Supplementary Information

Microbial Oxidation of Graphite by *Acidithiobacillus ferrooxidans* CFMI-1

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Methods

Synthesis of Graphite Oxide. Natural flake graphite was purchased from Qingdao Zhongtian Company (Qingdao, China). Graphene oxide (GO) was synthesized from natural graphite powders by a bio-oxidation method. A typical experiment procedure[1-3] is as follows: 3.0 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.1 g/L KCl, 0.5 g/L $MgSO_4 \cdot 7H_2O$ and 0.01 g/L of $Ca(NO_3)_2$ were added to 4 L water and the mixed aqueous solution was adjusted to a pH of 2.0. In convenience, the mixed aqueous solution was labeled as $9K_1$ medium. A 25 mL of 2% graphite suspension was added into 4 L $9K_1$ medium. Then the $9K_1$ medium was sterilized *via* autoclaving. 222.5 g of $FeSO_4 \cdot 7H_2O$ was added 1 L water and the aqueous solution was adjusted to a pH of 2.0, which was labeled as $9K_2$ medium. Then $9K_2$ medium was degermed by microfiltration membrane (0.22 μm) filtration. The above 4L $9K_1$ medium and 1L $9K_2$ medium were mixed in a 5 L glass reaction kettle, namely 9K medium. Bacteria (*Acidithiobacillus ferrooxidans* CFMI-1) were inoculated into 9K medium and grown at 30℃ with stirring speed 170 r/min for 3 days in a 5 L glass reaction kettle (Fig. S-2). Then the reaction was not ceased by adding some deionized water until the above systems became tawny and the precipitate appeared. Subsequently, diluted hydrochloric acid was added to dissolve the generated yellow precipitate with stirring for two days for enough dissolving. The slurry-like product was centrifugated and washed with distilled water five times to remove thallus and impurities, and dried in a vacuum oven at 60° for 12 h. Then, 0.37 g biologically converted graphite oxide samples were obtained. In order to improve the bio-oxidation degree of the samples, the obtained samples were bio-oxidized two more times by above experiment procedures. Then, the product (bio-oxidation of 3 times) was labeled as BCGO. For comparison, CCGO was synthesized from purified natural graphite according to the method reported by Hummers and Offeman[4].

Characterization. Raman spectra was recorded from 500 to 4000 cm^{-1} on a Renishaw Invia Raman Microprobe using a 514 nm argon ion laser. Field-emission

Transmission electron microscopy (FE-TEM) images were taken with a JEOL JEM2100F microscope. Field-emission scanning electron microscopy (FESEM) was performed with a LEO1550 microscope. X-ray photoelectron spectra (XPS) was carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg Kα radiation (hν=1253.6 eV). The XPS peaks were deconvoluted using Lorentzian-Gaussian components after a shirley background subtraction. Energy Dispersive X-ray Detector (NORAN SYSTEM7, Thermo scientific) were performed at 250kV. Samples for AFM imaging were prepared by drop-casting the dispersive BCGO onto freshly cleaved mica substrates, which were then allowed to dry in air.

References

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2. T. Kai, T. Nagano, T. Fukumoto, M. Nakajima and T. Takahashi, *Bioresource Technology*, 2007, **98**, 460–464.

3. M. E. A. G. Oprime, O. Garcia and A. A. Cardoso, *Process Biochemistry*, 2001, **37**, 111–114.

4. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339.

