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Photo-electrochemical communication between cyanobacteria (*Leptolyngbia* sp.) and osmium redox polymer modified electrodes†

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Photosynthetic microbial fuel cells (PMFCs) are an emerging technology for renewable solar energy conversion. Major efforts have been made to explore the electrogenic activity of cyanobacteria, mostly using practically unsustainable reagents. Here we report on photocurrent generation (≈8.64 μA cm^{-2}) from cyanobacteria immobilized on electrodes modified with an efficient electron mediator, an $Os^{2+/3+}$ redox polymer. Upon addition of ferricyanide to the electrolyte, cyanobacteria generate the maximum current density of ≈48.2 μA cm^{-2}.

Photosynthetic microbial fuel cells (PMFCs) are an emerging prospective technology for CO_2 free renewable solar energy production and rely on photosynthesis for generation of electricity.[1] Cyanobacteria account for 20–30% of global photosynthetic productivity and convert solar energy into chemical energy.[2] They contain both respiratory and photosynthetic systems in their thylakoid membranes unlike higher plants and algae and any excess electrons generated in photosynthesis can be shared with the respiratory system.[3] Moreover, cyanobacteria have their own mechanism to prevent photo-damage at high light intensity and are able to survive under different environmental conditions, *e.g.*, at irregular levels of CO_2, diverse light exposure, and dryness,[4] which is supposed to give them a long stability in PMFCs.[5] Therefore cyanobacteria have the practical potential to harness solar energy in a versatile global area.

Studies have revealed that cyanobacteria may be exploited in photo-bioelectrochemical cells *via* direct electron transfer (DET) with electrodes.[2,6] They have been explored for biofuel generation[7] as well as heavy metal remediation.[8] Cyanobacteria have greater advantages over metal reducing bacteria, since external organic carbon sources are not needed for electricity generation.[2] Energy generation from isolated photosynthetic reaction centers, photosystem I (PSI), photosystem II (PSII), and thylakoids require complex isolation and immobilization techniques resulting in short-term stability that limits their use in applications.[5] Reports demonstrated different cyanobacteria in PMFCs, *e.g. Anabaena* sp.,[9] *Synechococcus* sp.[10] and *Synechocystis* sp.,[11] and mostly using artificial redox mediators to carry out the extracellular electron transfer from the cells to the electrode. However, the use of environmentally unfriendly, unsustainable and practically unfeasible artificial mediators in PMFCs limits their practical application currently.[6] In contrast, flexible osmium redox polymers (ORPs) have already been very successfully used in enzyme based reagentless biosensors,[12] where they fulfil the requirements of both supplying the system with a mediator (that does not diffuse away with time) and also forming a 3-D immobilization matrix (a hydrogel) for the enzyme. Besides that polymeric mediators draw attention due to their efficient shuttling properties, stable adsorption on the electrode surface and the possibility to form multiple layers of enzymes[13] as well as bacterial cells.[14,15]

Here we report on the electrochemical communication of *Lyptolyngbia* sp. (CYN82)[16] using an ORP modified graphite electrode. Cyclic voltammetry (CV) and chronoamperometry (CA) measurements have been used to record the photocurrent generation. To the best of our knowledge, this is the first time PMFCs with such a polymeric mediator have been reported. To measure the photocurrent density generated by cyanobacteria, the response registered under light off conditions is subtracted from that registered under light on conditions. All potentials mentioned here are referred to Ag|AgCl (sat. KCl) if not stated otherwise.

Cyanobacteria convert H_2O and CO_2 to glucose by photosynthesis and under dark conditions they consume glucose for survival. They can generate electricity from both the photosynthetic and the respiratory machinery that provide the foundation of PMFCs if these electrons are collected.[17]

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To investigate the presence of photosynthetic pigment inside CYN82, absorbance measurements of the extracted photosynthetic dye were made and it was confirmed that the most essential photosynthetic pigment responsible for current generation,[18] chlorophyll *a*, appeared at a wavelength of 665 nm. In addition other necessary pigments such as chlorophyll *b* and carotenoids were visible in the spectrum at ≈400 nm (ESI,† Fig. S1). The appearance of these pigments at their particular wavelengths confirms the necessary photosynthetic activity of CYN82.[19]

DET between the cells and electrodes may be preferable over mediated electron transfer (MET) for power generation, since it minimizes the over-potential in bio-electrochemical systems and simplifies the electrochemical cell design and operation. We investigated whether CYN82 can communicate with a solid bare graphite electrode directly without any mediator. It has been revealed that DET of cyanobacteria[5] is feasible *via* their naturally produced nanowires, proposed to be similar to that reported for metal reducing bacteria.[20] To investigate for the possibility for DET, CYN82 cells were adsorbed on a bare graphite electrode and illuminated with a fibre optic light source with a light intensity of 44 mW cm^{-2} (a light intensity where photosynthesis is no longer limited by light) and only pure electrolyte was present as an electron donor (Fig. 1).

