Electronic Supplementary Information (ESI)†

Conductive Artificial Biofilm Dramatically Enhanced Bioelectricity Production in *Shewanella*-Inoculated Microbial Fuel Cells

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1. Detailed experimental procedures

1.1 Materials and instruments

Hydrochloric acid (HCl), potassium ferricyanide ($K_3Fe(CN)_6$), potassium chloride (KCl), sodium polystyrene sulfonate (PSS, Sigma) and pyrrole were purchased from Sigma. Graphite was treated with acetone and 1 M HCl separately for 6 h, and pyrrole was distilled before usage. All the other chemicals were research graded and used as received. All the polymerization and electrochemical measurements were performed on a CHI660D workstation (CH Instruments, USA).

1.2 Bacteria culture and anode preparation

Shewanella Oneidensis MR-1 (*S. Oneidensis*, MR-1, obtained from Max-Planck-InstitutfürTerrestrischeMikrobiologie, Germany) was used as the anode respiration bacteria. After incubated in LB broth for 12 hours at 37 °C, the bacteria was inoculated in 120 ml LB broth containing 1.2 g graphite at 37 °C, 210 rpm for 24 h (OD_{600}=2 when no graphite incubated together). They were then kept at rest (without shaking) at 37 °C for 6 h, allowing further attachment of bacteria to the graphite powder. Finally, they were harvested by centrifuge at 9000 rpm for 5 min.

Carbon cloth (Gas Hub, Singapore) was used as the anode substrate. Before used, the carbon cloth was treated with acetone and HCl to make it more hydrophilic to facilitate the bacteria adhesion. It was then cut into 1 x 1.5 cm and one side of the cloth was sealed with silica rubber (Dow Corning).

2.3 Fabrication of the conductive artificial biofilm (CAB)

To obtain the CAB with different thickness, a layer-by-layer fabrication of graphite/bacteria composite and polypyrrol (PPy) on the carbon cloth (1.5 cm^2) was used[1]. Briefly, after centrifuge, the precipitate was redispersed in 3 ml of medium to obtain a mixture of graphite and bacteria. To prepare the CAB, 100 μL of the composite was spread onto the unsealed side of the carbon cloth and medially dried under room temperature. PPy was then electrochemically polymerized onto the composite of bacteria/graphite from a solution containing 0.2 M pyrrole and 0.1 M PSS under a constant potential of 0.8 V (*vs*. SCE) and 0.5 C of charge except that for the outmost layer where 2 C was used. These processes were repeated to fabricate CAB with different thickness (CAB-1 to -3 was fabricated with 1, 3, and 5 repeat of graphite/bacteria composite and PPy coating, respectively). The PPy monomer solution was

purge with pure nitrogen for 15 min and filtered with 0.2 μm membrane before used. After fabrication, CABs was characterized with FESEM.

1.4 MFCs operation

Two-chamber MFCs (5.5 x 5.5 x 6 cm for each chamber) with proton exchange membrane (PEM, GasHub, Singapore) was used in our study. The anolyte was a mixture of LB broth and M9 with 18 mM sodium lactate (1:19); and the catholyte consisted of 50 m$MK_3Fe(CN)_6$ and 50 mMKCl. Both anode and cathode contained 140 ml of electrolyte.

The MFC was subjected to a continuous discharging period of ~630 h with an external load of 2000 Ω. The polarization curve was conducted by varying external loads. The output voltage was measured using a digital multimeter (ESCORT3146A).

1.5 The CAB morphology characterized by FESEM and confocal microscopy

1.5.1 FESEM image of the CAB

Clean and treated carbon cloth, graphite, graphite/bacteria composite, CAB were observed with a JEOL field emission electron microscope (FESEM, JSM-6700F-FE-SEM, Japan) before and after the MFC operation.

