Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

SUPPLEMENTARY INFORMATION

Light-driven microbial dissimilatory electron transfer to hematite†

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Supplementary Methods

Microbial cultivation conditions. *G. sulfurreducens* DL-1 cells were routinely cultivated in modified DMSZ medium without vitamins and resazurin. The medium consisted of minerals solution, acetate and fumarate. The minerals solution contained: NH_4Cl 1.50 g/L, Na_2HPO_4 0.60 g/L, KCl 0.10 g/L, trace element solution 10 mL/L, and selenite-tungstate solution 1.0 mL/L. The trace element stock solution contained (per liter): Nitrilotriacetic acid 1.500 g, $MgSO_4 \cdot 7H_2O$ 3.000 g, $MnSO_4 \cdot H_2O$ 0.500 g, NaCl 1.000 g, $FeSO_4 \cdot 7H_2O$ 0.100 g, $CoSO_4 \cdot 7H_2O$ 0.180 g, $CaCl_2 \cdot 2H_2O$ 0.100 g, $ZnSO_4 \cdot 7H_2O$ 0.180 g, $CuSO_4 \cdot 5H_2O$ 0.010 g, $KAl(SO_4)_2 \cdot 12H_2O$ 0.020 g, H_3BO_3 0.010 g, $Na_2MoO_4 \cdot 2H_2O$ 0.010 g, and $NiCl_2 \cdot 6H_2O$ 0.025 g. The selenite-tungstate stock solution contained (per liter): NaOH 0.5 g, $Na_2SeO_3 \cdot 5H_2O$ 3 mg, and $Na_2WO_4 \cdot 2H_2O$ 4 mg. 20 mM acetate and 50 mM fumarate were individually supplemented as the electron donor and acceptor. The medium was boiled for 15 min, and sparged with N_2:CO_2 (80:20) to remove dissolved oxygen. After cooling down to room temperature, $NaHCO_3$ was dosed to adjust the pH to 7.0. Then, the medium was dispensed in serum bottles, and sealed with butyl rubber stoppers and aluminum tear-off seals before autoclaving.

Biofilm development in electrochemical cells. Single-chamber and three-electrode cells were used for biofilm development and electrochemical tests. Either ITO or α-Fe_2O_3/ITO electrode was used as working electrode (effective area of 4.91 cm^2).

Ag/AgCl (KCl saturated) and a platinum wire were respectively used as reference and counter electrodes. The chambers were autoclaved before use. In each electrochemical cell, 100-mL sterile culture medium without fumarate was added as the electrolyte, and de-aerated by bubbling with N_2:CO_2 (80:20). The medium pH was adjusted to 7 using $NaHCO_3$. Acetate of 20 mM was dosed as carbon source. Activated cells were seeded into the chambers (50%) and the potentials of the working electrodes were kept at a constant level (+0.0 V, +0.2 V, or +0.6 V) with a multi-potentiostat (CHI 1030A, CH Instruments Inc., China). The working electrodes served as the sole electron acceptor for microbial respiration at 30 °C.