It was shown (Fig. 1) that when the CYN82 cells were illuminated they generated a photocurrent of 1.30 μA cm^{-2} evaluated as the difference in registered current density between situations "light on" and "light off" (6.85–5.70 μA cm^{-2}). We anticipate interactions between oxygen containing functional groups on the surface of the graphite[21] and quinones present in the photosynthetic electron transfer chain (PETC) of CYN82. Previously it was reported that the plastoquinone pool in PETC is responsible for the direct electrogenic activity between the cells and the electrode.[5] The reason for current generation was attributed to photo-electrolysis of water by the PETC inside the CYN82 cells.

In contrast, when the light was turned off the photocurrent decreased, since in the absence of light no water-splitting can occur, which is the origin of the electrons that can be transferred to the electrode surface through the PETC (Fig. 1). It is proposed here that PETC in the CYN82 cells is responsible for the photocurrent generation. Control experiments with unmodified graphite electrodes yielded no photocurrent when illuminated.

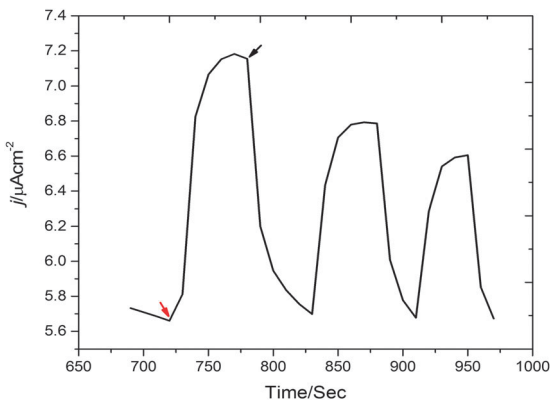
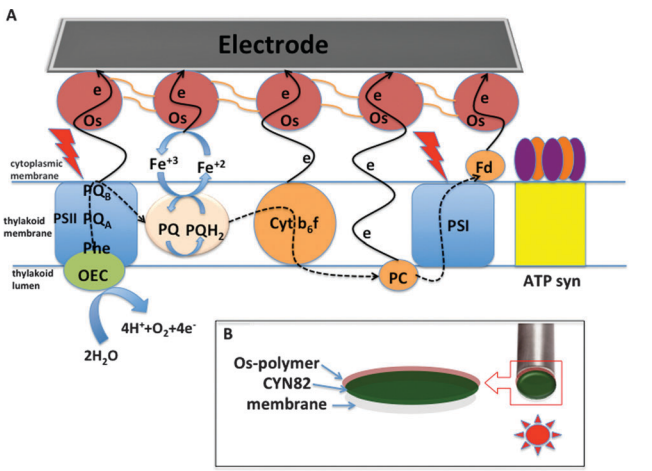


Fig. 1 DET between CYN82 (9.5 μg, wet weight) and a bare-graphite electrode. Electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl and 5 mM $MgCl_2$, applied potential: 350 mV *vs.* Ag|AgCl (sat. KCl), light intensity: 44 mW cm^{-2}, black and red arrows stand for light off and on, respectively.

To improve the photocurrent density we investigated four different cationic ORP[14,22] (ESI,† Fig. S2) denoted Os-A, Os-B, Os-C and Os-D, having different ligands to the metal center resulting in a range of redox potentials ($E^{\circ\prime}$) from −0.07 (Os-A), 0.12 (Os-B), 0.22 (Os-C) and 0.35 V (Os-D) *vs.* Ag|AgCl (sat. KCl). This potential window covers a large part of the potential range of PETC and therefore it is possible to extract electrons generated from PETC of the CYN82 cells at various positions. The approximate $E^{\circ\prime}$ of the participating redox complexes in the PETC are +1.0 (P680), −1.05 (P680*), +0.21 (P700), −1.52 (P700*), −0.47 (PQ_A), −0.3 (PQ_B), −0.11 (Cyt b_6f) and +0.11 (PC) V *vs.* Ag|AgCl (sat. KCl).[23]

In Scheme 1 the possible electron transfer sites are presented. Recently, Os-C was successfully used to "wire" heterotrophically grown *Rhodobacter capsulatus* cells,[24] where it forms a 3-D hydrogel through electrostatic interactions between the cationic ORP and the anionic bacterial cell membrane precipitating onto the electrode surface. A similar interaction is expected to take place between the ORP and the CYN82 cells.

