-Supporting Information-

Long-term Succession Shows Interspecies Competition of *Geobacter* in Exoelectrogenic Biofilms

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19 pages, including text (1165 words), 1 table, and 10 figures.

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time.

Figure S9. The growth rate of cell density of biofilms formed by *G. sulfurreducens* PCA and *G. anodireducens* SD-1 in pure culture or co-culture in cycle 1-5.

Figure S10. Transmission electron micrograph of (A) *G.sulfurreducens* PCA and (B) *G.anodireducens* SD-1 grown on electrodes.

Electrochemical and Chemical Analysis in MFCs

Cyclic voltammetry (CV) was conducted on MFC biofilm using a potentiostat (Autolab PGSTAT 302 N, Metrohm, Netherland) over a potential ranging from − 0.6 to 0.2 V at a rate of 1 mV/s, with a platinum plate (1 cm^2) counter electrode and an Ag/AgCl reference electrode.[1] Maximum power densities were obtained from polarization curves measured by varying external resistance from 1000 to 50 Ω with a 30 min of time interval. Open circuit potentials (OCP) of anode and cathode were recorded using Ag/AgCl as the reference electrode. All electrochemical tests were carried out monthly with sufficient substrates.

Substrate consumption was evaluated by measuring the change of chemical oxygen demand (COD) using a colorimeter (DR 1900, HACH Company) over one cycle.[2] The pH value was detected by a pHS-25 pH meter from Shanghai Leiji Instrument Factory. Dissolved oxygen (DO) in suspension was recorded using microsensors connected to a micromanipulator and a multimeter (MM-Meter, Unisense, Aarhus N, Denmark) every 5 min according to the standard protocol.[3]

Anaerobic and Aseptic Procedures in Co-culture Tests

Fresh medium was deoxygenated (N_2/CO_2, 80/20, v/v) for 40 min in serum vials (100 mL in volume), then sealed with butyl rubber and aluminum cover, and finally sterilized at 121 °C for 20 min. The reference electrodes were wiped with 75% ethanol and then sterilized under ultraviolet irradiation for 12 h. Other reactor components were

sterilized by autoclaving (121 °C, 20 min). The assembly and inoculation of reactors were completed in an anaerobic glove box (1029, Thermo Scientific, USA).

Selection of the Optimal Norspermidine (NP) Concentration

A batch of smaller single-chamber air-cathode MFCs (3 cm in diameter, 4 cm in length, volume of 28 ml) were constructed and operated as we previously described[4] to pre-explore the optimal concentration of NP. The electrochemical performance of biofilms remained stable in long-term operation, but there was little *Geobacter* in the outer layer, resulting in the inability of planktonic cells to generate current in L-BES (see the control group in Figure S1). At this time, reactors were supplemented with media containing different concentrations of NP (0, 1, 7, 20 and 70 mM), and were slightly shaken to bring NP into full contact with outer layers of the biofilms. After 12 hours of interaction, the media were excreted, accompanied by the shedding of part of the outer biofilm. In fact, bacterial adhesion related to exopolysaccharides was also severely damaged, so that the originally dense biofilms easy to fall off in the following cycles. Hence, reactors were cultured in a NP-free medium for at least 3 cycles until there were no visible biofilms shedding, indicating that the effect of NP on biofilm has been eliminated. To determine the impact of NP on the inner *Geobacter*, the planktonic cells exfoliating from the inner biofilm exposed after NP elution were also detected for electroactivity in L-BESs.

After flushing with ≤ 20 mM NP, cell voltages remained steady or gradually

recovered (Figure S1A). The planktonic cells after outer layer removal also restored the electroactivity in L-BESs (Figure S1B). Considering the starting current and CV limiting current of L-BESs, the inner layers remaining after flushing with 7 mM NP had the highest electrochemical performance (Figure S1B and S1C), which showed that 7 mM NP treatment maintained the advantages of *Geobacter* in biofilms to the greatest extent. Therefore, the optimum concentration of NP was determined to be 7 mM.

