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Single Microbe Photoelectrochemical Device using Scanning Electrochemical Microscopy

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Electronic Supplementary Information

Experimental section

Strain and culture conditions of Synechocystis pevalekii:

Synechocystis pevalekii strain BDUHKU21304 was purchased from the National Facility for Marine Cyanobacteria (NFMC – Tamil Nadu, India). The microbe was cultured photoautotrophically in aerobically condition in Artificial Seawater Nutrient Media III (ASN III) (32.4 g L^{-1}, PT153-5L) and Agar Powder Bacteriological grade (GRM026 - 500G). Sterile conditions were maintained by handling and processing bacterial samples in a laminar airflow hood (Equitron). Bacteria were grown in rotatory incubator (Orbitek) at 120 rpm, at 27±2 ˚C, pH 7.3±2 and with a light intensity of 1500 lux. The bacterial sample was kept in 16 hours light and 8 hours dark as per the manual information. At the latter stage we have conditioned the cyanobacterial samples for 2 hours light and 2 hours dark. The bacterial samples were collected each day and centrifuged in Remi R-24 at 8000 rpm for 8 minutes. The supernatant was removed, and the pellet is dispersed with 0.85% saline solution (Sodium Chloride Hi-AR (98.50-102.00%, GRM853-500G). The bacterial OD (growth) were recorded using UV-vis spectrophotometer (V-630 from JASCO) using Deuterium lamp and a Halo cathode lamp as a light source. OD was taken at $OD_{730nm.}$

Electrode preparation:

Fluorine-doped tin oxide (FTO) as anode: FTO conducting glass plates (TEC 7 grade) were procured from Greatcell Solar Industries (Australia). 2.5 cm x 2.5 cm were cut out from the FTO glass plate and then cleaned by ultrasonication in isopropyl alcohol, followed by dilute HCL (Qualigens) and IPA for 20 minutes each. These FTOs were then sintered at 500˚C for an hour on the hotplate.

Platinum coated FTO as cathode: 10 mg of H_2PtCl_6 (Chloroplatinic acid hexahydrate (37.5% Pt basis) was purchased from Sigma-Aldrich) in 10 ml of Ethanol (Changshu Hongsheng, Fine Chemical) were prepared. One drop of the same was added to the conductive surface of FTO and heated at 475 ˚C for 15 minutes.

Proton exchange membrane activation:

For activation of the Nafion membrane (Nafion-117, purchased from Fuel Cell Stores, USA), the membrane is cut short into 1 cm x 1 cm. 3 % H_2O_2 (30 % w/w Sigma) in a beaker, and the cut-short pieces of the membrane are heated at 85 ˚C for 45 minutes. After heating, membranes were dipped in a petri dish containing DI water. Again, membranes are heated at 85 ˚C for 45 minutes in a DI water beaker. After heating with DI water, the membrane was heated in 0.5 M H_2SO_4 (Qualigens) at 85 ˚C for 45 minutes. After heating, membranes were dipped in a petri dish containing DI water. After heating with H_2SO_4, it is again heated at 85 ˚C for 45 minutes in a DI water beaker. The activated Nafion membrane was subsequently enclosed in a petri dish filled with water.

Spacers (Gasket):

Silicone gasket rubber spacers were used and cut into pieces with the inner side of 1cm x 1cm and an outer side of 2 cm x 2 cm.

Device fabrication:

The device is comprised of two FTO plates (2.5 cm x 2.5 cm) coated with a compact TiO_2 layer as photoanode (1 cm x 1 cm), and platinum-coated FTO can act as a counter electrode (cathode). The two electrodes were separated by a silicone gasket as a spacer 1 mm thick. Nafion-117 membrane was used between the photoanode and the spacers.

Microbe injection

Synechocystis Pevalekii ($O.D_{680}$ nm = 2.0) was injected using a 1ml syringe along with mixture of ASN-III (50 µl) media and 2mM Potassium hexacyanoferrate (II) trihydrate (Sigma ≥ 99.0 %) in between the photoanode and Nafion membrane. The total sample injected was 0.5 ml in each device. In the case of NaCl as a media, same protocol is followed, instead of ASN.

Biophotovoltaics measurement and illumination:

The microbe's electrochemical characteristics were analyzed using a Biologic potentiostat (SP-150) through cyclic voltammetry and chronoamperometry. Measurements were conducted at the oxidation potential of potassium ferricyanide. Current was monitored continuously over a 12-hour period alternating between light and dark conditions. The system was initially stabilized in darkness for 2 hours, followed by 2 hours of illumination using the Holmarc Dual Gooseneck LED Illuminator, each arm providing 2000 lumens of white LED light with intensity control, totaling a maximum output of 4000 lumens.