Supplementary Figures

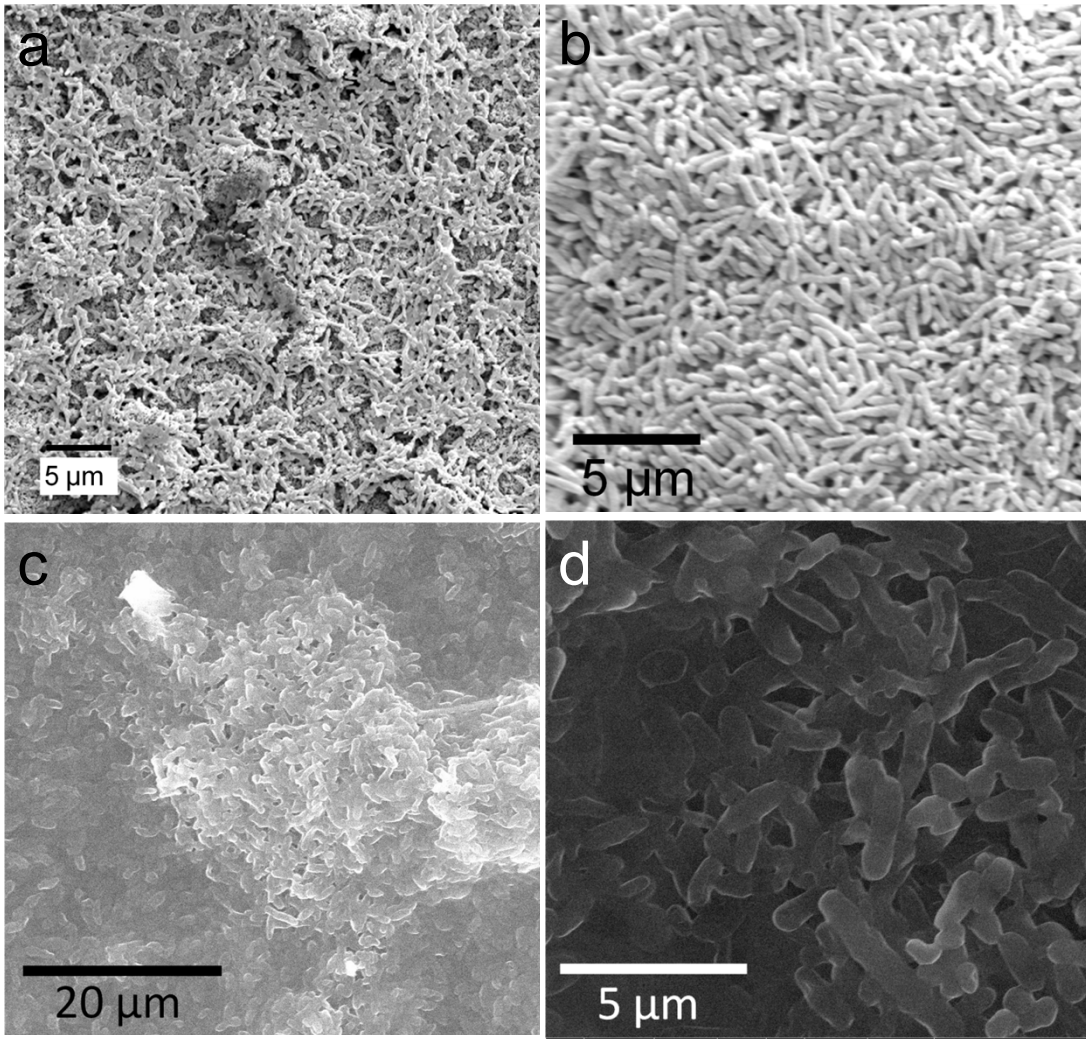

Figure S1 Microscopic morphology of *G. sulfurreducens* cells on α-Fe_2O_3 electrode. (**a**) SEM image of loose cells layer at anodic current of 5 µA; (**b**) SEM image of dense cells layer at anodic current of 1 mA; (**c** and **d**) ESEM images of dense cells layer on the α-Fe_2O_3 surface.