Tolerance Tests of Two *Geobacter* Strains to NP

To explore the tolerance of two *Geobacter* strains to NP, sterile NP at a final concentration of 7 mM was added to the modified acetate-ferric citrate (FcA) medium (10 mM sodium acetate, 20 mM ferric citrate) and inoculated with these two *Geobacter* strains. Similar to the interaction between MFC biofilms and NP, *Geobacter* cells were transferred to NP-free FcA media with an optical density at 600 nm (OD_{600}) of 0.1 after interacting with NP for 12 h. The cell growth curve was obtained by measuring the Fe(II) change over time through the phenanthroline method. When the electron acceptors in the system were all reduced, bacteria in the liquid were collected by centrifugation (13,700 ×g, 10 min), and then subjected to alkaline lysis (0.3 M NaOH) to obtain a total protein extract. The effect of NP on the biomass of these two *Geobacter* strains was evaluated by measuring the protein content.

Extracellular Polymeric Substances (EPS) Extraction

A modified cation exchange resin (CER) method was used to extract EPS from biofilm samples by physical friction.[5] The CER (Na ion exchange resin, strongly acidic, 20-50 mesh) used in this test was purchased from Sigma-Aldrich. Prior to use, CER (1.25 g) was placed in a 10 mL sterile centrifuge tube, and washed with 10 mL PBS in a rotating incubator at a speed of 40 r/min for 2 h. Then, PBS was poured away and the collected biofilm suspension was added to the centrifuge tube. The sample and CRE interacted in the rotating incubator (40 r/min) for 12 h at 4 °C. The supernatant obtained by centrifugation (9,000 ×g, 10 min, 4 °C) was the extracted EPS.

qPCR Settings

A 20 μL reaction system of qPCR included 10 μL 2 × AceQ qPCR SYBR Green Master Mix (Q111-02, Vazyme, Nanjing, China), 7 μL DNase-free water, 0.5 μL primers (20 mM) and 2 μL template DNA. The amplification was conducted by the following program: pre-denaturation at 94 ℃ for 5 min and then 40 cycles of 94 ℃ for 30 s, 57 ℃ for 30 s and 72 ℃ for 30 s. As a last step, a melting curve from 55°C to 94°C was applied to verify the purity of the qPCR products.

131 **Table S1**. Sequences of primers used for qPCR.

Primer name	Sequence (5' to 3')	Product length
GA-F (*Geobacter anodireducens* SD-1 for)	CCCTTTGCCATAATCAGC	128
GA-R (*Geobacter anodireducens* SD-1 rev)	ACCGACAGGAGGTAAATCG	
GS-F (*Geobacter sulfurreducens* PCA for)	ACCATCAATCTCTGTCTGGAG	130
GS-R (*Geobacter sulfurreducens* PCA rev)	TCTTGCCTTCGGTCACAT	