Scanning electrochemical microscopy (SECM) for single microbes:

Localized single-microbe electrochemical analysis was conducted using SECM (Sensolytics GmbH) fitted with Autolab electrochemical workstation. A 1 µm Pt tip was used as the working electrode and Pt wire as the counter electrode. The analysis utilized a built-in electrochemical cell where microbes were immobilized on an agar-based biofilm along with a ferricyanide-based redox mediator. A dual gooseneck white light LED served as the light source.

Additional details on metabolic modeling

The genome scale metabolic model of Synechocystis PCC 6803, as well as the Synechocystis strain used in the bio-photoelectrochemical device, both belong to the genus Synechocystis, the set of core metabolic reactions for both of them are essentially the same. The metabolic pathways operating for photosynthesis, amino acid metabolism, biomass generation, oxidative phosphorylation etc. in both Synechocystis (modeling and experiments) are common, consisting of the same biochemical reactions. As there are no existing genome-scale metabolic models of *Synechocystis pevalekii,* we have utilized existing *Synechocystis* metabolic models for simulating the metabolism.

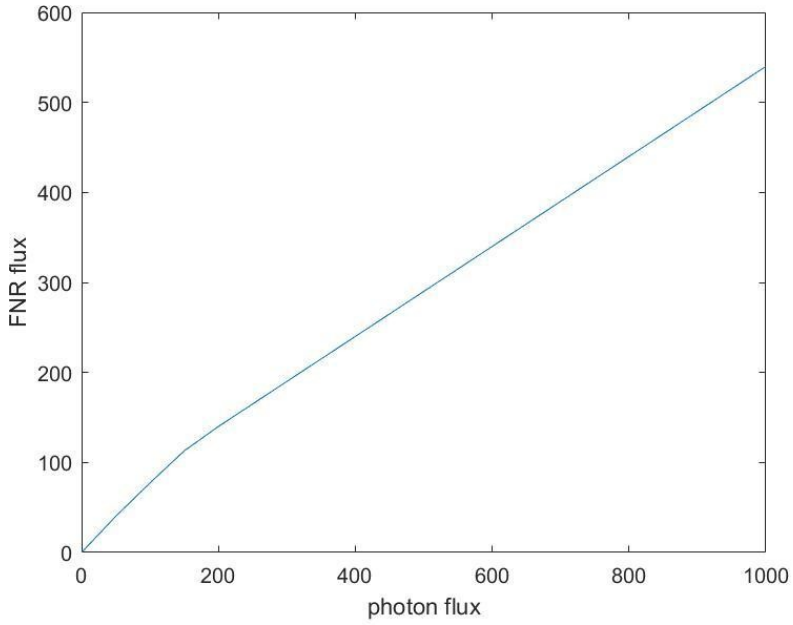

Figure S1. Effect of increasing light intensity on the FNR flux. The OD of the culture was fixed at 2. The concentration of the redox mediator was varied from 0.2 mM (varying concentration has no effect on the FNR flux).