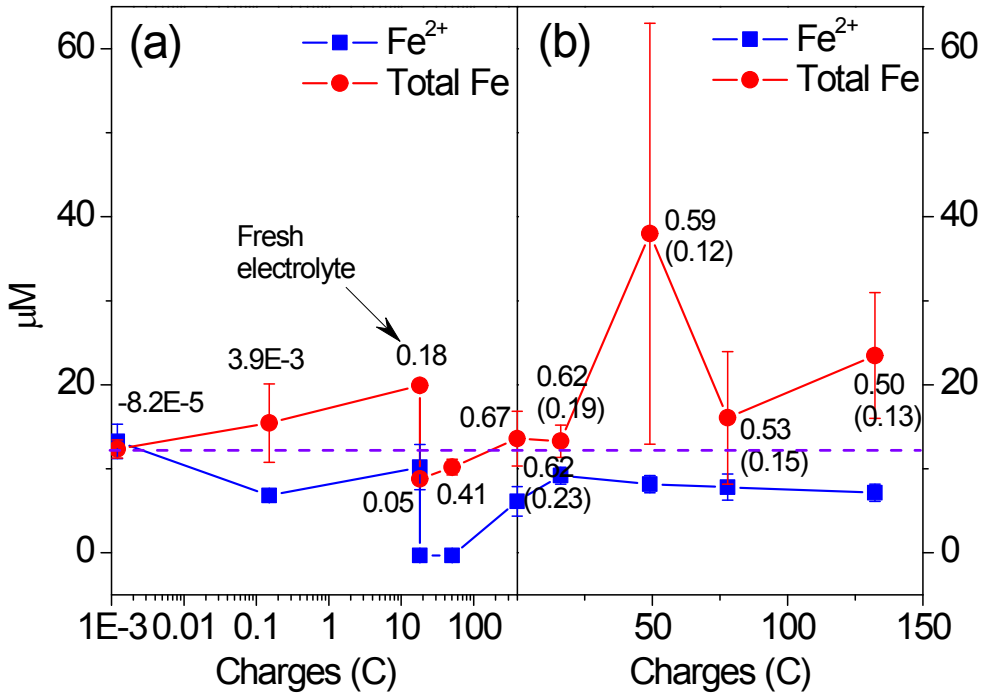

Figure S2 Dynamics of Fe(II) and total Fe concentrations in the electrolyte during the biofilm development and current evolution periods. The Fe concentration (μM) was plotted to integral charges (I×t, Coulombs) for: (**a**) the cells/α-Fe_2O_3 system under dark and (**b**) then under illumination. Total Fe = Fe(II) + Fe(III). The corresponding values of the electrochemical current (mA) were marked for each point. In Figure (**b**): I_{dark} (I_{photo}). The background of total Fe in electrolyte is shown with a dash of violet. The data were the average of the values from three different electrochemical cells.

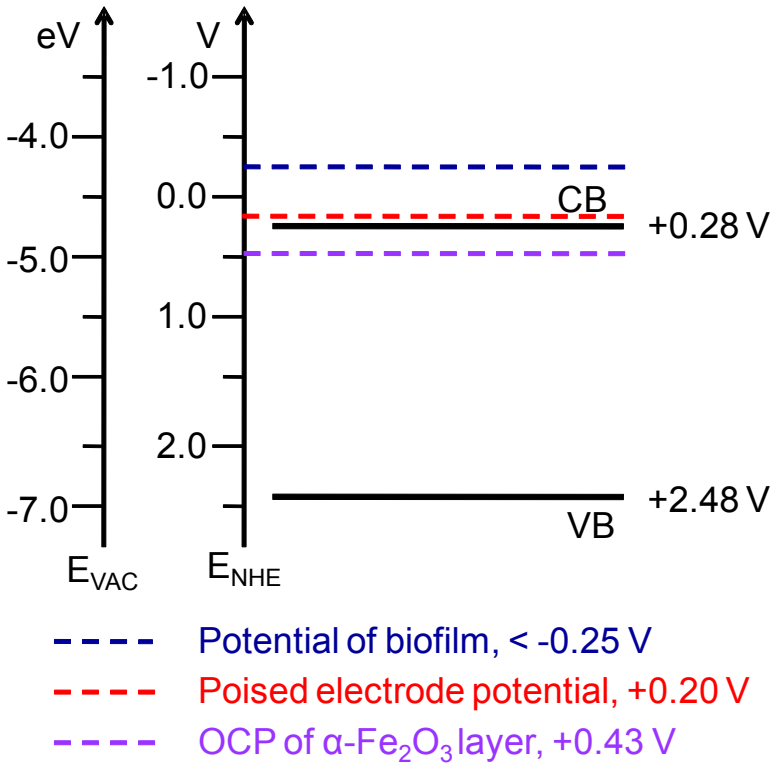

Figure S3 Relative energy diagram of n-type hematite (α-Fe_2O_3). The open-circuit potential (OCP) of α-Fe_2O_3 electrode shows the initial energy state of α-Fe_2O_3 layer before cell colonization. The representative potential of the mature, active biofilm is also shown. VB, valence band; CB, conduction band. Light of 563.4 nm wavelength is needed to excite the electrons to Fe(3d) orbitals (CB) and leave positive charges (holes) in O(2p) orbitals.