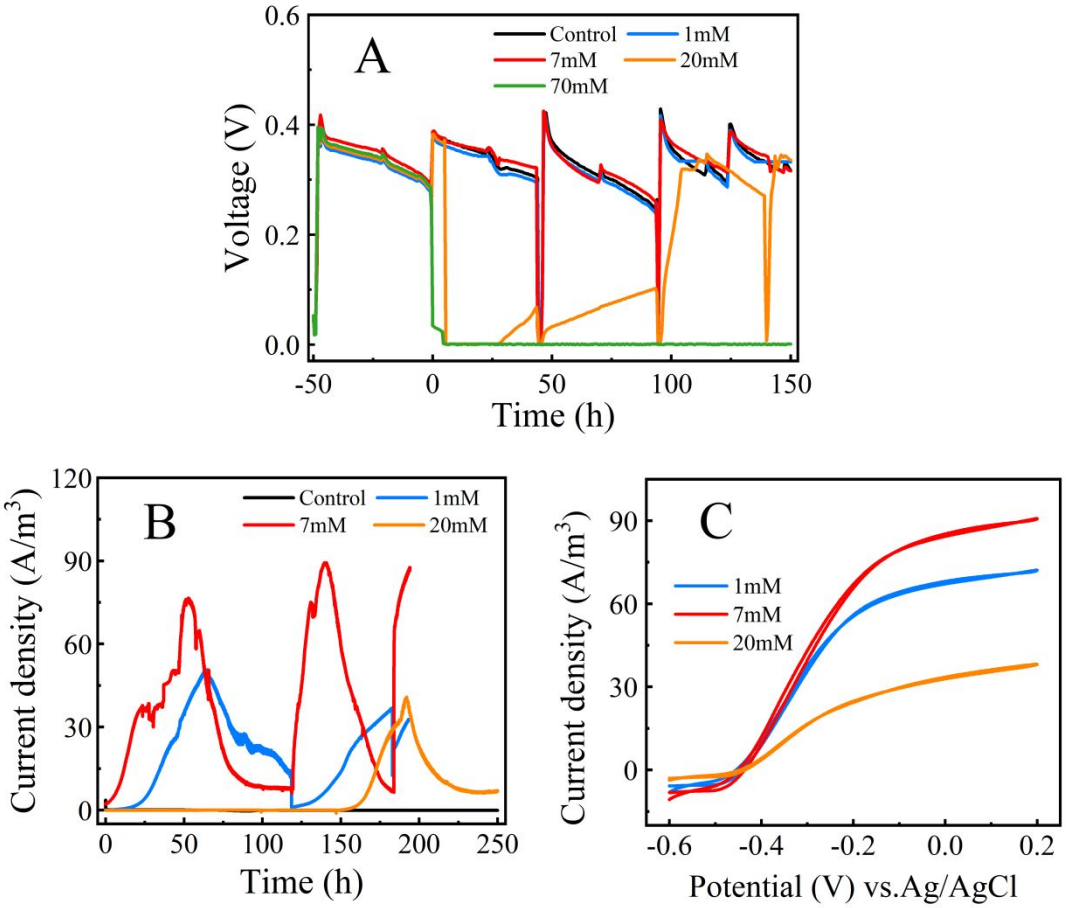
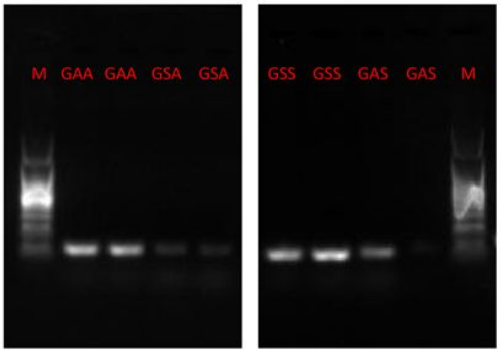


Figure S1 Effect of different concentrations (0, 1, 7, 20, 70 mM) of NP on MFC biofilms. (**A**) Cell voltages of smaller MFCs before and after NP flushing. (**B**) Time-current curves and (**C**) CV curves of L-BESs inoculated with planktonic cells of smaller MFC treated with NP.



Validation of primers GA-F/GA-R and GS-F/GS-R.
GAA: *G. anodireducens* DNA amplified by GA-F/GA-R;
GSA: *G. sulfurreducens* DNA amplified by GA-F/GA-R;
GSS: *G. sulfurreducens* DNA amplified by GS-F/GS-R;
GAS: *G. anodireducens* DNA amplified by GS-F/GS-R;
Two replicates were done for each combination.
M: 100 bp Marker.

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139 **Figure S2** Image of agarose gel electrophoresis of amplification products.