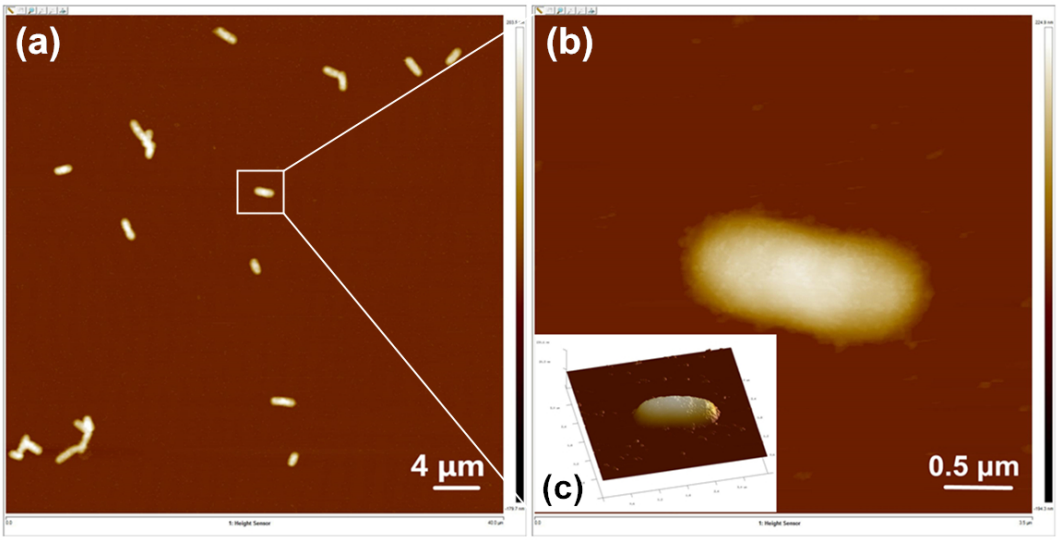

Figure S-1. Atomic force microscope images of *Acidithiobacillus ferrooxidans* CFMI-1.

Figure S-2. Bio-oxidation of graphite by *Acidithiobacillus ferrooxidans* CFMI-1 using a 5 L glass reaction kettle.

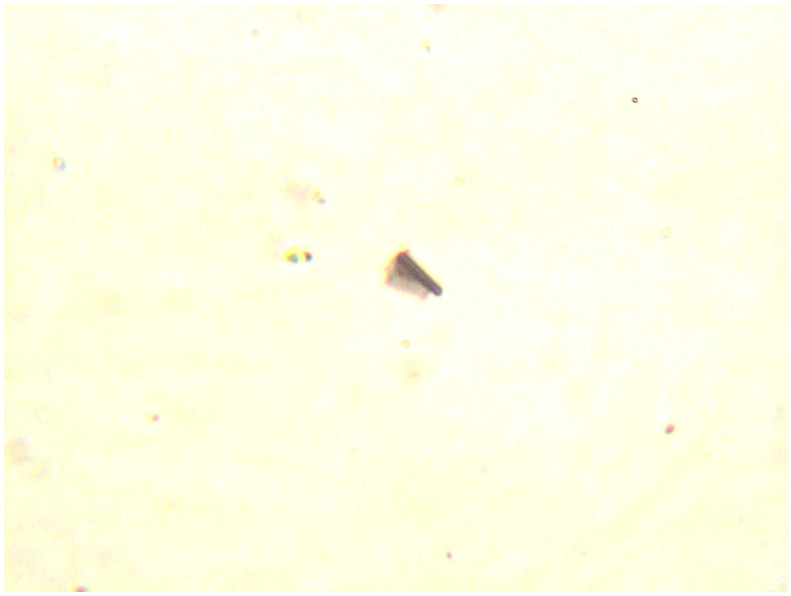

Figure S-3. Microscope photographs (×800) of graphite oxide after bio-oxidation by *Acidithiobacillus ferrooxidans* CFMI-1.

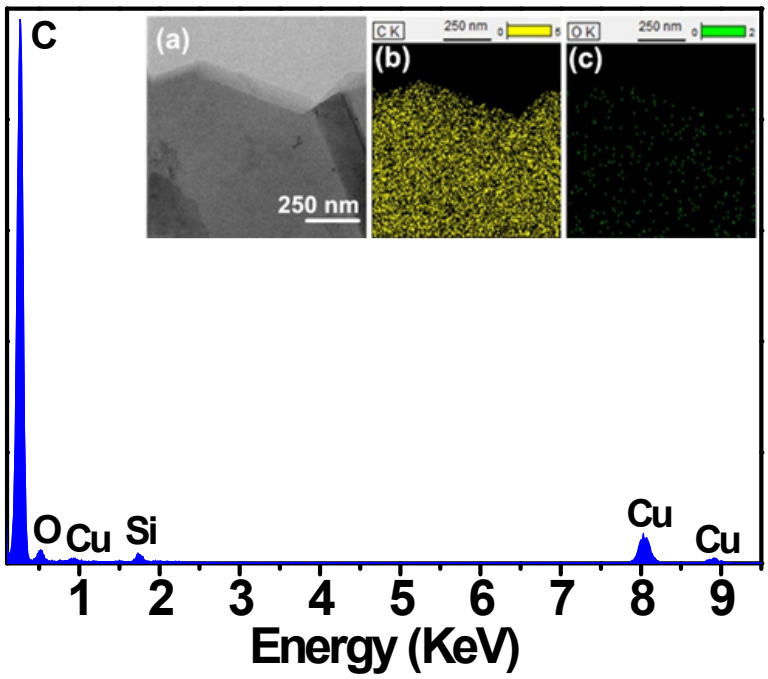

Figure S-4. EDX spectrum of BCGO. The area ratio of C peak curve integral and O peak is 30:1. (inset: TEM image of BCGO (a), mapping of C (b) and mapping of O (c).)