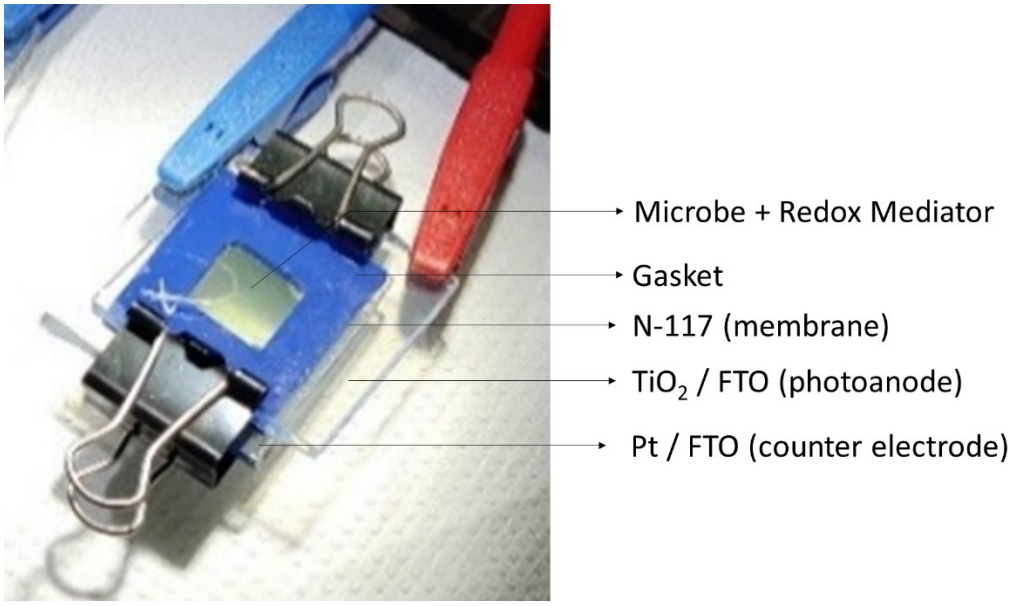

Figure S2. DSSC based device architecture.

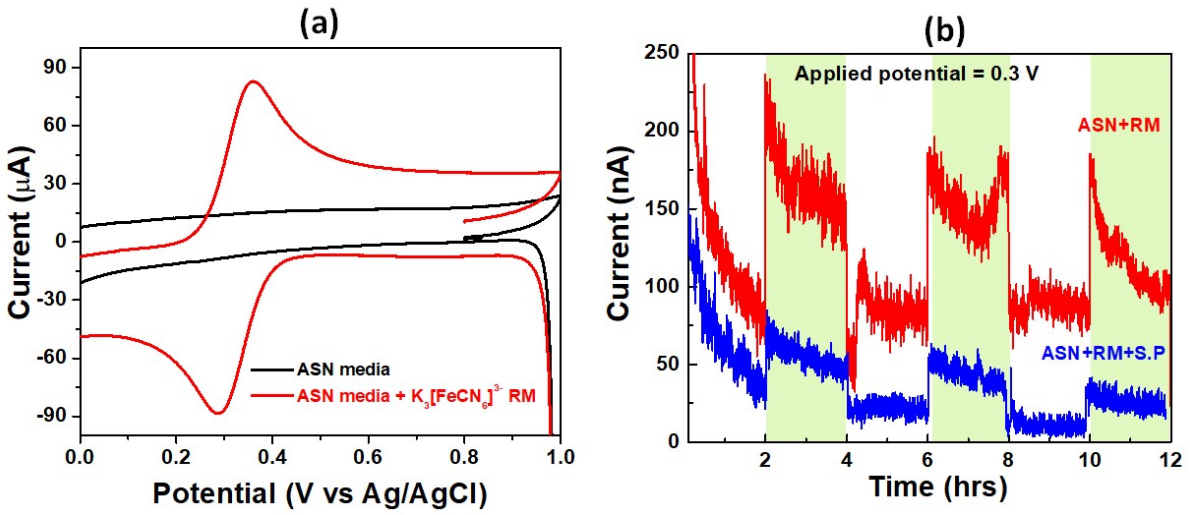

Figure S3. (a) CV curve of ASN media and ASN media + Redox mediator, performed in 2-electrode electrochemical system. (b) Chronoamperometry curves of ASN media + Redox mediator (Blank) and ASN media + Redox mediator + *Synechocystis Pevalekii* microbes at an applied potential of 0.3 V under dark and light conditions for 12 hrs.