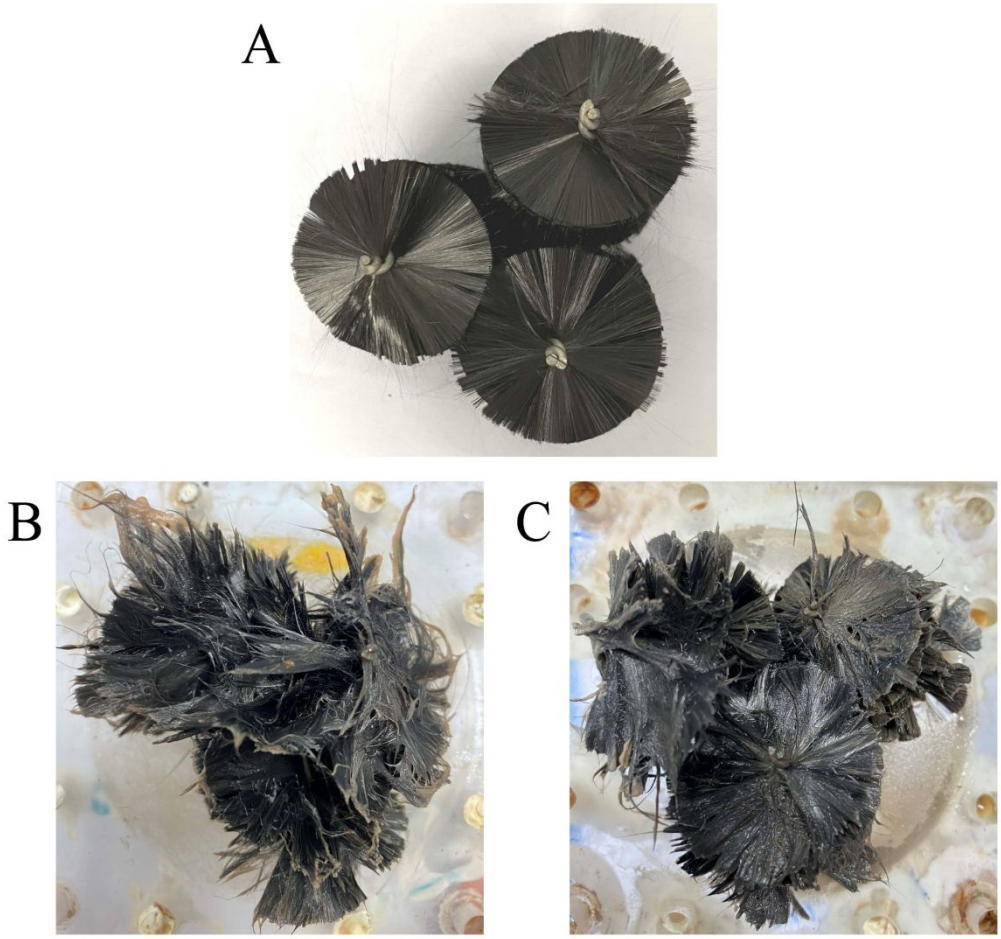


Figure S3 Physical images of MFC carbon fiber brushes anodes in different time. (**A**) Clean carbon fiber brush without biofilms. At the end of month 11, the representative carbon fiber brushes with biofilms (**B**) before NP treatment and (**C**) after 7 mM NP treatment.

