrane [16]. This energy fall is harnessed to transport protons through the membrane. Having in mind that cytochrome *c* type molecules are responsible for EET in the MFCs, it may be assumed that plant cytochromes could also participate in the EET processes in the PPFC. The two oxidation peaks on the CV (Fig. 5), recorded during PPFC irradiation, support this hypothesis.

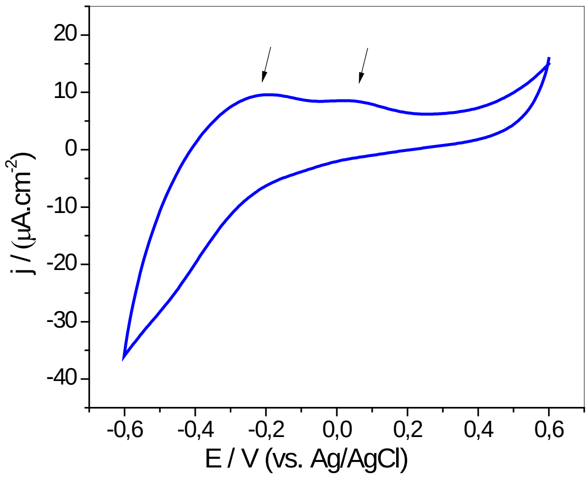


Fig. 5. CV of PPFC3 during irradiation with polarized light of visible spectrum.

CONCLUSIONS

Photo-induced charge transfer across solid-solid (anode and plants) or solid-liquid interfaces in PPFC is studied for first time. The processes of light-dependent phase of photosynthesis, responsible to photon energy absorption, are related with direct transferring of the harvested electrons to the anode of the plant fuel cell, which explains the previously established higher electrical current generated by PPFC during light periods of the day/night cycle. The results obtained by EIS suggest that the irradiation with high-energy blue-violet light, as well as polarized visible light leads to a huge accumulation of electrical charges within the plant cells, while the natural non-polarized visible light facilitates in highest extent the electron transfer from duckweeds to the PPFC anode. These findings are a prerequisite for implementation of further analyses, aiming at elucidation of the plant redox complexes contribution to extracellular electron transfer in a plant fuel cell.

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REFERENCES

1. M. Mitov, Y. Hubenova, *Chemistry: Bulg. J. Sci. Edu*., **24**, 404 (2015).
2. Y.V. Hubenova, in: Encyclopedia of Interfacial Chemistry: Surface Science and Electrochemistry, Klaus Wandelt (ed), Elsevier, 2018, p. 537.
3. D.P.B.T.B. Strik, H.V.M. Hamelers, J.F.H. Snel, C.J.N. Buisman, *Int. J. Energy Res*., **32**, 870 (2008).
4. N. Kaku, N. Yonezawa, Y. Kodama, K. Watanabe, *Appl. Microbiol. Biotechnol*., **79**, 43 (2008).
5. M. Helder, D.P.B.T.B. Strik, H.V.M. Hamelers, A.J. Kuhn, C. Blok, C.J.N. Buisman, *Bioresour. Technol*., **10**, 3541 (2010).
6. R.A. Timmers, D.P.B.T.B. Strik, H.V.M. Hamelers, C.J.N. Buisman, *Appl. Microbiol. Biotechnol*., **86**, (2010).
7. R.A. Timmers, M. Rothballer, D.P.B.T.B. Strik, M.E.S. Schulz, M. Schloter, A. Hartmann, B. Hamelers, C. Buisman, *Appl. Microbiol. Biotechnol*., **94**, 537 (2012).
8. P. Bombelli, D.M.R. Iyer, S. Covshoff, A.J. McCormick, K. Yunus, J.M. Hibberd, A.C. Fisher, C.J. Howe, *Appl. Microbiol. Biotechnol.*, **97**, 429 (2013).
9. A.J. McCormick, P. Bombelli, R.W. Bradley, R. Thorne, T. Wenzele, C.J. Howe, *Energy Environ. Sci*., **8**, 1092 (2015).
10. L. De Schamphelaire, L. Van den Bossche, H.S. Dang, M. Höfte, N. Boon, K. Rabaey, W. Verstraete, *Environ. Sci. Technol*., **42**, 3053 (2008).
11. P. Bombelli, D. Ross, F. Felder, M. Cooper, D.M.R. Iyer, J. Royles, S.T.L. Harrison, A.G. Smith, C.J. Harrison, C.J. Howe, R. Soc. Open Sci., 3, 160249 (2016).
12. Y. Hubenova, M. Mitov, *Bioelectrochemistry*, **87**, 185 (2012).
13. Y. Hubenova, M. Mitov, *Bioelectrochemistry*, **106**, 226 (2015).
14. S. Liu, H. Song, X. Li, F. Yang, *Int. J. Photoenergy*, 2013, Article ID 172010 (2013).
15. S. Jung, *Int. J. Electrochem. Sci*., **7**, 11091 (2012).
16. K.J. Appenroth, K. Krech, Á. Keresztes, W. Fischer, H. Koloczek, *Chemosphere*, **78**, 216 (2010).