Photocurrent generation with Os-A, Os-B, Os-C, and Os-D exhibited 1.32, 4.24, 8.64 and 6.33 μA cm^{-2} (Fig. 2). The photocurrent increases linearly with an increased $E^{\circ\prime}$ of ORP except for Os-D. It is expected that when increasing the $E^{\circ\prime}$ a higher photocurrent is to be exhibited as the thermodynamic driving force is increased for donation of electrons to the ORP. However, variation in accessibility of the redox complex to the electron-donating site



Scheme 1 (A) Schematic potential electrons transfer sites of cyanobacterial cells immobilized on a graphite electrode *via* different redox complexes in the PETC *e.g.*, PSII, plastoquinone (PQ), cytochrome b_6f (Cyt b_6f), plastocyanin (PC), PSI, and ferridoxin (Fd). OEC, Phe, PQ_A, PQ_B, PQH_2 and ATP syn represent oxygen evolving complex, pheophytin, plastoquinone A, plastoquinone B, plastoquinol and ATP synthase respectively. PSI and PSII refer to the photosynthetic reaction centres and their respective pigments are P680 (P680*) and P700 (P700*), where * signifies the excited state. (B) The immobilization of cyanobacteria on an ORP modified graphite electrode surface and illumination approach.

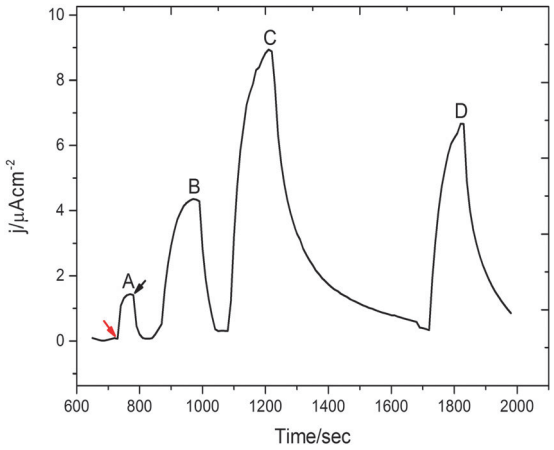


Fig. 2 Comparison of background corrected (light off conditions) photocurrent generation mediated with (A) Os-A, (B) Os-B, (C) Os-C and (D) Os-D; the $E^{\circ\prime}$ of Os-A, Os-B, Os-C and Os-D was −0.07, 0.12, 0.22 and 0.35 V *vs.* Ag|AgCl, respectively, applied potential: +130 mV > $E^{\circ\prime}$ of each ORP, electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl and 5 mM $MgCl_2$, light intensity: 44 mW cm^{-2}, black and red arrows stand for light off and on respectively. The results of four different experiments with the four different ORPs have been combined in this figure.

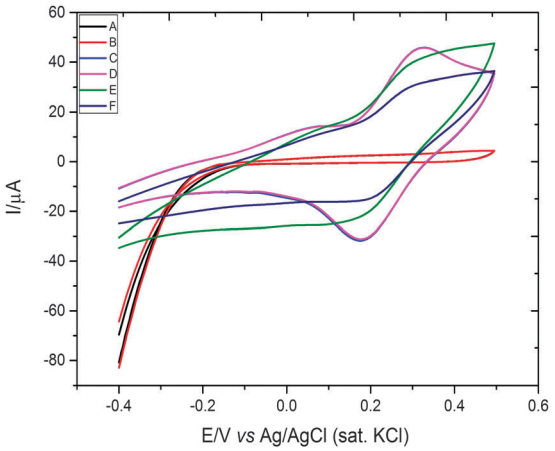


Fig. 3 CVs of a (A) bare graphite electrode with light off, (B) bare graphite electrode with light on, (C) Os-C modified electrode with light off, (D) Os-C modified electrode with light on, (E) CYN82 immobilized on Os-C modified electrode with light on, (F) CYN82 immobilized on Os-C modified electrode with light off, applied potential: 350 mV *vs.* Ag|AgCl (sat. KCl), light intensity: 44 mW cm^{-2}. Electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl, and 5 mM $MgCl_2$.

in the PETC should also be of importance. The lower $E^{\circ\prime}$ values of P680*, P700*, PQ_A, PQ_B, PQ, and Cyt b_6f compared to the $E^{\circ\prime}$ values of the ORPs indicate that they should be able to donate electrons to the ORP. Control experiments with the ORPs but with the absence of cells revealed no photocurrent when illuminated.