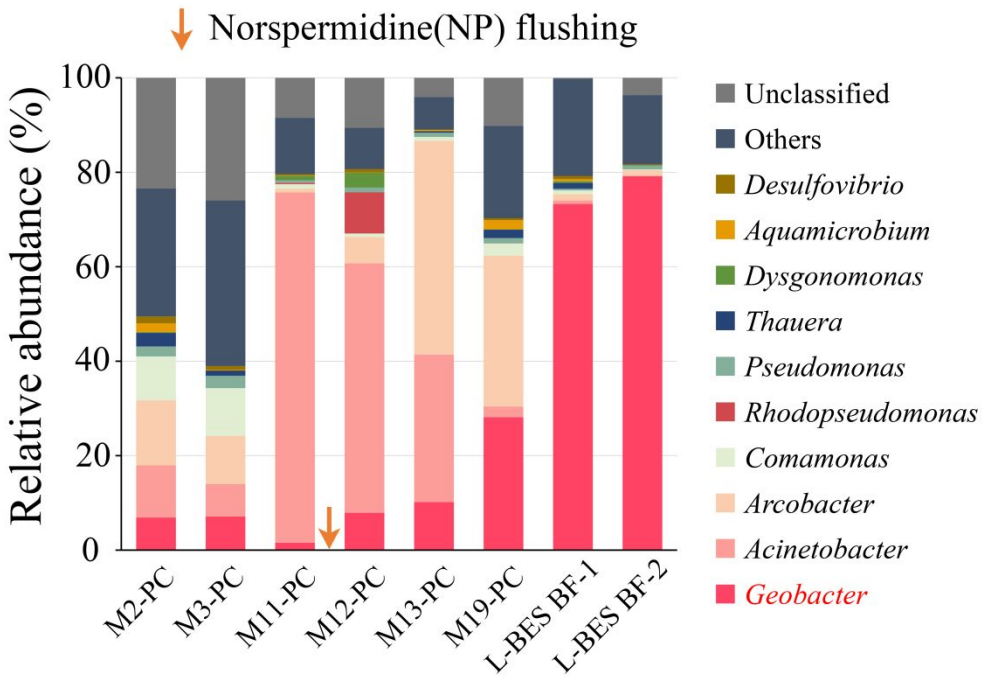
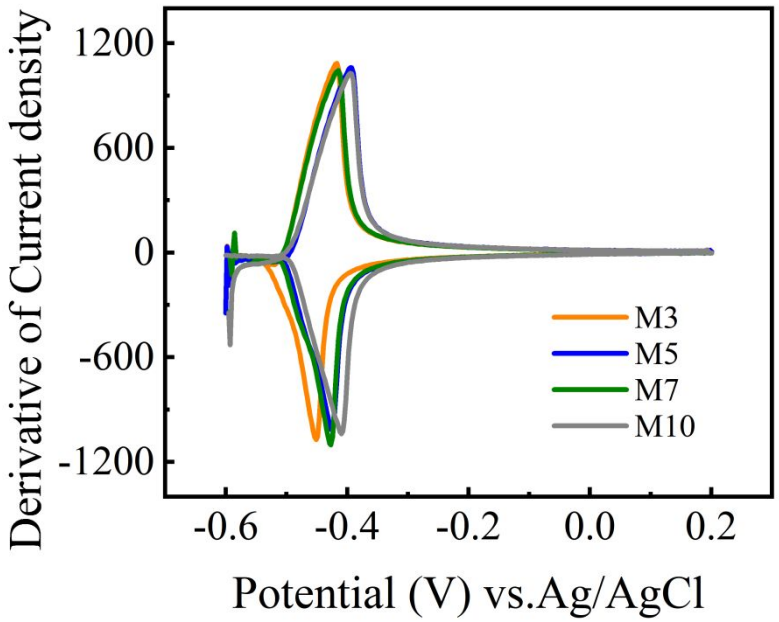


Figure S4 Taxonomic classification of microbial communities in MFC planktonic cells and L-BES biofilms at the genus level (Top 10). M2-PC to M19-PC indicate results of MFC planktonic cells from month 2 to 19. L-BES BF-1: the biofilm formed by inoculating L-BES with planktonic cells in the first four months. L-BES BF-2: the biofilm formed by inoculating L-BES with planktonic cells after NP flushing (after month 11). The orange arrow represents the 7 mM NP treatment.



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153 **Figure S5** First order derivative on turnover CVs of anodic biofilms in MFCs from
154 month 3 (M3) to month 10 (M10).

