Supplementary material

S1 Materials and methods

S1.1 Inoculation, acclimation and operation

The nutrient solution in the anodes of both MFCs and MECs contained (mg·L^{-1}) NH_4HCO_3, 4.9; $KHCO_3$, 1.5; $NaH_2PO_4 \cdot 2H_2O$, 21.2; $Na_2HPO_4 \cdot 12H_2O$, 28.8; $MgSO_4 \cdot 7H_2O$, 0.15; vitamins 12.5 mL·L^{-1} and minerals 12.5 mL·L^{-1} [1].

For the initial 4 batch cycles of MFCs and MECs, the anode chambers of MFCs and MECs were inoculated from the anode chambers of operating MFCs running on acetate that were originally inoculated using primary clarifier effluent from the Lingshui Wastewater Treatment Plant (Dalian, China), and the inoculation solution was equivalently combined with the nutrient solution. The inoculation was then omitted from the anolyte medium in all subsequent experiments. The same nutrient medium was used in the biocathode chambers of MECs except for the addition of sodium acetate (1.0 g·L^{-1}) in the anode chambers of both MFCs and MECs, and Co(II) (40 mg·L^{-1}) in the cathode chambers of MECs, respectively at a same solution conductivity (SC) of 8.3 mS·cm^{-1} (adjusted by 0.1 mol·L^{-1} NaCl). In the cathode chambers of MFCs, however, $CuCl_2$ solution (concentration as desired) was added at a same SC of 8.3 mS·cm^{-1} (adjusted by 0.1 mol·L^{-1} NaCl). The cathode chambers of MECs were in particular inoculated from the biocathode chambers of operating MECs with Co(II) removal [2] and the inoculation solution was similarly and equivalently combined with the nutrient solution during the initial 4 batch cycle acclimation period. This inoculum was then omitted from the catholyte medium in all subsequent tests. During the entire acclimation period, the MFCs were always connected with an external resistance of 1000 Ω due to the spontaneous occurrence of the reactions between the anodes and the cathodes whereas the biocathode MECs were applied at an external voltage of 0.2 V due to a favorable half cell redox potential of Co(II) reduction at –0.23 V (vs. SHE). Both the anolyte and the catholyte were renewed every 2–3 days. After 7 batch cycles and the potentials of anodes and cathodes in both MECs and MFCs were unchangeable, indicating the finish of MFCs and MECs acclimation. The external resistance of 1000 Ω in MFCs and the external voltage of 0.2 V were then removed from MFCs and biocathode MECs, respectively, and connected each other. A small resistor of 10 Ω was always used to collect the circuit electrical data. The so-called MECs with biocathodes and driven by MFCs were used for subsequent experiments. Prior to add the solutions into the anode and the cathode chambers of both MFCs and MECs, the solutions were sparged with N_2 gas for 15 min. The reactors were run at temperature of 22℃ ± 2℃. The initial pHs in the catholyte of MFCs and MECs were always 2.0 and 6.2, respectively [2,3]. All reactors were operated in

fed-batch mode and all tests were conducted in duplicate. All of the inoculation and solution replacements were performed in an anaerobic glove box (YQX-II, Xinmiao, Shanghai, China).

To illustrate the roles of circuit current and/or microorganisms in Cu(II) and Co(II) removals, two controls were operated: one was run in the open circuit conditions (OCC-cathode/biocathode controls) to examine changes in Cu(II)/Co(II) concentration in the absence of circuit current; the other was operated in the closed circuit conditions with no presence of biofilm on the cathodes of MECs (CCC-abiotic controls) to exclude the role of biofilm on Co(II) removal.

S1.2 Measurements and analyses

Cu(II) and Co(II) were measured by an atomic absorption spectrophotometer (AAnalyst 700, PerkinElmer, USA). Hydrogen produced in the headspace of biocathode chambers of MECs was analyzed using a gas chromatograph (GC7900, Tianmei, Shanghai, China) and a molecular sieve column (TDX-01, 60-80, 4 mm × 2 m) with argon as the carrier gas. Chemical oxygen demand (COD) in the anode chambers of MFCs and MECs was measured using standard methods (APHA). Organics in the biocathode chambers of MECs were tentatively defined as the COD value of the catholyte after filtered through 0.22 μm Millipore membrane. Biomass in the biocathode chambers of MECs was defined as COD difference before and after the catholyte was filtered through 0.22 μm Millipore membrane.