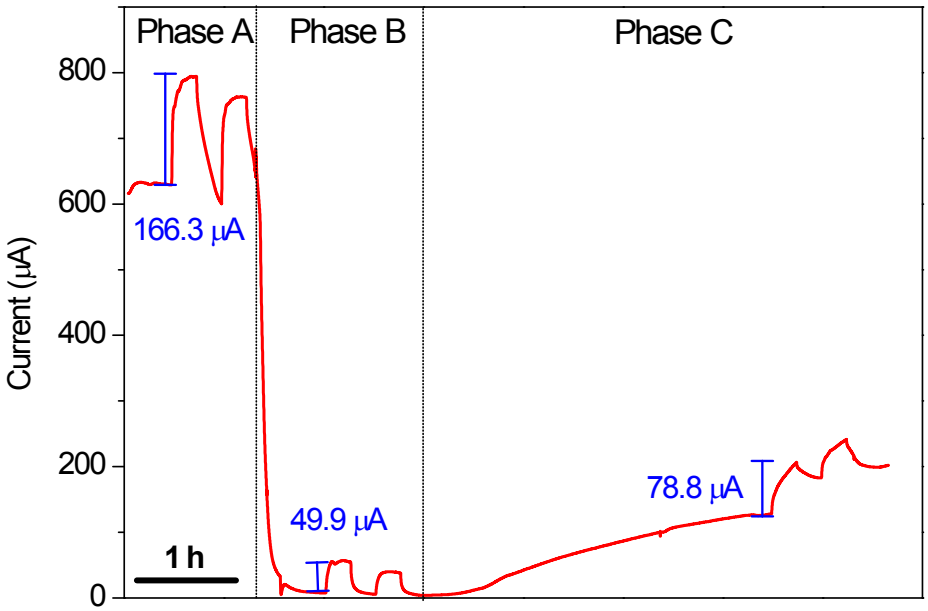

Figure S4 Suppression of photocurrent with CO binding to cytochrome c in biofilm. The mature biofilm on the α-Fe_2O_3 electrode with steady current was used. Photocurrent production at +0.2 V was examined with two cycles of intermittent illumination on the α-Fe_2O_3 layer before CO was used (Phase A), after bubbling with CO for 10 min (Phase B), and after eliminating the inhibition of CO by bubbling with N_2 for 3 h (Phase C).