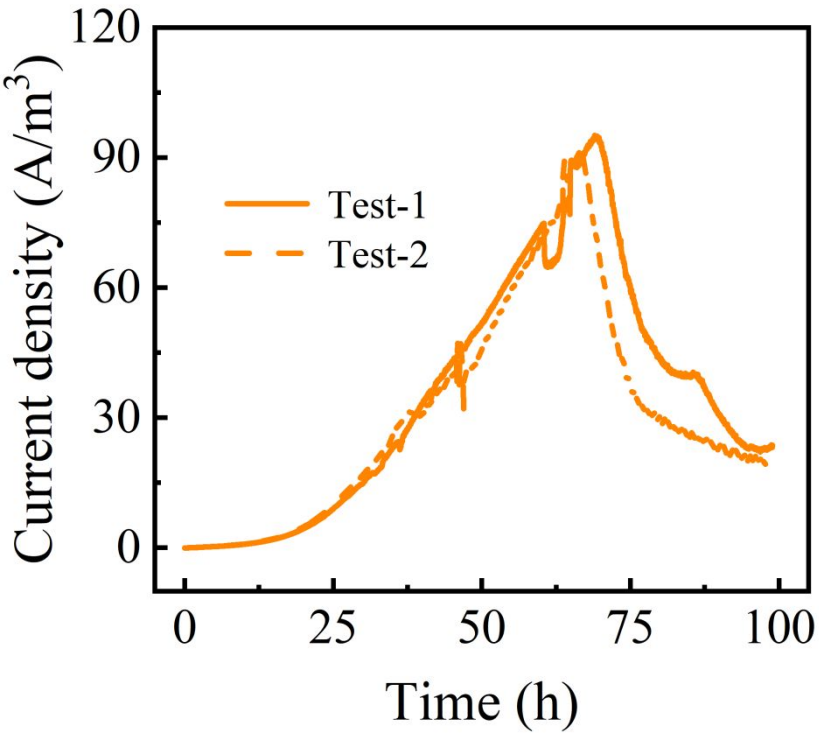

Figure S6 The representative time-current curves of L-BESs inoculated by planktonic cells in MFCs collected after NP treatment (from month 12). Test-1 and Test-2 are two parallel experimental groups.