Current density in polarization curves was normalized either to the cathode project area ($A \cdot m^{-2}$) or working volume in the cathode chambers of MFCs ($A \cdot m^{-3}$) whereas power density was calculated based on per working volume in the cathode chambers of MFCs ($W \cdot m^{-3}$).

S1.3 Calculations

Anodic CEs in MFCs ($CE_{MFC,an}$, %) and MECs ($CE_{MEC,an}$, %) were calculated as the ratios of the cumulative coulomb number flowing across the MFCs and biocathode MECs, and the equivalent COD consumption in the anodic MFCs and MECs, respectively at an operational period of 6 h (Eqs. (1)–(2)). Biomass in the biocathodic MECs was defined as COD difference before and after the catholyte was filtered through 0.22 mm Millipore membrane whereas organics were calculated as COD difference of the filtered catholytes at an initial and the end of system operation. Yields of copper (Y_{Cu}, $mol \cdot mol^{-1}$ COD), cobalt (Y_{Co}, $mol \cdot mol^{-1}$ COD), hydrogen (Y_{H2}, $mol \cdot mol^{-1}$ COD), organics (Y_{org}, $mol \cdot mol^{-1}$ COD), and biomass (Y_{bio}, $mol \cdot mol^{-1}$ COD) were calculated based on either Cu(II) and Co(II) removal or productions of hydrogen, organics and biomass ($mol \cdot L^{-1}$) divided by the change of CODs in the anolyte of either MFCs or MECs ($mol \cdot L^{-1}$) within a 6 h operational period (Eqs. (3)–(7)). Since Cu(II) and Co(II) were always undetectable in the anolyte in the abiotic cathode controls during an entire operation period of 6 h, diffusion of Cu(II) and Co(II) ions from catholyte to anolyte was negligible. Similarly, no acetate was observed in the

catholyte of the abiotic cathode controls after a fed batch cycle, demonstrating no occurrence of acetate diffusion from anolyte to catholyte. Accordingly, system efficiency (η_{sys}, %) was calculated according to Eq. (8).

$$CE_{\mathrm{MFC,an}} = \frac{\int Idt}{96485 \times \frac{4 \times \Delta COD_{\mathrm{MFC,an}} \times V_{\mathrm{MFC,an}}}{32}} \times 100\%, \quad (1)$$

$$CE_{\mathrm{MEC,an}} = \frac{\int Idt}{96485 \times \frac{4 \times \Delta COD_{\mathrm{MEC,an}} \times V_{\mathrm{MEC,an}}}{32}} \times 100\%, \quad (2)$$

$$Y_{\mathrm{Cu}} = \frac{\frac{Cu_{\mathrm{i}} - Cu_{\mathrm{t}}}{64} \times V_{\mathrm{MFC,ca,l}}}{\frac{\Delta COD_{\mathrm{MFC,an}}}{32} \times V_{\mathrm{MFC,an}}}, \quad (3)$$

$$Y_{\mathrm{Co}} = \frac{\frac{Co_{\mathrm{i}} - Co_{\mathrm{t}}}{59} \times V_{\mathrm{MEC,ca,l}}}{\frac{\Delta COD_{\mathrm{MEC,an}}}{32} \times V_{\mathrm{MEC,an}}}, \quad (4)$$

$$Y_{\mathrm{H_2}} = \frac{n_{\mathrm{H_2,t}} \times V_{\mathrm{MEC,ca,g}}}{\frac{\Delta COD_{\mathrm{MEC,an}}}{32} \times V_{\mathrm{MEC,an}}}, \quad (5)$$

$$Y_{\mathrm{bio}} = \frac{\Delta COD_{\mathrm{MEC,bio}} \times V_{\mathrm{MEC,ca,l}}}{\Delta COD_{\mathrm{MEC,an}} \times V_{\mathrm{MEC,an}}}, \quad (6)$$