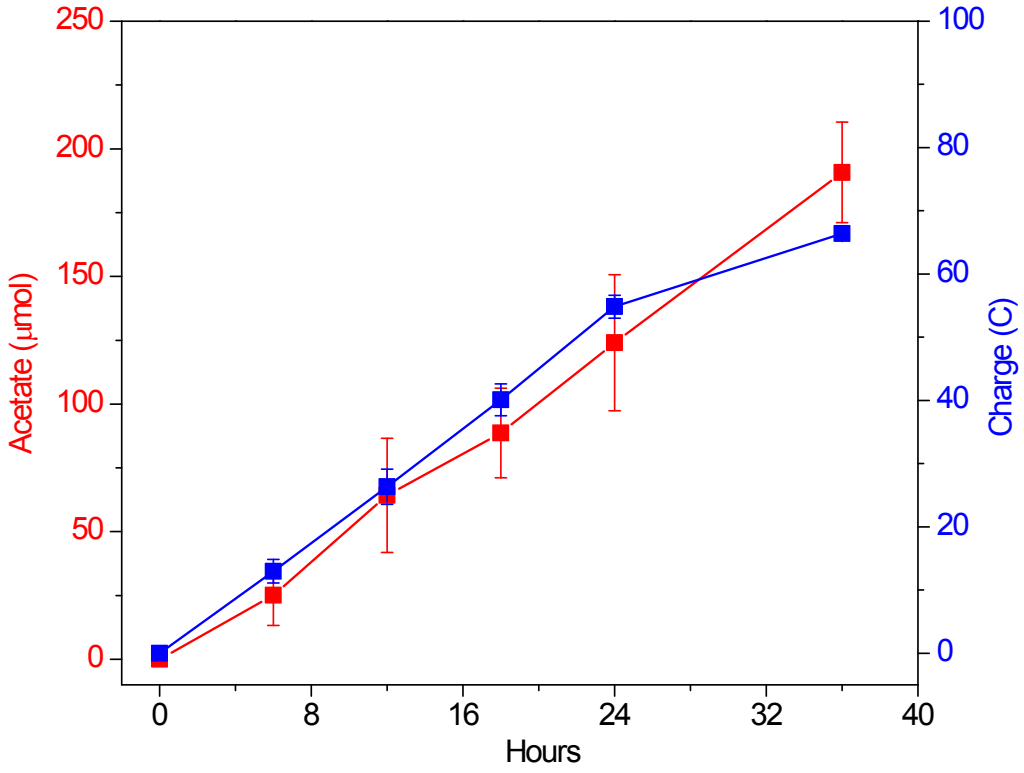

Figure S5 Relationship between the acetate consumption and the charge generation of the mature biofilms on the ITO electrodes at +0.2 V. The acetate consumption was calculated from the remaining concentrations in the electrolyte. The electric quantities were calculated by integrating currents with time. No illumination was provided.

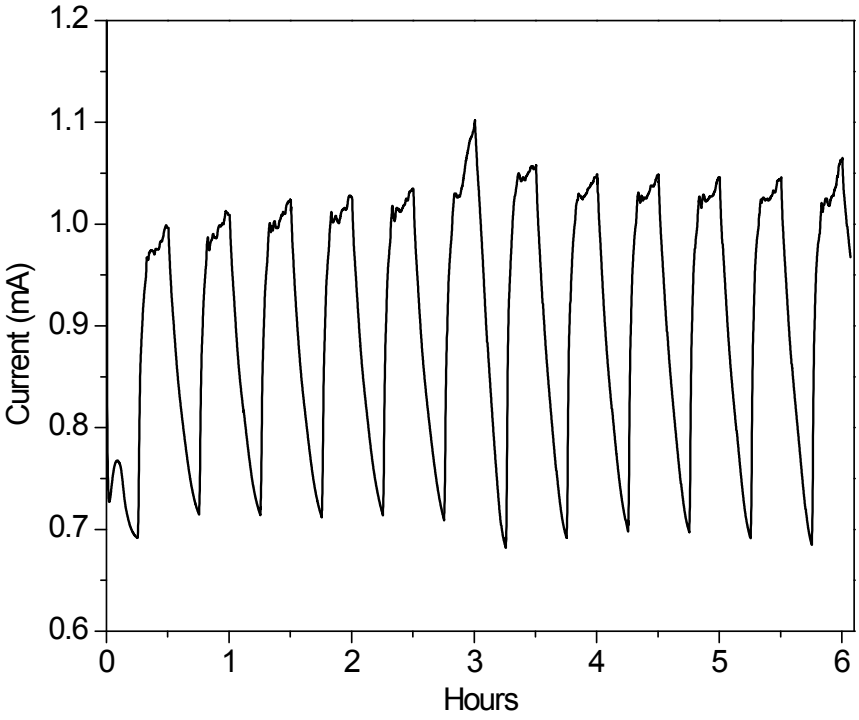

Figure S6 Representative photocurrent and operation procedures in the acetate consumption tests. Intermittent illumination (dark/light, 15 min/15 min) was applied to the mature biofilms on the α-Fe_2O_3 electrodes at +0.2 V. The experiment was temporarily interrupted every 6 h for sampling. A fluorescent mercury lamp was used as light source.