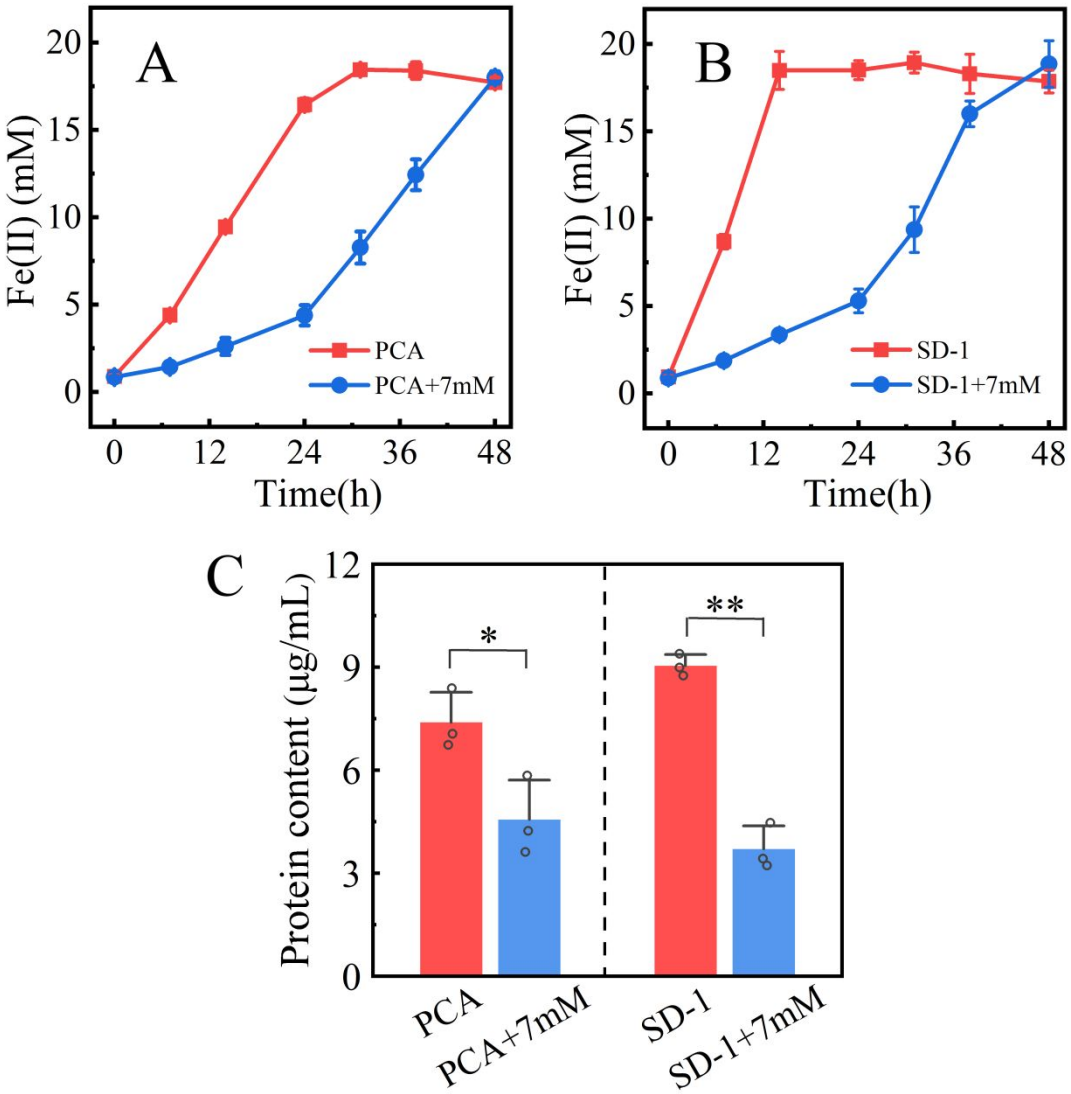

Figure S7 The effect of NP on two *Geobacter* strains. The variation of Fe(II) content of (A) *G. sulfurreducens* PCA and (B) *G. anodireducens* SD-1 in modified FcA media over time. (C) Biomass of these two *Geobacter* strains at 48 h. Group PCA+7mM / SD-1+7mM means that *Geobacter* cells interacted with NP for 12 h before being inoculated into the FcA media. Group PCA / SD-1 was the control.

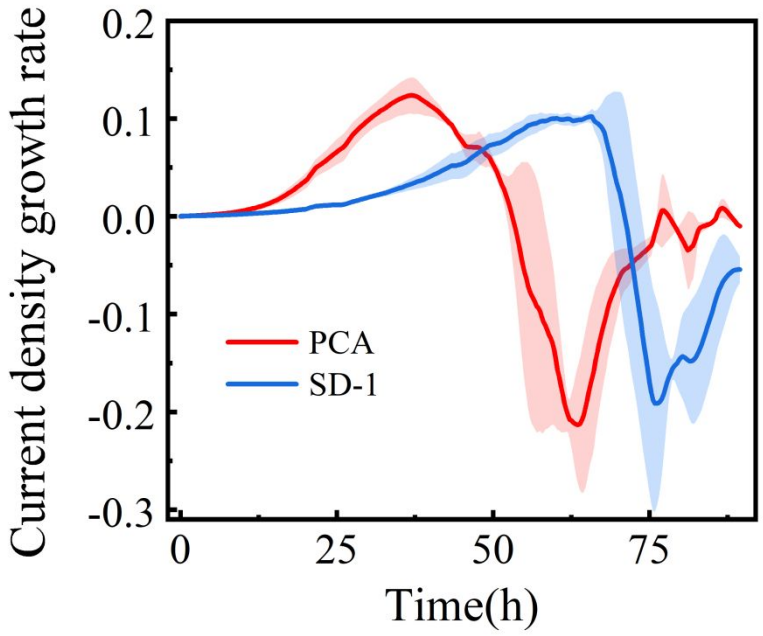

Figure S8 The change of current density growth rate of biofilms formed by *G. sulfurreducens* PCA and *G. anodireducens* SD-1 in pure culture in the first cycle over time.

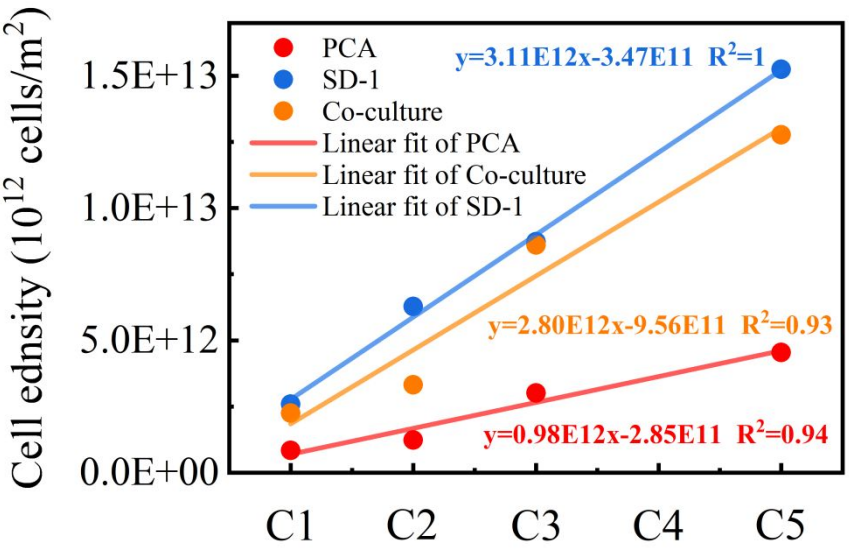

Figure S9 The growth rate of cell density of biofilms formed by *G. sulfurreducens* PCA and *G. anodireducens* SD-1 in pure culture or co-culture in cycle 1-5.

