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Supplementary Materials for

Silver nanoparticles boost charge-extraction efficiency in *Shewanella* microbial fuel cells

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Materials and Methods

Synthesis of reduced graphene oxide/Ag nanoparticles (rGO/Ag) and rGO composites

Typically, 2 ml of graphene oxide (3 mg/ml) solution is mixed with 2 ml of silver nitrate ($AgNO_3$ 0.1 M) aqueous solution and 6 ml of DI water. 20 mg of sodium borohydride ($NaBH_4$) is dissolved in 10 ml DI water. Under vigorous stirring, the 10 ml $NaBH_4$ solution is added dropwise, slowly. After 10 min, the entire solution becomes dark black. Stirring is continued overnight at room temperature. The rGO/Ag composite is washed three times with the centrifuge and sonication with the DI water. Finally, the composite is dissolved in 10 ml ethanol for further use. For rGO, the entire process is the same except no $AgNO_3$ is added.

Fabrication of carbon paper/rGO/Ag nanoparticles and carbon paper/rGO anode

The carbon paper (CP) is cut into small pieces, 2 cm^2 (1 cm × 2 cm). 1 ml of the rGO/Ag solution is dropped on carbon paper to form a uniform layer. After that, the sample is dried in air. For carbon paper/rGO anode, the fabrication process and drying are the same.

Bacteria culture

Shewanella oneidensis MR-1 is first inoculated with 20 ml of LB solution. The entire flask together with the LB solution is put in a 30 ℃ shaker for at least 15 h (usually overnight). 200 μL of bacteria colonies are taken out and put in another flask containing 20 ml of fresh LB solution. Before any test, 5 ml of bacteria colonies are taken out, centrifuged and washed 3 times with *Shewanella* medium. The final bacteria solution's OD_{600} is about 1.0.

Shewanella medium ingredient

The *Shewanella* medium contains (per liter of deionized water): 9.07 g PIPES buffer ($C_8H_{18}N_2O_6S_2$), 3.4 g sodium hydroxide (NaOH), 1.5 g ammonium chloride (NH_4Cl), 0.1 g potassium chloride (KCl), 0.6 g sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), 18 mM 60% (v/v) sodium DL-lactate ($C_3H_6O_3$) solution as the electron donor, 10 mL of 100× amino acids stock solution, and 10 mL 100× minerals stock solution. This base medium is adjusted to an initial pH of 7.2 using HCl and NaOH and then sterile filtered using 0.22 μm vacuum driven disposable bottle top filters (Millipore). Finally, before the medium is used, 0.5 mL 100 mM ferric NTA ($C_6H_6FeNO_6$) stock solution is added per liter of medium. The 100× amino acids stock solution contains (per liter of deionized water) 2 g L-glutamic acid ($C_5H_9NO_4$), 2 g L-arginine ($C_6H_{14}N_4O_2$), and 2 g DL-serine ($C_3H_7NO_3$). The 100× minerals stock solution contains (per liter of deionized water): 1.5 g nitrilotriacetic acid ($C_6H_9NO_6$), 3 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.5 g manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$), 1 g sodium chloride (NaCl), 0.1 g ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), 0.1 g calcium chloride dehydrate ($CaCl_2 \cdot 2H_2O$), 0.1 g cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), 0.13 g zinc chloride ($ZnCl_2$), 10 mg cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), 10 mg aluminum potassium disulfate dodecahydrate ($KAl(SO_4)_2 \cdot 12H_2O$), 10 mg boric acid (H_3BO_3), 25 mg sodium molybdate dehydrate (Na_2MoO_4), 24 mg nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), and 25 mg sodium tungstate (Na_2WO_4). All stock solutions are sterile filtered prior to use.

Half-cell microbial fuel cell experiment

The three electrodes are placed in a three-neck flask. The working electrode is carbon paper or carbon paper/rGO or carbon paper/rGO/Ag. The reference electrode is 1 M KCl Ag/AgCl

electrode, and the counter electrode is platinum wire. After adding all the solutions, the entire system is purged with ultra-pure N_2 gas for 30 min to remove dissolved oxygen. In a typical current-time (*I-t*) measurement, a positive potential (200 mV vs. reference electrode) is added on the working electrode and the corresponding electrochemical current is recorded.