$$Y_{\mathrm{org}} = \frac{\Delta COD_{\mathrm{MEC,org}} \times V_{\mathrm{MEC,ca,l}}}{\Delta COD_{\mathrm{MEC,an}} \times V_{\mathrm{MEC,an}}}, \quad (7)$$

$$\eta_{\mathrm{sys}} = \frac{\frac{Cu_{\mathrm{i}} - Cu_{\mathrm{t}}}{64} \times V_{\mathrm{MFC,ca,l}} \times \mathrm{a}_1 + \frac{Co_{\mathrm{i}} - Co_{\mathrm{t}}}{59} \times V_{\mathrm{MEC,ca,l}} \times \mathrm{a}_2 + \frac{4}{32} \times (\Delta COD_{\mathrm{MEC,bio}} + \Delta COD_{\mathrm{MEC,org}}) \times V_{\mathrm{MEC,ca,l}} + n_{\mathrm{H_2,t}} \times V_{\mathrm{MEC,ca,g}} \times \mathrm{a}_3}{(V_{\mathrm{MFC,an}} \Delta COD_{\mathrm{MFC,an}} + V_{\mathrm{MEC,an}} \Delta COD_{\mathrm{MEC,an}}) \times \frac{4}{32}} \times 100\%, \quad (8)$$

$Cu_{\mathrm{i}}$and $Co_{\mathrm{i}}$are the initial Cu(II) and Co(II) concentrations in the catholytes whereas $Cu_{\mathrm{t}}$and $Co_{\mathrm{t}}$are the Cu(II) and Co(II) concentrations at an operation time of 6 h ($g \cdot L^{-1}$); $n_{\mathrm{H2,t}}$ is hydrogen concentration in the headspace of the biocathode chambers of MECs at 6 h ($mol \cdot L^{-1}$); $V_{\mathrm{MFC,an}}$ and $V_{\mathrm{MEC,an}}$ are anolyte volumes in MFCs and MECs, respectively (L); $V_{\mathrm{MFC,ca,l}}$ and $V_{\mathrm{MEC,ca,l}}$ are catholyte volumes in MFCs and MECs, respectively (L); $V_{\mathrm{MEC,ca,g}}$ is volume of headspace in the biocathode chambers of MECs (L); a_1 ($2\ mol \cdot mol^{-1}$), a_2 ($2\ mol \cdot mol^{-1}$) and a_3 ($2\ mol \cdot mol^{-1}$) are the number of electrons required for Cu(II) and Co(II) reduction as well as hydrogen evolution, respectively. $\Delta COD_{\mathrm{MFC,an}}$ and $\Delta COD_{\mathrm{MEC,an}}$ are the cumulative *COD* consumptions over a set period of 6 h in the anode chambers of MFCs and MECs, respectively ($g \cdot L^{-1}$); $\Delta COD_{\mathrm{MEC,biomass}}$and

$\Delta COD_{MEC,organics}$ are the changes of biomass and organics in the catholyte of MECs at a set period of 6 h ($g \cdot L^{-1}$). I is circuit current (A); 64, 59, and 32 are the atom/molecule weights of Cu, Co and O_2, respectively ($g \cdot mol^{-1}$); 4 is the molar number of electrons required for oxygen reduction ($mol \cdot mol^{-1}$); 96485 is Faraday constant ($C \cdot mol^{-1}$ e^-).

S2 Results and discussion

S2.1 Development of individual MFCs and MECs with microbial cathodes

Following inoculation and at an external resistance of 1000 Ω, the difference in anode and cathode potentials of MFCs became larger with acclimation time, in addition to the gradually more negative anode potentials in MFCs (Fig. S1(a)), implying the development of exoelectrogens on the anodes of MFCs [2–4]. After 7 batch cycles and around 15 days acclimation, the anode and cathode potentials in MFCs stabilized at –0.098 V and –0.25 V. Similarly, at an applied voltage of 0.2 V, both anode and biocathode potentials in MECs experienced a gradual evolution, achieving repeatable and stable values of –0.197 V and –0.367 V, respectively at 7th batch cycle acclimation (Fig. S1(b)). These results imply the finishing of individual MFCs and biocathode MECs acclimation. LSV analysis indicates both open circuit potential (OCP) and maximal power density in MFCs experienced gradual increase with acclimation time, achieving the similar 0.64 V and 4.2–4.6 $W \cdot m^{-3}$ at 9 and 12 d (Fig. S1(c)), also reflecting the end of MFCs acclimation.