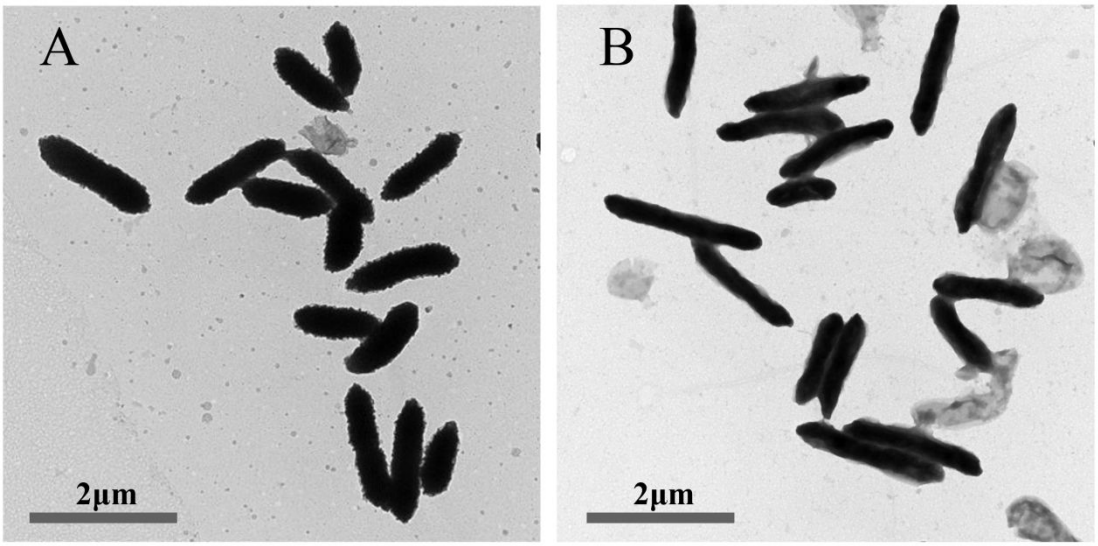

Figure S10 Transmission electron micrograph of (**A**) *G.sulfurreducens* PCA and (**B**) *G.anodireducens* SD-1 grown on electrodes.

References

(1) Li, T.; Zhou, Q.; Zhou, L.; Yan, Y.; Liao, C.; Wan, L.; An, J.; Li, N.; Wang, X., Acetate limitation selects *Geobacter* from mixed inoculum and reduces polysaccharide in electroactive biofilm. *Water Res.* **2020,** *177*, 115776.

(2) Wan, Y.; Huang, Z.; Zhou, L.; Li, T.; Liao, C.; Yan, X.; Li, N.; Wang, X., Bioelectrochemical ammoniation coupled with microbial electrolysis for nitrogen recovery from nitrate in wastewater. *Environ. Sci. Technol.* **2020,** *54*, 3002-3011.

(3) Zhou, L.; Yan, X.; Yan, Y.; Li, T.; An, J.; Liao, C.; Li, N.; Wang, X., Electrode potential regulates phenol degradation pathways in oxygen-diffused microbial electrochemical system. *Chem. Eng. J.* **2020,** *381*.

(4) An, J.; Li, N.; Wan, L.; Zhou, L.; Du, Q.; Li, T.; Wang, X., Electric field induced salt precipitation into activated carbon air-cathode causes power decay in microbial fuel cells. *Water Res.* **2017,** *123*, 369-377.

(5) Liao, C.; Zhao, Q.; Wang, S.; Yan, X.; Li, T.; Zhou, L.; An, J.; Yan, Y.; Li, N.; Wang, X., Excessive extracellular polymeric substances induced by organic shocks accelerate electron transfer of oxygen reducing biocathode. *Sci. Total Environ.* **2021,** *774*, 145767-145767.