Full cell microbial fuel cell experiment

The H-shaped two-chamber MFC is constructed by connecting two 120 ml chambers with a proton exchange membrane (PEM) Nafion 211 separator. The diameter of the chamber channel is 5.5 cm. The cathode is carbon cloth (2 cm × 4 cm) coated with 40% Pt/C with oxygen purging. Before the tests, two sterilization steps are conducted to ensure the device is fully sterilized. Before constructing the devices, all the plastic chambers are soaked in aqueous detergent solution, and sufficiently rinsed with deionized water. After the devices and membranes are fully constructed, the entire device is immersed into boiling water for 15 min for further sterilization. Before the test, the anodic solution is purged with pure nitrogen gas for 30 min to remove the dissolved oxygen. The anodic chamber is tightly sealed to maintain the anaerobic condition during MFC operation. All MFC experiments are operated at ~30 °C. At MFC steady state, the polarization curves are obtained by varying the external resistor. The output voltage is recorded with a multi-meter. The output current is calculated from Ohm's Law: $I=V/R$ (R is the value of external resistor). The output power is calculated by $P=IV=V^2/R$. For long-standing tests and the EIS tests, the cathode solution is changed to potassium ferricyanide ($K_3[Fe(CN)_6]$, 50 mM) and potassium chloride (KCl, 50 mM).

Bacteria number determination

The bacteria numbers on different electrodes are determined with the combination of hemocytometer counting method and total nitrogen analysis method. In a typical hemocytometer counting process, bacterial solutions after calibrated dilution are dripped on the hemocytometer counting chamber with the cover glass. The volume of a 1 mm grid is 1×10^{-4} ml. Total nitrogen analysis is conducted through HACH total nitrogen reagent set (high range, persulfate digestion method). The electrode after an MFC cycle is sonicated to break the biofilm from the electrode for nitrogen analysis.

Scanning electron microscope (SEM) measurement

After MFC testing, the entire anode with the biofilm is fixed by 2.5% glutaraldehyde ($C_5H_8O_2$) solution overnight at 4 °C and chemically dehydrated using gradient concentration ethanol aqueous solutions (50, 70, 80, 90, and 95% each one time, then 100% twice). The sample was dried in the vacuum chamber and finally sputter-coated gold for further SEM imaging.

Transmission electron microscope (TEM) measurements

After MFC testing, the bacterial solution is taken out, centrifuged and fixed by 2.5% glutaraldehyde ($C_5H_8O_2$) solution overnight at 4 °C and 2% osmium tetroxide (OsO_4) aqueous solution at 4 °C for 4 h. After fixation, the bacteria pellet is chemically dehydrated using gradient concentration of ethanol (50, 70, 80, 90, and 95% once each, then 100% twice) and 100% acetone. The bacteria pellet is finally embedded in the resin (Araldite 502) and polymerized in the oven at

60 ℃ for at least 36 h. Ultrathin sections of 70 nm are cut by an ultratome and deposited on carbon coated copper grids for TEM imaging.

Electrochemical surface area (ECSA) measurement

The electrochemical surface area (ECSA) of our samples is determined by testing the double layer capacitance (C_{dl}), in which C_{dl} is linearly proportional to the active surface area in the electrochemical capacitor research. In this process, cyclic voltammetry (CV) measurements were conducted in the medium buffer solution without bacteria within the non-faradaic potential range (0.1-0.2 V vs. Ag/AgCl), which is dominated by surface adsorption/desorption. Then C_{dl} can be calculated by plotting the current difference between anodic and cathodic sweep ($\Delta J = J_a - J_c$) at 0.15 V against the different scan rate, where the slope is twice that of C_{dl}. In order to determine the exact value of the ECSA, we also determined the C_{dl} of a flat surface (e.g., glassy carbon electrode) with known geometry and surface area (0.196 cm^2).

Electrochemical impedance spectroscopy (EIS) measurement

For the R_{ct} of different electrodes without the bacteria, the measurement is conducted in a three-neck flask with bacteria buffer medium. The three electrodes carbon paper, rGO, and rGO/Ag are set as working electrodes. The Pt wire and Ag/AgCl are set as the counter electrode and the reference electrode, respectively. EIS is measured within the frequency range of 10^5 to 0.5 Hz at the voltage of 0.2 V vs RHE. For the full MFC, the EIS test is conducted after 2 days of MFC setup. The cathode is the potassium ferricyanide ($K_3[Fe(CN)_6]$, 50 mM) and potassium chloride (KCl, 50 mM). The anode is set as the working electrode and the cathode is connected with both the counter and reference electrode.

Confocal laser scanning microscopy (CLSM) measurement

The L7007 live/dead bacterial viability kit is used to evaluate the bacteria viability on the anode. The anode with biofilm after MFC test is rinsed with PBS and stained with SYTO 9 dye and propidium iodide (PI) mix solution. The stained cells are examined using a Leica TCS SP8 II confocal/multiphoton laser scanning microscope (Germany). The alive-dead assay solution is prepared by diluting SYTO 9 dye solution (1.67 mM solution in dimethyl sulfoxide) and propidium iodide (1.67 mM solution in dimethyl sulfoxide) stock solutions in PBS at final concentrations of 30 μM and 30 μM. In living bacteria, the SYTO 9 dye will emit strong green fluorescence. In dead bacteria, the PI will bound to DNA and emit red fluorescence.

