Ferrous Ion Regulated Extracellular Electron Transfer: towards self-Suppressed Microbial Iron(III) Oxides Reduction

Yonghua Yao, Xia Huang*

State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China

1. Experimental Details

Microbe preparation: *Shewanella loihica* PV-4 were cultured aerobically in marine broth (MB, 20 g/L) at 30 °C for 24 h firstly. Then microbes were collected and washed for three times with defined media (DML: contains NaHCO₃ (2.5 g), CaCl₂•2H₂O (0.08 g), NH₄Cl (1.0 g), MgCl₂•6H₂O (0.2 g), NaCl (10 g), and HEPES (7.2 g) per liter with sodium lactate (10 mmol/L) as the carbon source, pH 7.8) and finally was put into fresh DML medium for further aerobic cultivation at 30 °C for 24 hours during which process the number of microbe would not increase because lacking of yeast extract. Then the microbes were collected by centrifuging and were transferred into the electrochemical cell. The concentration of the cell suspension in the electrochemical cell was set to be the optical density at 600 nm of 2.0.

Electrochemical characterization: A single chamber, three-electrode system was used to monitor the microbial extracellular electron transfer behavior with the ITO working electrode (the electric active area of 3.14 cm²) as the only external electron acceptor. An Ag/AgCl (sat. KCl) and a platinum wire were used as the reference and counter electrode, respectively. DML (4 mL) was used as electrolyte and was deaerated thoroughly by N₂ bubbling for 30 min before measurements, which enables the poised ITO electrode acting as the only electron acceptor. The temperature was maintained at 30 °C during the whole electrochemical process.

The electrochemical measurement was performed using electrochemical workstation CHI 1030B (CH Instruments, USA).

2. The effect the exogenously addition of FeSO₄ on the current generation from bacterial *S. loihica* PV4 at a constant potential of -200mV. Upon FeSO₄ injection, the EET current
immediately decreased drastically, suggesting the alternation of EET pathway that might be triggered by a fast interfacial physicochemical process.

![Graph showing FeSO₄ injection effect](image1.png)

Figure S1: The curve of current generation versus time for strain *S. loihica* PV4 at a constant potential of -200 mV. The EET current keep increase at the initial 20 h, as previously reported. The FeSO₄ (the final concentration in the system is controlled as 50 µM) was added into the system in the moment indicated by the arrow, leading to an immediate and drastic current drop. It clearly suggested that the addition of FeSO₄ could largely suppress the EET current.

3. Whole-cell cyclic voltammogram (CV) of strain *S. loihica* PV4 taken after electrochemical culture at -200 mV for 30 h in the absence (black) and presence (red) of ferrous ions with scan rate of 50mV/s. A couple of asymmetric redox peaks was clear shown, which is attributable to the continuously electron injection into the OMCs from the respiratory chain. To be noticed, the *Eₜ* information based on the asymmetric redox peaks cannot fully reflect the redox nature of OMCs.

![Whole-cell CV graph](image2.png)

Figure S2. Whole-cell CV of strain *S. loihica* PV4 taken after electrochemical culture at -200 mV for 30 h in the absence (black) and presence (red) of ferrous ions with scan rate of 50mV/s.
4. Whole-cell CV of pure FeSO₄ solution (500 µM) taken with the scan rate of 20V/s. A clear redox peak with the Eₛ of -0.33V was shown, which is assignable to the oxidation of Fe²⁺ and reduction of Fe³⁺. To be noticed, the redox active specie of Fe²⁺/Fe³⁺ gives the Eₛ of -0.33V (vs Ag/AgCl), which is very different from the Eₛ of OMCs (within the range of -5 mV to -105 mV vs Ag/AgCl).

5. The linear sweep voltammogram obtained for the system with different concentration of ferrous ions, and the scan rate is 10 mV/h. In the presence of ferrous ion, the Eₛ show clearly negative shifting, and moreover the Eₛ shifts negatively with the increasing of the concentration of ferrous ions. The cross points of the dashed line and LSV curves indicated the EET pattern at each conditions when the concentration of ferrous ions varies. When the concentration of ferrous ions is small (0 µM, 10 µM or 30 µM), the EET current is liable to keep increasing with time because the reduced OMCs are involved (as indicated by the black, green and blue dots on the LSV curves). Whereas with the time prolonging, the accumulation of ferrous ions keeps increase until the concentration over than 50 µM, the EET pattern changes drastically where the EET current will be suppressed aroused by the oxidized OMCs.

Figure S3. Whole-cell CV of pure FeSO₄ solution (500 µM) taken with the scan rate of 20V/s

Figure S4 (a) The linear sweep voltammogram obtained with a scan rate of 10 mV/h for the system without (black) and with different concentration of ferrous ions (green: 10µM; blue: 30µM; red: 50µM). (b) The dependence of the Eₛ on the concentration of ferrous ions.
6. Whole-cell CVs of strain *S. loihica* PV4 in the absence of ferrous ions obtained with a scan rate of 50 mV/h before and after exogenous addition of riboflavin.

![Figure S5](image1.png)

Figure S5. Whole-cell CVs obtained with a scan rate of 50 mV/h at the moment of 3 (before the riboflavin addition) and 4 (about 20 h after riboflavin addition) of strain *S. loihica* PV4 cultured electrochemically at -200 mV in the absence of ferrous ions (Fig. 2c, black curve).

7. The effect of ferric ions on the bacterial EET behaviour was monitors by measuring EET current and whole cell CV in the presence of ferric ions, which suggesting that the ferric ions do not show any inhibition effect on the bacterial EET current.

![Figure S6](image2.png)

Figure S6. (a) Current generation versus time of strain *S. loihica* PV4 at a constant potential of -200 mV for the system without (black) and with ferric ions (50 μM, blue). (b) Whole-cell cyclic voltammograms of strain *S. loihica* PV4 taken after electrochemical culture at -200 mV for 30 h in the absence (black) and presence (blue) of ferric ions.