Supporting Information

Photo-enhanced Electrochemical Interaction between *Shewanella* and a Hematite Nanowire Photoanode

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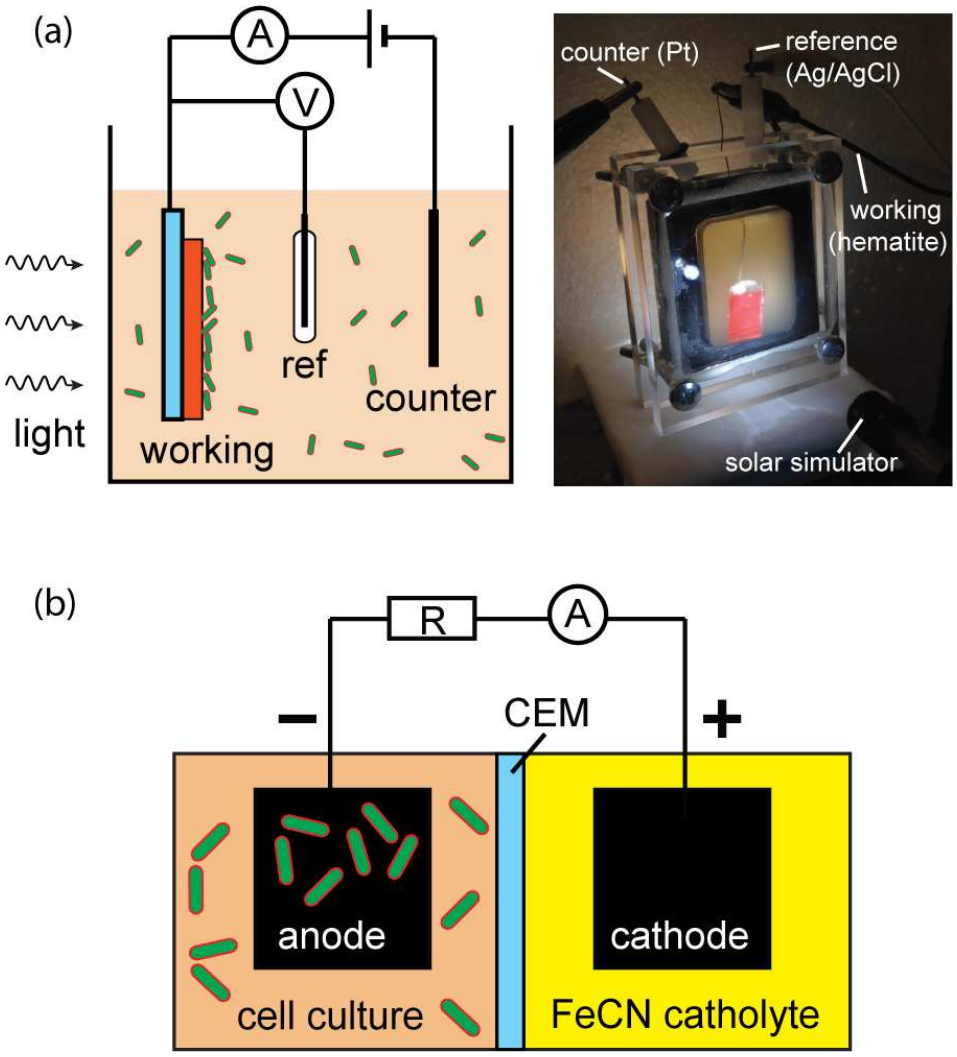

Figure S1 (a) Scheme and device picture of the solar microbial photoelectrochemical system (solar MPS), configured as a three-electrode photoelectrochemical cell. (b) Scheme of the microbial fuel cell (MFC) device. CEM represents cation exchange membrane.

Experimental Section

Materials

Fluorine-doped tin oxide (SnO_2:F) glass substrates were purchased from Wuhan Geao Company. Ferric chloride ($FeCl_3 \cdot 6H_2O$, 99%) was purchased from Acros Organics. HCl (36.5%–38% by weight), sodium chloride (NaCl, >99.5%), MOPS (>99.5%, cell culture tested), HEPES sodium salt (98%, cell culture reagent grade) and sodium nitrate ($NaNO_3$, 99.4%) were purchased from Fisher Scientific Company. Sodium DL-lactate solution (syrup, 60 % w/w, cell culture reagent grade), sodium fumarate (>99%), hydroxylamine hydrochloride (>99.999%) and Ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt) were purchased from Sigma Aldrich. SYTO® 16 Green fluorescent nucleic acid stain (1 mM in DMSO) was purchased from Life Technologies.

Synthesis of Hematite (α-Fe_2O_3) Nanostructures

The hematite nanowire arrays were prepared by previously reported hydrothermal method [1,2]. 20 ml aqueous solution containing 0.15 M of ferric chloride and 1 M sodium nitrate at pH 1.5 (adjusted by HCl) was added into a 30 mL stainless steel autoclave with a Teflon liner. A cleaned FTO glass substrate was put into the autoclave, and then heated at 95 °C for 4 hrs. The autoclave was cooled down at room temperature. The substrate was covered with a uniform layer of iron oxyhydroxide nanowire (β-FeOOH) film (yellow color). The β-FeOOH coated substrate was washed with deionized water, and then air dried. To obtain crystalline hematite, the β-FeOOH nanowire arrays were calcined in a horizontal quartz-tube furnace. The tube furnace was first vacuumed to a pressure of 15 Torr, and then filled with ultrahigh purity N_2 at 550 °C for 2 hr (99.998%,

Praxair) at 740 Torr pressure with a N_2 flow rate of 50 sccm. Herein, the initial oxygen content is estimated to be 0.43% (O_2/(O_2 + N_2), volume%)[3]. The color of the film changed from yellow to red. For those to be used in control experiment in Fig 5, the β-FeOOH nanowire arrays was first calcined in air at 550 °C for 2 hr, then subsequently was annealed at 800 °C for additional 20 min. The β-FeOOH nanowires were converted into hematite nanowires [2].