Coulombic efficiency (QE) and turn-over frequency (TOFs) calculations

The Coulombic efficiency is evaluated by the following equations S1-S3(*14*). In equation S1, the η is the Coulombic efficiency, C_{output} is the amount of experimental electric quantity in the microbial fuel cell, in Coulombs. In equation S2, t is the total time in seconds. In equation S3, C_{total} is the total theoretical amount of charge (Q, in Coulombs) transferred in the lactate oxidation process. $C_{lactate}$ is the lactate concentration of the bacteria buffer medium. $V_{solution}$ is the total volume of the medium. n: The number of electrons transferred during the oxidation of one molecule of lactate. F is Faraday's constant (96,500 C/mol).

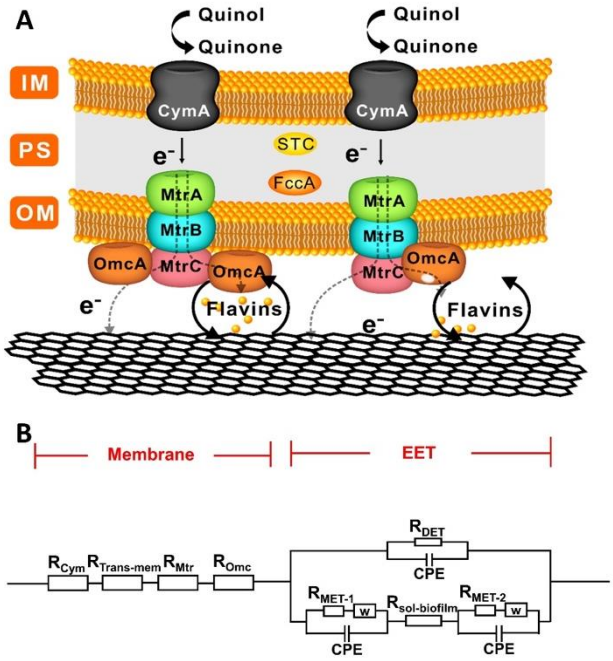
$$\eta = C_{output}/C_{total} \quad (S1)$$

$$C_{output} = \int (I \cdot t) \quad (S2)$$

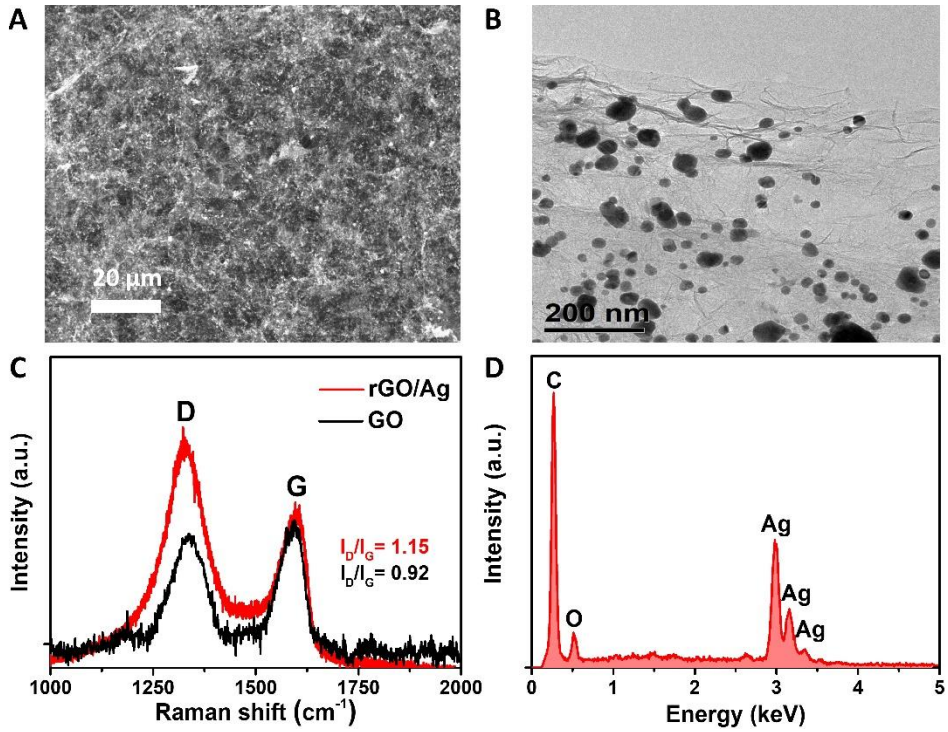
$$C_{total} = C_{lactate} \cdot V_{solution} \cdot n \cdot F \quad (S3)$$

The turnover frequency of single bacteria is evaluated using equation S4. Here, $n_{substrate}$ is the total number of lactate molecules that have been oxidized, and t is the total time in seconds. $n_{catalyst}$ is the number of catalysts (and also the bacteria number).