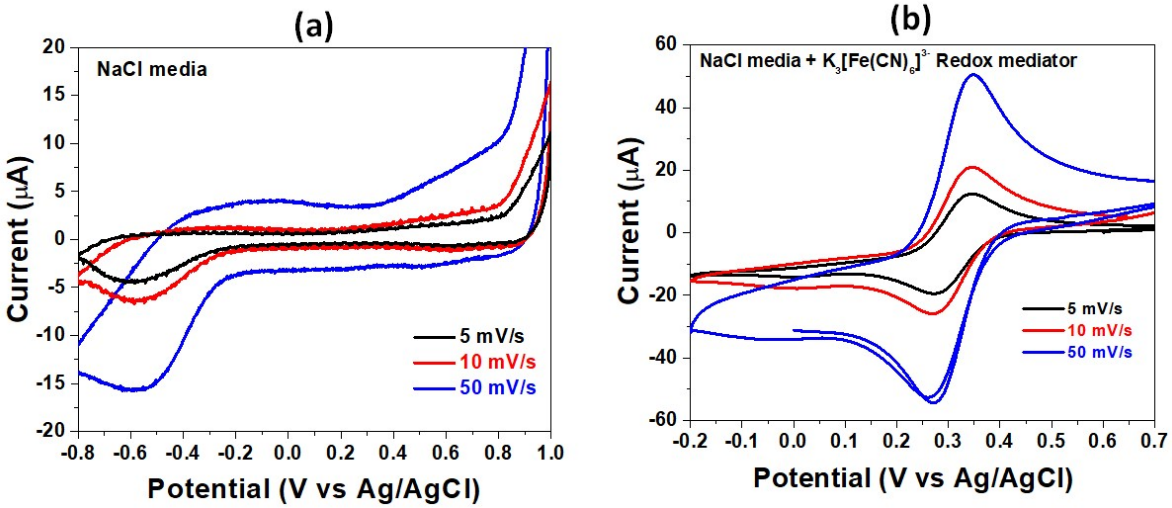

Figure S4. CV measurements of (a) NaCl media and (b) NaCl+FeCN+Redox-mediator performed in 2-electrode electrochemical system at different scan rates.

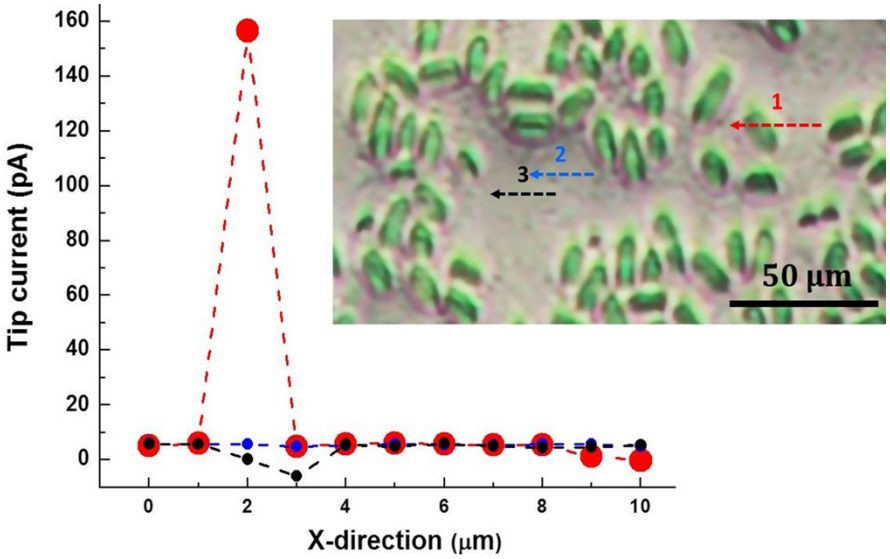

Figure S5. SECM line-scan showing performed on Agar-based bio-film with immobilized *Synechocystis pevalekii* microbes showing photocurrent response on the single microbe.

In the SECM experiment, using an illumination area that encompasses the entire electrode can compromise the accuracy of photocurrent measurements for individual *Synechocystis pevalekii* cells. This is due to the potential for other microalgae or components within the illuminated zone to also generate electrons, which could be transferred through the biofilm, thereby affecting the overall current measurements. To mitigate this issue, we assessed the photoresponse in various regions of the cell, independent of the illumination area in a SECM and the lines are depicted in Figure S5. The analysis revealed that when a 1 µm Pt-microelectrode tip was scanned across the microbe (point 1 – red arrow in inset), a photocurrent of 155 pA was recorded. In contrast, when the tip was moved across the biofilm at points 2 (blue arrow) and 3 (black arrow), no photocurrent was detected. This occurred because of the insulating properties of the biofilm. High-magnification microscopy was used to accurately to distinguish between microbe and non-microbe areas for precise measurements. The experiment demonstrates that the area of light illumination does not influence the photoresponse recorded from individual microbe.

Table S1. Name of the reactions and their subsystem provided in Figure 1.

Rxn no.	**Reaction name**	**Subsystem**
Rxn 1	Pyruvate-ferredoxin oxidoreductase	Pyruvate metabolism
Rxn 2	Dihydrolipoamide:NAD+ oxidoreductase	Citrate cycle (TCA cycle)
Rxn 3	(S)-2-Hydroxy-acid:oxygen 2-oxidoreductase	Glyoxylate and dicarboxylate metabolism
Rxn 4	Formate:tetrahydrofolate ligase (ADP-forming)	Glyoxylate and dicarboxylate metabolism, One carbon pool by folate
Rxn 5	NAD(P)H dehydrogenase (plastoquinone-8 & 3 protons) (periplasm)	Oxidative phosphorylation
Rxn 6	Succinate dehydrogenase (thylakoid)	Oxidative phosphorylation