Photoelectrochemical (PEC) Measurements

Linear sweep voltammograms were measured in a three-electrode configuration with a platinum wire as counter electrode, Ag/AgCl (in 1 M KCl) as reference electrode, and hematite nanowire-arrayed substrate as working electrode, at a scan rate of 10 mV/s. Hematite photoanode was fabricated by soldering a Ti wire onto the bare edge of FTO glass substrate, and then sealing the FTO glass substrate and metal contact region with insulating epoxy resin. The exposed effective area for the hematite working electrode is 1.0-1.5 cm^2. All I-V measurements were recorded by an electrochemical workstation (Model CHI 660D, CH Instruments, Inc., Austin, TX); A 150 W Xe lamp (Newport 6255) coupled with an AM 1.5 global filter (Newport 81094) was used as the light source. The intensity of the incident light was measured with a digital power-meter and was controlled at 100 mW/cm^2. Amperometric I-t curves were collected with the same setup as linear sweep voltammograms, and recorded by an electrochemical workstation (Model CHI 1040B, CH instruments, Inc., Austin, TX) with a light intensity of ca.45 mW/cm^2. Cyclic voltammogram studies of the whole cell suspension were carried out under an anaerobic condition where the chamber was pre-purged and continuously

purged with nitrogen gas during the measurement.

Bacterial Strain and Culture Conditions

All glassware, solutions and media were autoclaved for sterilization prior to use. *Shewanella oneidensis* strain MR-1 (ATCC 700550) was purchased from American Type Culture Collection (ATCC, Manassas, VA), and cell cultures were started from replicate frozen stocks. For conventional (an)aerobic culture, cells were incubated in *Shewanella* basal medium with sodium lactate (15 mM) as the electron donor[4]. Sodium fumarate (40 mM) was added as the electron acceptors for anaerobic culture. Cells were grown at 30°C and with shaking at 220 rpm for 12-24 h (aerobic) or 24-48 h (anaerobic). For semi-anaerobic culture, cells were cultured in trypticase soy broth (TSB, B.D. Biosciences, Inc., San Jose, CA) at either room temperature or 30°C for 12-24 h with shaking at 100 rpm. For MFC and MPS studies, the cell culture was transferred into the anode chamber of either device. For electrochemical studies, the cell culture was first centrifuged to obtain a cell pallet, which was subsequently re-suspended in 10 mM MOPS (pH=7) and 8.5 mM NaCl solution[5].

Bacterial Fixation and Imaging

For fluorescent imaging, the bacteria-colonizing electrode was rinsed gently with PBS buffer, and then immersed with 1 μM SYTO stain in PSB buffer solution for 30 min. The sample was rinsed with PBS and mili-Q water again to remove extra dyes, dried at room temperature and imaged with a fluorescent optical microscope (Olympus BX51). For SEM imaging, the photoelectrode was treated using standard glutaraldehyde and osmium

tetroxide fixatives before electron microscopic studies[6]. In short, the photoanode was first rinsed with mili-Q water to remove non-adherent cells, then fixed in 2% glutaraldehyde solution overnight at 4 ºC, rinsed with water again, and finally stained with 1% osmium tetroxide solution for 2 hrs. After a final rinsing with water, the samples were dehydrated by serial, 10 min transfers through 50, 70, 90 and 100% ethanol. As-fixed samples were investigated under a field-emission SEM (Hitachi S-4800 II).

Colorimetric Iron Determination Assay

The iron retention was determined by a modified Ferrozine method[7]. First, working curve was obtained by using a series of standard $FeSO_4$ aqueous solutions from 0.1 to 5 mM. 0.5 mL of each $FeSO_4$ standard solution was added to 0.5 mL hydroxylamine/1 M HCl solution (saturated hydroxylamine hydrochloride in 1 M HCl aqueous solution), vortex for 10 s and incubated at room temperature for 1 h. Subsequently, a 20 μL of this solution was added to 200 μL of ferrozine (0.05 %) in 50 mM HEPES buffer at pH 7.0, followed by adding 1 mL of mili-Q water. The absorbance at 563 nm was measured and correlated with the known Fe(II) concentration to plot a working curve.

To determine the possible iron dissolution from the photoanode during the long-term culture, all the hematite substrates used were cut into halves, for quantifying the iron mole on the substrate before and after the long-term solar MPS operation. Two different hematite substrates (annealed at 550°C or at 800°C) were tested either without a bias or with an applied bias of 0.8 V *vs.* Ag/AgCl. For each condition, the experiment was run in triplicate. The substrate was incubated with 2 ml hydroxylamine/ HCl solution until the

iron on the substrate was dissolved and reduced completed, which was then treated as described above and compared with the working curve to determine the iron mole on the substrate.

References:

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