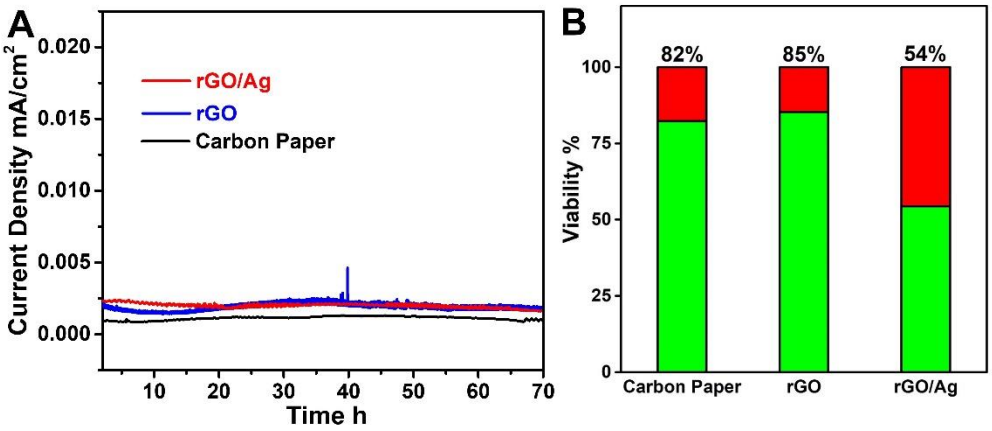
$$\text{Turn-over frequency} = n_{substrate} / t \cdot n_{catalyst} \quad (S4)$$



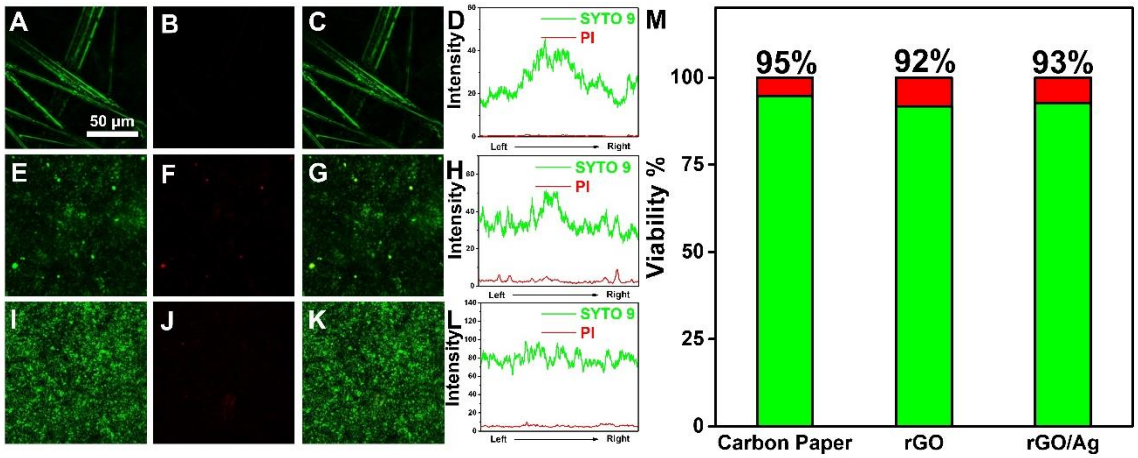
Supplementary Fig. 1. Scheme of extracellular electron transfer. The metabolic electrons in the cytoplasm are first transferred through the CymA on the inner membrane from which they cross the periplasm by hopping from the CymA cytochromes redox centers to the periplasmic c-Cyts FccA and small tetrahaem cytochrome (STC), as represented in R_{Cym} and $R_{\text{Trans-mem}}$ in the equivalent circuits. On the outer membrane, the electrons are transferred via membrane proteins MtrA, MtrB, MtrC, and OmcA (R_{Mtr} and R_{Omc}) to the electrode surface through extracellular electron transfer (EET) process that includes two parallel paths: across proteins MtrC and OmcA to the electrode via direct electrical contact as represented by direct electron transfer resistance (R_{DET}), and indirect redox shuttling process mediated by the bacteria secreted flavins as represented by mediated electron transfer resistance (R_{MET})