However, the short lifetime of P700* and P680* makes them unlikely to be possible electron donors to ORP rather than for the natural electron acceptors in the electron transfer pathway of the photosystems. The increase in photocurrent generation from Os-A to Os-C indicates that $E^{\circ\prime}$ of the ORP plays an important role in accepting electrons from PETC. Reduced plastoquinone (PQH_2), known for having a long life time and predominant presence in PETC,[25] makes it a good electron donor, whereas Cyt b_6f and PC could also be used. Here, Os-C generates the highest photocurrent (8.64 μA cm^{-2}) possibly because of better the combination of accessibility to the PETC in the lipid bilayer membrane, higher $E^{\circ\prime}$, and greater solubility.[13] Therefore, the rest of the experiments were conducted with this polymer.

The concentration of CYN82 on the electrode surface was optimized and it was found that 9.5 μg (wet weight) shows the highest photocurrent (ESI,† Fig. S3). When the concentration increased (>9.5 μg) the photocurrent goes down, possibly due to the formation of too thick a cell layer, where light does not reach through the entire layer of cells. Therefore, all the experiments presented here were conducted with this optimized concentration.

To investigate the effect of illumination CVs were recorded for bare, Os-C and CYN82 with Os-C modified electrodes. There is an insignificant influence of light either on the bare graphite electrode (Fig. 3A and B) or on the Os-C polymer modified electrode (Fig. 3C and D). The $E^{\circ\prime}$ of Os-C is, from the CV, 0.22 V in agreement with the previously determined value.[26] When CYN82 cells were immobilized on Os-C modified electrodes and in the absence of light (Fig. 3F), the intensity of both the anodic and the cathodic peak currents goes down since the CYN82 cells retard the redox response of the osmium redox centers of Os-C due to the strong electrostatic interactions. A similar change in response was observed for electrodes modified with redox polymers with and without different kinds of bacterial cells.[14]

The most significant response was observed for electrodes modified with both CYN82 cells in combination with Os-C when illuminated (Fig. 3E) as the anodic and cathodic current increases. The $Os^{2+/3+}$ redox centres in the polymer matrix are reduced by available electrons from photo-electrolysis of the electrolyte and re-oxidized at the electrode surface polarized at a higher potential (E_{appl} > $E^{\circ\prime}$ of Os-C). It can be assumed from these CVs that the Os^{3+} moieties can easily accept electrons produced during the photosynthetic event and shuttle them to the electrode.

The influence of light intensity on the generation of photocurrent was investigated and the results are shown in ESI,† Fig. S4. Studies showed that the light intensity has a significant influence on the photosynthetic carbon reduction cycle, however, too much light may destroy the photosynthetic apparatus, especially that of PSII.[27] It is known that the light intensity to saturate photosynthesis is obtained for a light intensity of 25 mW cm^{-2}. In our experiments the photocurrent increases from 2.32 to 9.21 μA cm^{-2} when increasing the light intensity from 44 to 680 mW cm^{-2}. A similar response was observed for thylakoid membranes isolated from spinach.[28] This is attributed to the fact that while the light intensity increases, a larger portion of the plastoquinone pool in PETC gets reduced by the electrons available from photolysis of the electrolyte and will become oxidized at the electrode resulting in a higher photocurrent. To avoid any kind of photo-damage of the photosynthetic machinery of the CYN82 cells, it was decided to conduct all the experiments at 44 mW cm^{-2}.