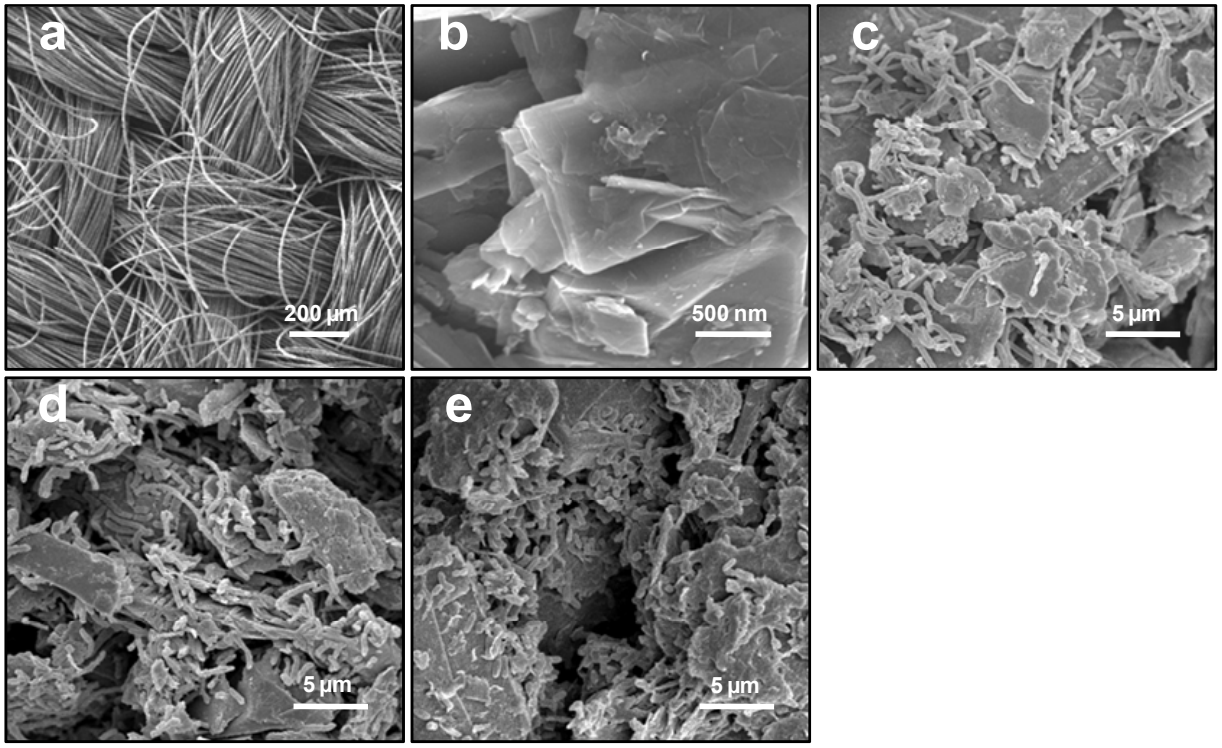

Fig. S1 (a) FESEM image of carbon cloth, **(b)** graphite, **(c)** graphite and bacteria composite, and the cross section view of the CAB-2 before **(d)** and after **(e)** the MFC operation.

1.5.2 Characterization of CAB by fluorescence confocal scanning microscopy (FCSM)

To investigate the viability of bacteria in the CAB after the MFC operation, the surface and interior of the CAB-2 was characterized with FCSM. For the interior of the CAB, a certain thickness of the coating was carefully removed from the surface to expose the inner layer of CAB. Samples were then dyed with the LIVE/DEAD BacLight Bacterial Viability Kit (L7012,

Invitrogen) which labels live cell with green color and dead cell with red color and proceeded to confocal microscopy observation with Zeiss LSM 510 Meta confocal microscope (Fig. S2).