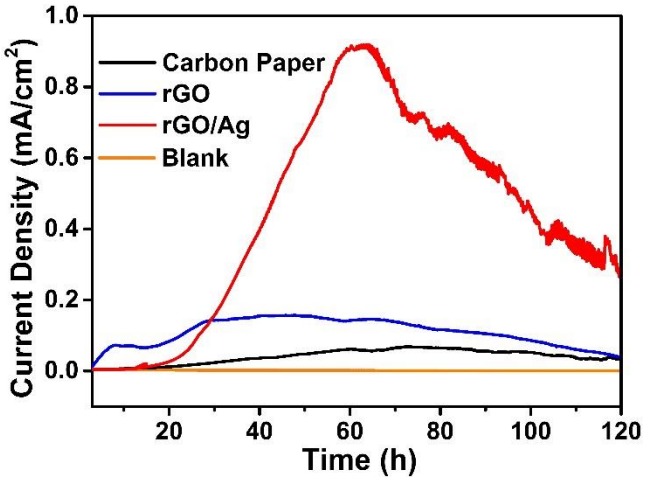
Supplementary Fig. 2. Physical characterization of rGO/Ag. (**A**) Scanning electron microscope image of rGO/Ag. (**B**) Transmission electron microscope image of rGO/Ag, which shows that the rGO is covered with Ag nanoparticles. The Ag nanoparticles are all attached on the rGO sheets rather than being free-standing. (**C**) Raman spectrum of GO and rGO/Ag, which reveals that the graphene oxide (GO) is reduced with higher I_D/I_G ratio. (**D**) EDS analysis of rGO/Ag (a.u., arbitrary units).



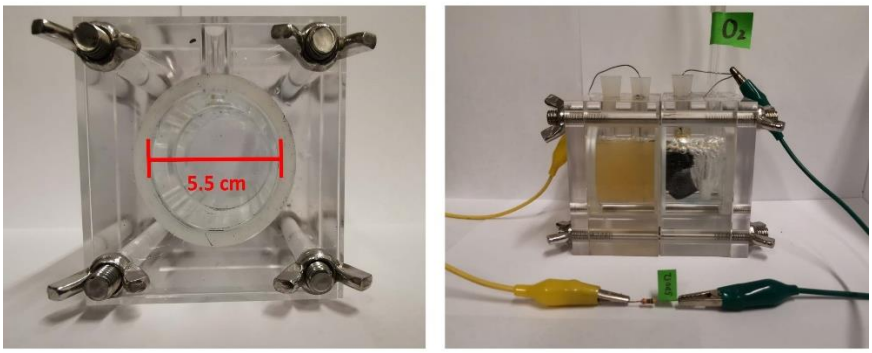
Supplementary Fig. 3. Microbial fuel cell with *E. coli* and viability tests. (**A**) I-t curves of different electrodes with *E. coli*; (**B**) Viability tests of different electrodes after the microbial fuel cell tests in *E. coli*. With the introduction of Ag and antibacterial features, the *E. coli* does not have the ability to reduce the Ag^+ and the viability is undermined. We found that the viability of *E. coli* decreases from ~80% to ~50% with the presence Ag loading on the electrode.



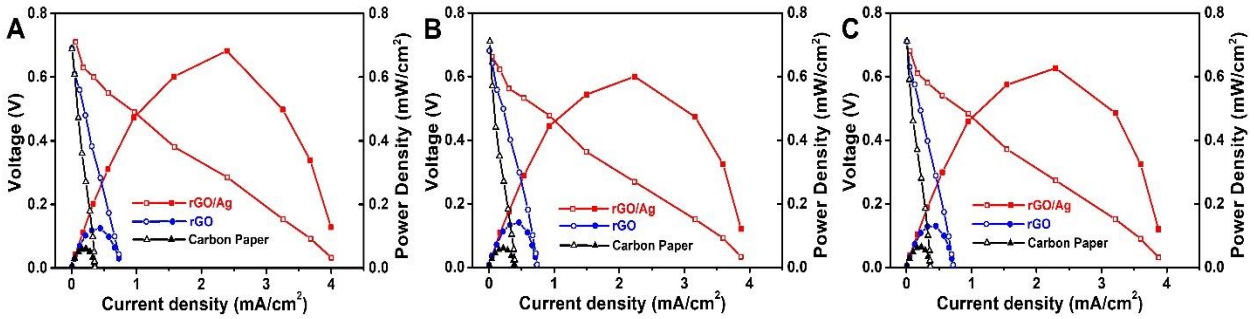
Supplementary Fig. 4. *Shewanella* Viability tests based on live/dead kit and confocal laser scanning microscopy (CLSM). The combination of fluorescence dye SYTO 9/propidium iodide (PI) can differentiate live/dead cell under CLSM. Under CLSM images, we observe that on all three electrodes, the green fluorescence intensity is much stronger than the red fluorescence. (**A**-**C**) Carbon paper biofilm fluorescence of SYTO 9 (green), PI (red) and their overlap; (**D**) Green/red fluorescence intensity comparison; (**E-H**) rGO biofilm green/red fluorescence and intensity; (**I-L**) rGO/Ag biofilm green/red fluorescence and intensity; (**M**) Bacterial viability evaluation determined from the CLSM fluorescence results. These results show that the existence of Ag does not undermine the bacterial viability (rGO/Ag: 93%) compared with the other two electrodes (carbon paper: 95%; rGO: 92%).