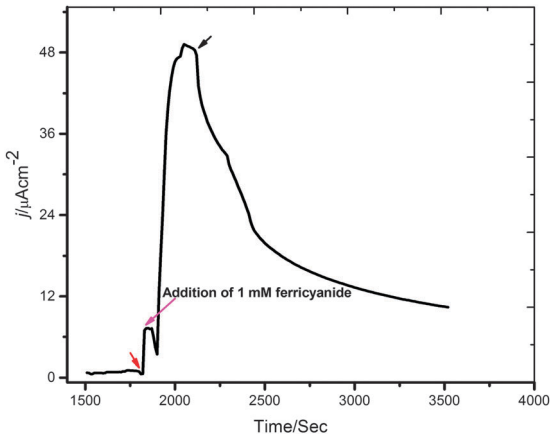


Fig. 4 Improvement of photocurrent generation with a double mediator. The figure shows background corrected (light off conditions) current density. CYN82 immobilized on Os-C modified electrode, [ferricyanide]: 1 mM, applied potential: 350 mV *vs.* Ag|AgCl (sat. KCl), light intensity: 44 mW cm^{-2}. Electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl, and 5 mM $MgCl_2$, black and red arrow stands for light off and on respectively.

Ferricyanide is known to mediate electron transfer from multiple photosynthetic reaction centers to electrodes and can diffuse easily through the cell membranes and is a suitable choice because of its low inherent photo activity compared to any quinone derivatives that are also commonly used as mediators.[18] To explore the effect of ferricyanide on the photocurrent, it was added to the electrolyte while the CYN82 cells were immobilized on the surface of a bare graphite electrode. The results show that CYN82 cells generated 5.92 μA cm^{-2} in the presence of 1.0 mM ferricyanide when the light was turned on (ESI,† Fig. S5).

To boost up the generation of the photocurrent, one soluble (ferricyanide) and one polymeric mediator (Os-C) were used together. Ferricyanide is known to be an efficient electron acceptor for both PSI and PSII,[29] and Os-C is known to exhibit efficient electron transfer properties with bacterial cells.[14] When the CYN82 cells were immobilized on the Os-C modified electrode, the photocurrent upon addition of 1 mM ferricyanide increased from 6.74 to 48.15 μA cm^{-2} (Fig. 4).

The reason can be attributed to the low molecular weight ferricyanide having higher diffusing capability into the membrane than the Oc-C polymer and results in more efficient electron transfer from the cells to the electrode. A similar increase in response was demonstrated when the cyanobacterial cells were treated with another soluble mediator *p*-benzoquinone.[5] A higher catalytic response is also observed for *Saccharomyces cerevisiae* cells when using a double mediator system.[30] Control experiments with ferricyanide in solution and with bare graphite exhibited no significant photocurrent when illuminated.

The source of photocurrent generation is of great importance to discover, and especially, which particular photosynthetic pigment is responsible for donating electrons to the ORP. Among all photosynthetic inhibitors, diuron is the most widely used and known particularly for inhibiting PSII, blocking the electron transfer from PSII to plastoquinone (PQ) by binding with either PQ_A or PQ_B. When diuron binds with PQ_B the electron transfer is shut down entirely, whereas binding with PQ_A it slows down the electron transfer rate.[31] The effect of inhibition by diuron at different concentrations as well as comparison with non-inhibited photocurrent is displayed in ESI,† Fig. S6. When the concentration of diuron was increased gradually from 0 mM to 0.4 mM, the photocurrent generation went down from 8.52 to 1.20 μA cm^{-2} and at 0.5 mM, more than 90% of the original photocurrent is inhibited. It can be inferred from this phenomenon that diuron binds with PQ_B. A reasonable suggestion is thus that photo-electrolysis of the electrolyte by PSII is the major source of photocurrent in this entire system.

Conclusions

In this work both direct and mediated electrogenic activity of cyanobacterial cells have been confirmed as the source of photocurrent. Of the four investigated ORPs, Os-C yields a significant photocurrent generation of 8.64 μA cm^{-2}, possibly because of a combination of a high $E^{\circ\prime}$, a greater accessibility to the membrane of the cyanobacterial cells, and a better solubility. When ferricyanide was added to the electrolyte in combination with the ORP the photocurrent reaches a maximum of 48.15 μA cm^{-2}. We believe this observation has substantial implication for future photosynthetic solar energy conversion. No optimization of the electrode with any conductive nanomaterials and engineering of the cyanobacterium has been attempted to enhance the photocurrent density. However, for further progress of power generation future work should focus on the use of three-dimensional electrode material, greater design of the electrochemical cell, and an improved immobilization technique. An understanding of the photosynthetic light harvesting complex on the molecular level and a detailed investigation of its electron transfer mechanism would be useful to reveal nature's own finely tuned energy generation process.

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