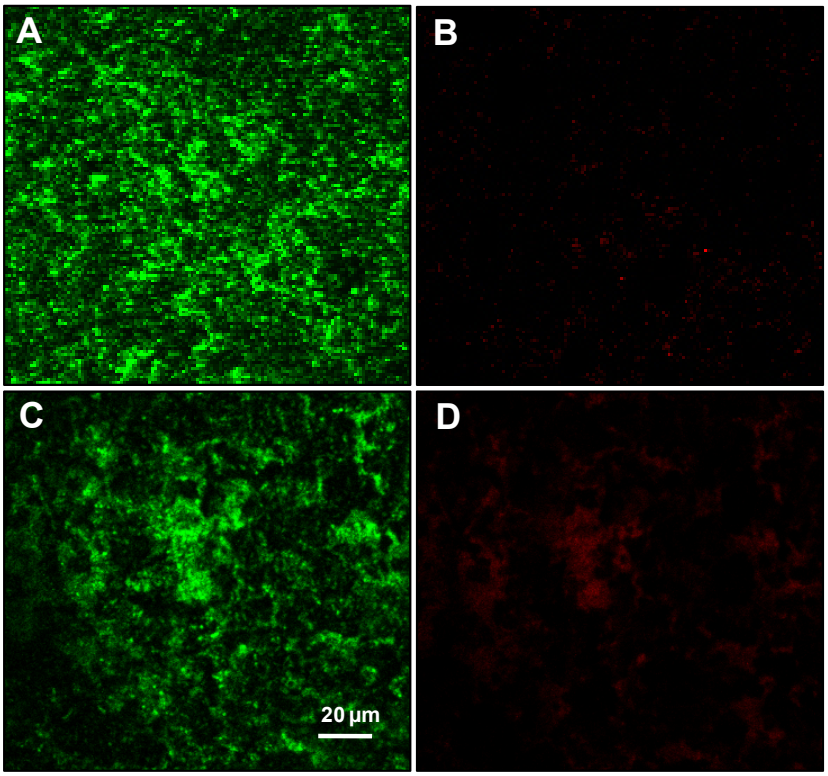

Fig. S2 FCSM images of the exterior **(A, B)** and interior **(C, D)** of the CAB-2. Live cells were dyed with green color **(A and C),** while dead cells were dyed with red color **(B and D)**.

1.5.3 Four electrode measurement of resistance of the CAB

The conductivity of the CAB was measured using a four-probe measurement system, which consisted of Summit series probe station (Cascade Microtech, Beaverton, OR, USA) and E5270B semiconductor analyzer (Agilent, Santa Clara, CA, USA). Result was calculated using the uniform thin sheet model[2]:

$$\rho = \frac{V}{I} * \frac{\pi t}{In\ 2}$$

where t is the thickness of the material. In this study, the thickness of the CAB-2 was estimated as ~ 0.45 mm using optical microscope (Olympus BX51, QES. PTE. LTD., Singapore).

2. Comparison of power output between the CABs and other control experiments

Fig. S3 compared the voltage output of the first MFC operation cycle between CAB-2 and several control experiments (*i.e.*, natural biofilm, PPy and bacteria composite, graphite and bacteria composite without PPy).

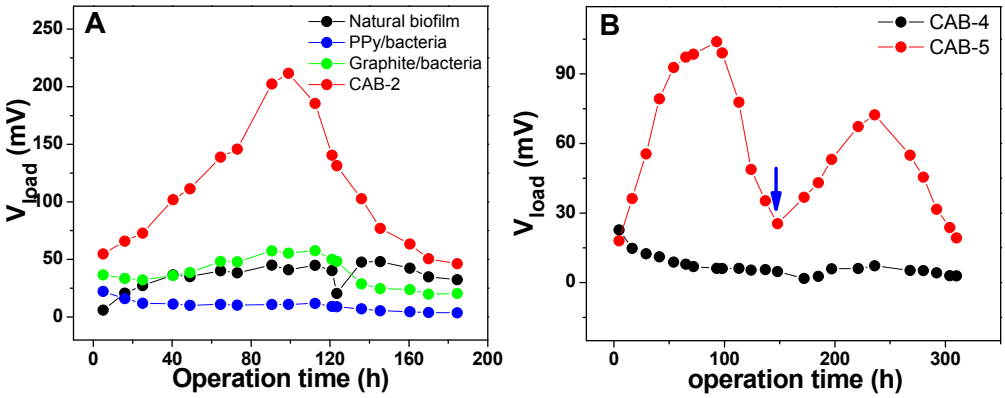

Fig. S3. (A) Voltage output of the CAB-2 (red), natural biofilm (black), CAB without graphite (blue), and CAB without PPy (blue). **(B)** Voltage output of the CABs with less graphite. CAB-4 and -5 were prepared with the same procedure of CAB-1 and -2 but with only 1/5 of graphite mass. Blue arrow represents medium refreshment.

Reference

1. H. Chen, L. Guo, A. R. Ferhan and D.-H. Kim, *The Journal of Physical Chemistry C*, 2011, **115**, 5492-5499.
2. L. J. Swartzendruber, *Solid-State Electronics*, 1964, **7**, 413-422.