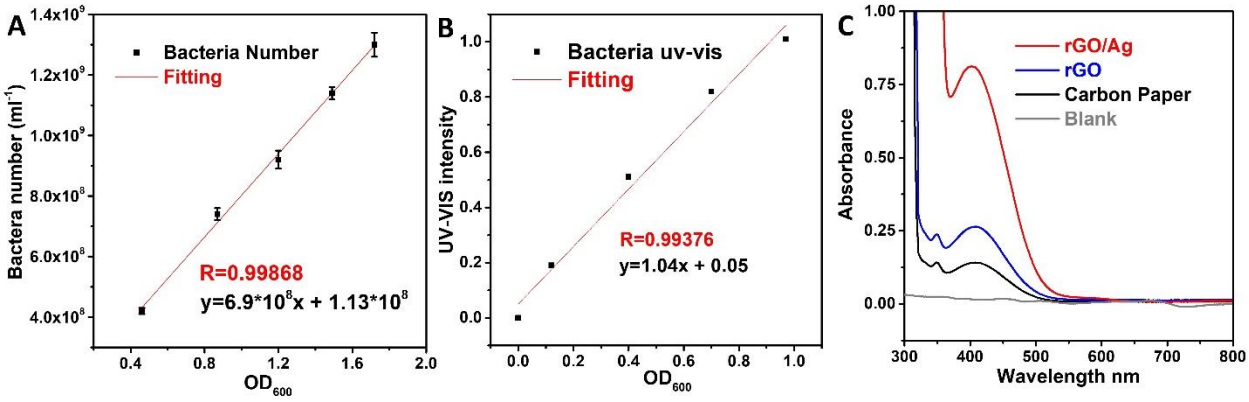
Supplementary Fig. 5. The half-cell MFC tests of different anode electrode as the current vs. time (*I*-*t*) curves. A positive potential (+0.2 V vs. Ag/AgCl in 1 M KCl solution) is applied on the working electrode. The output current density (*I*) vs. time (*t*) is recorded as *I*-*t* curves. The current output of the blank test (with no bacteria added) shows negligible current value throughout the testing period, while the anodic current density of different anodic materials with bacteria shows increasing current with time due to the gradual bacteria attachment and biofilm formation.



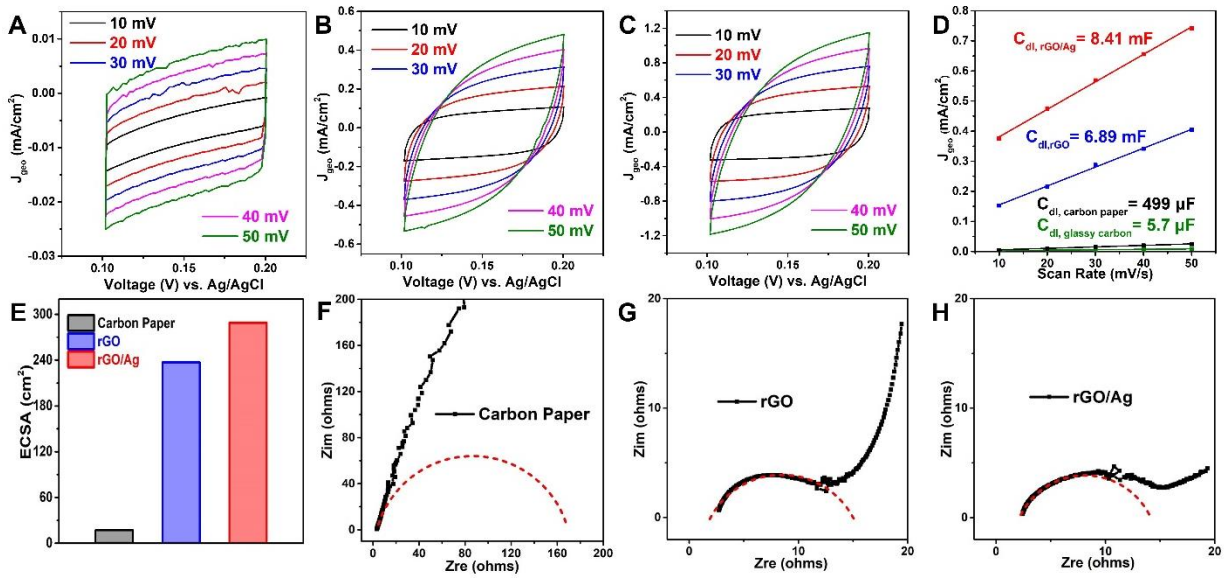
Supplementary Fig. 6. The setup of the double chamber microbial fuel cell (MFC). The H-shaped two-chamber MFC is constructed by connecting two 120 ml chambers with 5.5 cm diameter channels. The carbon paper, rGO and rGO/Ag anodes are positioned in the anodic chamber fed with buffer medium contains 18 mM sodium lactate. The cathode is carbon cloth (2 cm × 4 cm) coated with 40% Pt/C with oxygen purging. The anodic solution is purged with ultra-pure nitrogen gas for at least 30 min to remove the dissolved oxygen. The anodic chamber is tightly sealed to maintain anaerobic conditions during MFC operation. The anodic and cathodic chambers are separated with the proton exchange membrane (PEM) Nafion 211. The anode and cathode are connected to a 500 Ω resistor in parallel with a multi-meter to record the output voltage. All MFC experiments are operated in the static incubator at a temperature of 30 °C.