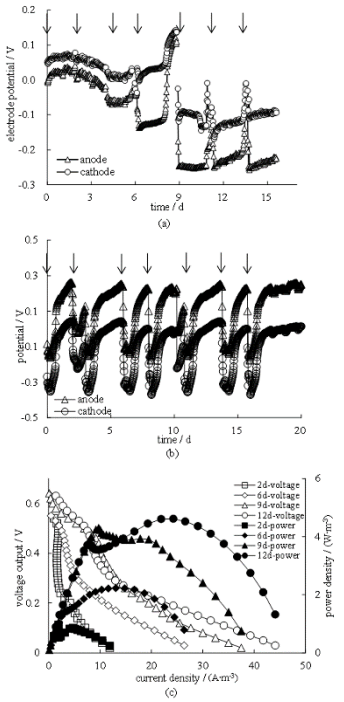

Fig. S1 Acclimation of individual (a) MFCs and (b) biocathode MECs, and (c) polarization curve of MFCs periodically (a resistance of 1000 Ω was used in the MFCs; an external voltage of 0.2 V was applied in the biocathode MECs; arrows indicate when anode and cathode chambers were fed with fresh medium)

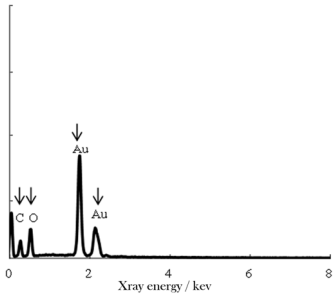

Fig. S2 EDS analysis on the surface of biocathodes with no bacteria covered

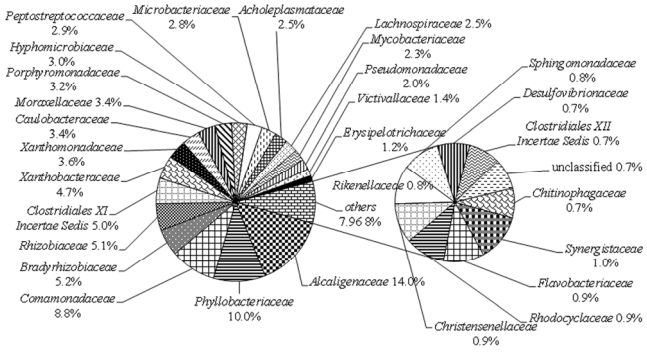

Fig. S3 Relative abundance of bacterial reads retrieved from the biocathodes of MECs classified at the family level. Families that represented less than 0.5% of the total bacterial community composition were classified as "*others*"

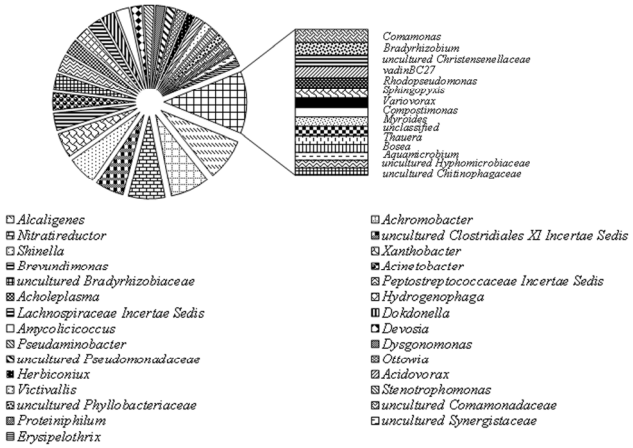

Fig. S4 Relative abundance of bacterial reads retrieved from the biocathodes of MECs classified at the genus level. Genera that represented less than 0.5% of the total bacterial community composition were classified as "*others*"

References

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