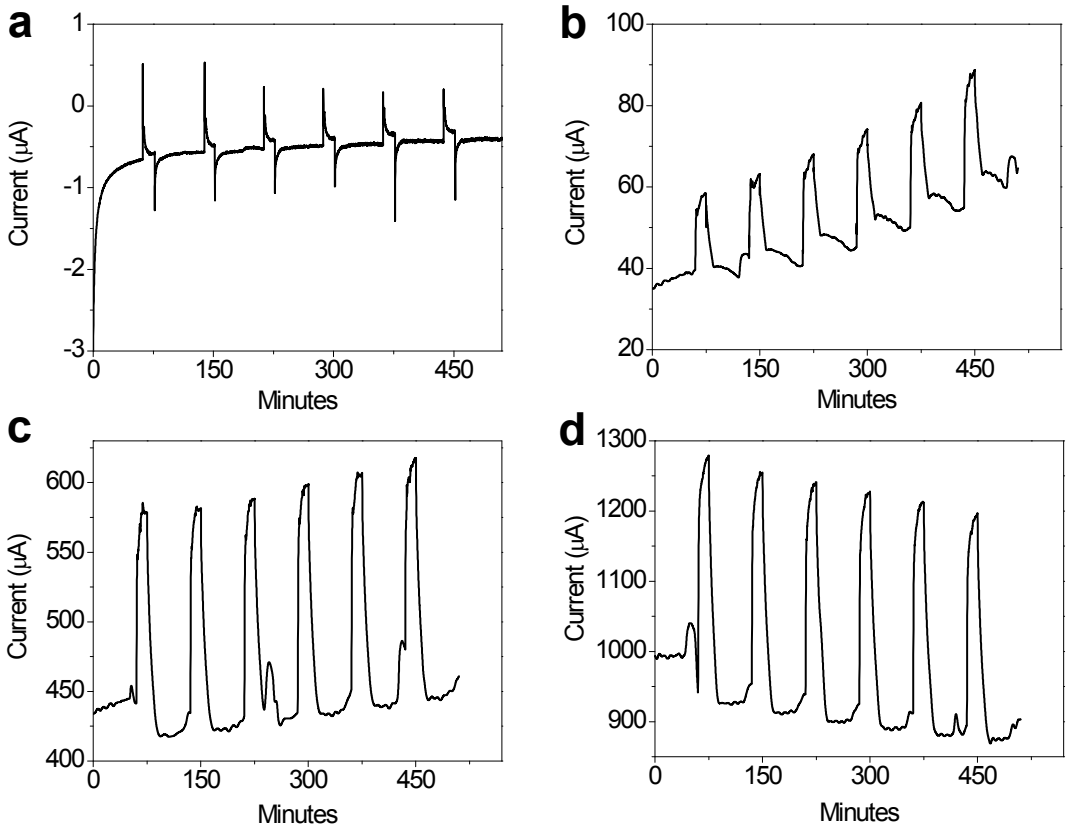

Figure S7 Responses of α-Fe_2O_3 to the intermittent illumination at different stages of biofilm formation at +0.2 V. Dynamics of photocurrent before microbial adhesion to α-Fe_2O_3 (**a**), at thin biofilm coverage (**b**), at thick biofilm coverage (**c**), and at fully mature stage (**d**).