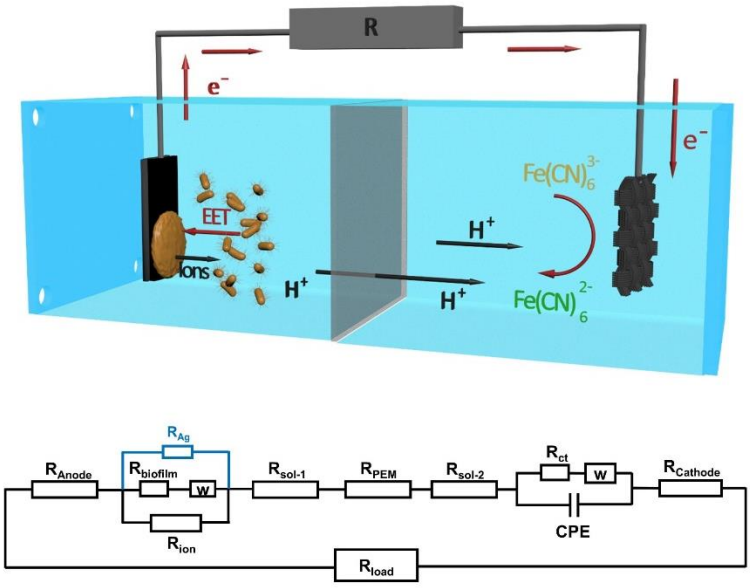
Supplementary Fig. 7. Multi-test of microbial fuel cells shows power output ranges from 0.60 mW/cm^2 to 0.68 mW/cm^2.



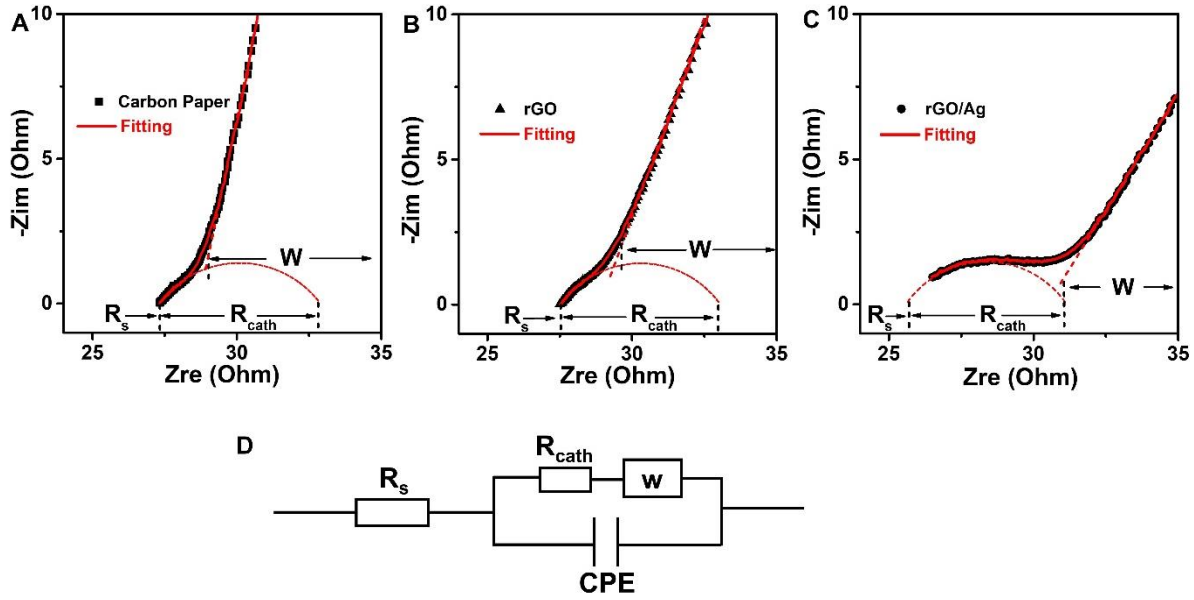
Supplementary Fig. 8. The characterization of bacteria numbers on different electrodes. (**A**) The OD_{600}-Bacteria number standard deviation line based on hemocytometer counting method. (**B**) The OD_{600}-Absorbance standard deviation line based on total nitrogen analysis. (**C**) The absorbance of different number of bacteria on different electrode in the total nitrogen analysis. The testing solution is diluted 3.3 times from the original electrode bacteria solution.