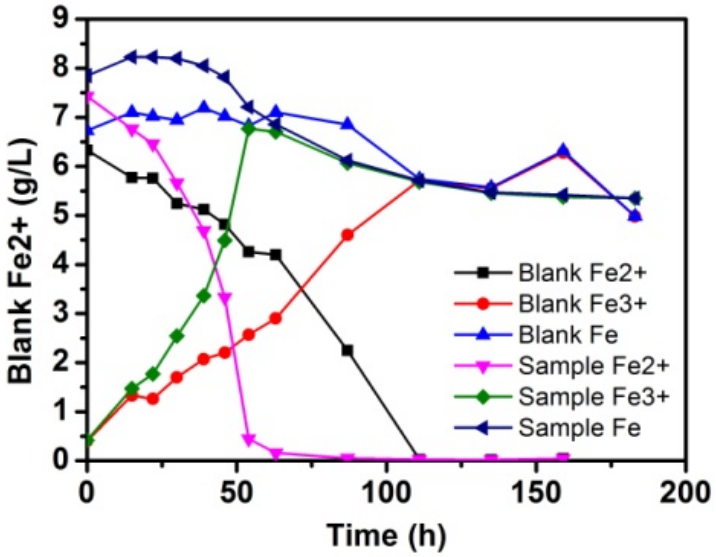

Figure S-5. Variation of concentration of various valence of Fe with the bio-oxidation time by *Acidithiobacillus ferrooxidans* CFMI-1. (Blank-without graphite; Sample-with 10 mL of 2% graphite suspension in 1 L 9K medium (SI, Methods))

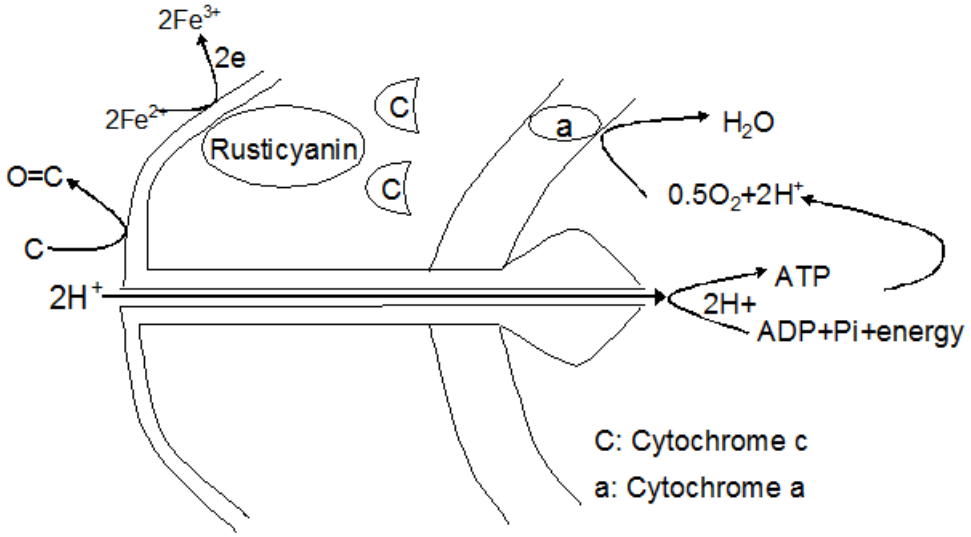

Figure S-6. The oxidation mechanism schematic of Fe^{2+} and graphite of *Acidithiobacillus ferrooxidans* CFMI-1. The enzymes and cytochrome participate in oxidation of Fe^{2+} and graphite can be described as follows: Fe^{2+} goes into biological respiratory chain located before cytochrome C after the ubiquinone bond with rusticyanin, then linked with cytochrome C (electron transport chain), the electron transferred to oxygen by cytochrome a finally.

Table S-1. I(D)/I(G) ratio of the Raman spectra of graphite, BCGO and CCGO.

Samples	I(D)/I(G)
Graphite	0.05
BCGO	0.23
CCGO	0.78

Table S-2. XPS data of C1s of CCGO and BCGO. The results of the four main peaks are tabulated below as binding energies and area percentages relative to C-C bonds (in parentheses).

Samples	C-C	C-OH	C-O-C	HO-C=O
CCGO	285.24 (100)	286.45 (22)	287.38 (180)	288.25 (14)
BCGO	284.52 (100)	285.22 (46)	286.19 (44)	290.60 (12)

Table S-3. Oxygen atomic percent of BCGO prepared by *Acidithiobacillus ferrooxidans* CFMI-1.

Sample	BCGO	CCGO	Graphite
C% (atom %)	92.8	52.8	99.6
O% (atom %)	7.2	47.2	0.4