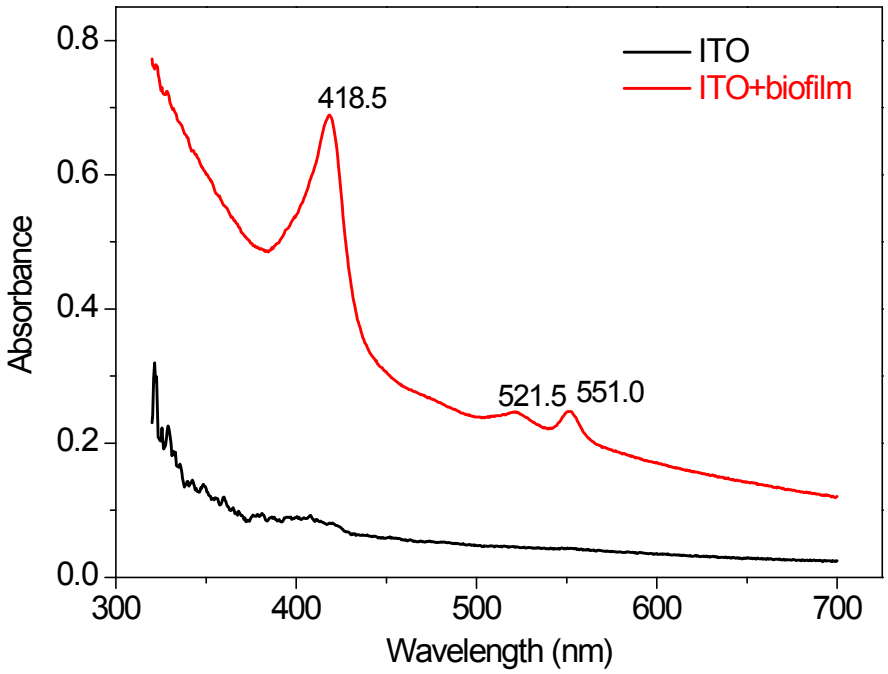

Figure S8 UV-Vis absorbance of *G. sulfurreducens* biofilm on the ITO electrode. Absorbance of the α-Fe_2O_3 layer was deducted.

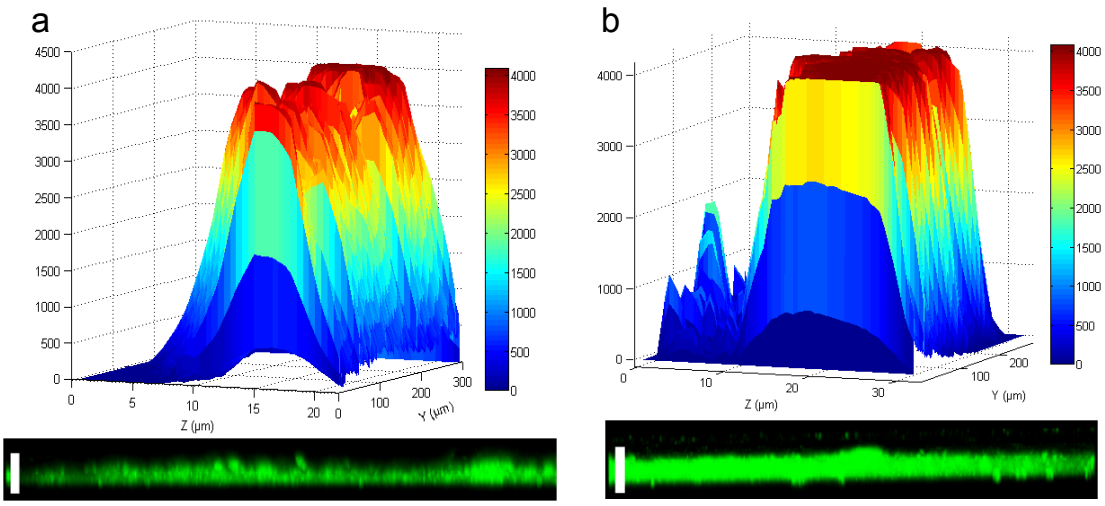

Figure S9 3-Dimentional distribution of the reductive enzymes in the biofilms. Active biofilms on the α-Fe_2O_3 electrodes were stained with RedoxSensor™ Green reagent. The dye can be reduced by the reductive enzymes in biofilms and yields green fluorescence under 490-nm light irradiation. Representative 3D images of biofilms were obtained with CLSM, and the spatial distribution of the reduced enzymes within biofilms is represented by fluorescence intensity. The fluorescence intensities in the X direction are superposed here, while those in the Y and Z directions of biofilms are shown. The interface between biofilms and α-Fe_2O_3 is defined as Z = 0. (a) The fluorescence intensities of an unilluminated biofilm. (b) Illuminated biofilm. The images at the bottom are cross-section views of biofilm with α-Fe_2O_3. Scale bars: (A) 20 μm; (B) 25 μm.