Supplementary Fig. 9. C_{dl} and corresponding ECSAs of the three different electrodes. (**A-C**) CV curves of the carbon paper, rGO and rGO/Ag electrode in the non-faradic range in the medium buffer solution; (**D**) C_{dl} results of the various electrodes obtained from CV; (**E**) ECSA values of the various electrodes: carbon paper: 17 cm^2; rGO: 235 cm^2; rGO/Ag: 284 cm^2. (**F-H**) EIS tests of different electrodes in the medium buffer solution. the difference in ECSA for three different electrodes matches well with (and accounts for) different ion absorption R_{ct} values determined for carbon paper (~166 Ω), rGO (14 Ω), and rGO/Ag (12 Ω) in the medium buffer solution. The rather similar R_{ct} values for rGO and rGO/Ag electrodes suggest that the Ag nanoparticles in rGO electrode architecture is not playing a notable role in the charge transfer process and cannot explain the much higher TOFs observed in the biofilms on rGO/Ag electrode.



Supplementary Fig. 10. The equivalent circuit of the double chamber microbial fuel cell (MFC). The R_{anode} and $R_{cathode}$ represent the Ohmic resistance of anode and cathode. R_{sol} and R_{PEM} are the solution resistance and proton exchange membrane resistance, respectively. $R_{biofilm}$ corresponds to the *Shewanella* biofilm extracellular electron transfer (EET). R_{ion} is related to the ion transfer across the *Shewanella* biofilm. R_{ct} is the charge transfer resistance of the cathode reaction.



Supplementary Fig. 11. The EIS tests of MFC blank tests. In the blank control tests (**A**-**C**), the setup is the same as for the MFC tests except no bacteria are added in the anodic chamber. The system is dictated by the cathode reaction as illustrated in the equivalent circuit diagram (**D**). The red solid lines are the best fitting line for each EIS data. The R_s is the sum of the MFC Ohmic resistance. The R_{cath} represents the value of cathode ferricyanide reduction reaction. The values of R_s and R_{cath} are listed in Table S4.

Supplementary Table 1. The comparison of the current density of different half-cell MFCs.

Anode (*Shewanella* MR-1)	Current Density mA/cm^2
Graphene/PANI*(17)*	0.58
CNT Hydrogel*(19)*	0.5
Porous PANI gel*(33)*	0.396
rGO/Ag (This work)	**0.92**

Supplementary Table 2. The comparison of the current and power density of double chamber MFCs with different anodic materials and bacteria species.

Anode	Bacteria	Current Density mA/cm^2	Power Density mW/cm^2
Graphite fiber brush*(11)*	Mix[#]	0.8	0.143
Carbon paper*(11)*	Mix[#]	0.28	0.05
VA-CNT*(18)*	*Geobacter*	0.26	0.083
CNT textile fiber*(16)*	Mix[#]	0.5	0.11
rGO/Pt*(14)*	*Shewanella* MR-1	0.69	0.148
3D chitosan hydrogel*(15)*	*P. aeruginosa*	0.55	0.153
Graphite felt(*43*)	*Shewanella* DSP10	1.1	0.4
rGO/Ag (This work)	***Shewanella* MR-1**	**3.85**	**0.66**

[#]The "Mix" means more than two kinds of unspecified bacteria in the system.

Supplementary Table 3. The comparison of the Coulombic efficiency (QE) of MFCs with different anodic materials and bacteria species.

Anode	Bacteria	QE
Steel/CNT[#] *(20)*	*Geobacter*	16%
CNT Hydrogel *(19)*	Mix	32%
VA-CNT* *(18)*	*Geobacter*	61%
rGO/Pt *(14)*	*Shewanella* MR-1	69%
rGO/Ag (This work)	***Shewanella* MR-1**	**81%**

[#]"CNT" means carbon nanotube. *"VA" means vertically aligned.

Supplementary Table 4. The resistance values determined from the blank and MFC EIS tests. The anodic electrodes are carbon paper, rGO and rGO/Ag.

Blank	carbon paper	rGO	rGO/Ag
R_s (Ω)	27.2	27.6	26.3
R_{cath} (Ω)	6.2	5.8	5.9

MFC	carbon paper	rGO	rGO/Ag
R_s (Ω)	36.5	27.6	27.0
R_{cath} (Ω)	6.8	6.9	6.8
$R_{biofilm}$ (Ω)	482	102	16