Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supplementary information:

Hierarchical micro/nano structures of carbon composite as anodes for microbial fuel cells

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1. Effects of the GF pretreatment on the performances of MFC

In order to examine if the doped CNTs were responsible for the improved power outputs from MFC (Fig. 4), GFs were subjected to the pretreatment (the sulfuric-acid treatment and water/ethanol wash) as described in section "Experimental" (without the CNT synthesis) and used as anodes for MFCs inoculated with rice paddy-field soil. The MFC experiment was performed as described in section "Experimental".

IV and IP curves were drawn for MFCs with the bare-GF and pretreated-GF anodes (Fig. S1), and their performances were compared. It is shown in this graph that the pretreatment of the GF anode did not exert substantial positive effects on the MFC performance, indicating that the doped CNTs were responsible for the increased power outputs from MFCs presented in Fig. 4.

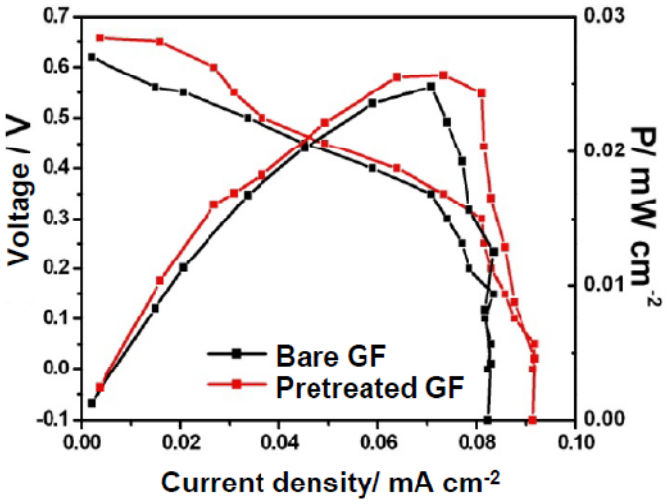

Fig. S1. IV and IP curves for MFCs with the bare-GF and GF

2. Comparison of protein amounts

In order to compare amounts of microbes attached to the bare GF and CNT-GF anodes, proteins were extracted from the anodes and quantified using a bicinchoninic acid-assay (BCA) system (Newton, *et al*., *Appl. Environ. Microbiol.*, 2009, **75**, 7674). A piece of anode (1 cm^2) was put in a bacterial protein extraction reagent (Pierce) and incubated at 40ºC for 1 hour. After the anode was removed from the reagent solution, it was centrifuged at 15000 rpm. A resultant supernatant was subjected to the BCA assay according to the manufacturer's instruction, and an amount of protein was estimated according to a standard curve made using bovine serum albumin solutions.

It was revealed that proteins extracted from the CNT-GF anode was approximately 30% more than those from the bare GF anode (Fig. S2). This result indicates that the 5-fold increase in power output with the CNT-GF anode (compared to that with the bare GF anode) was not ascribable to the increase in affinity between anode and microbes.

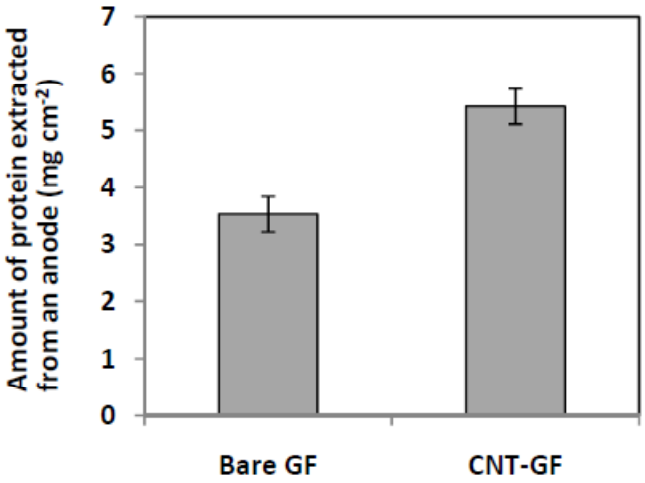

Fig. S2. Amounts of proteins extracted from anodes.

3. Pure-culture MFC experiments

In order to better understand mechanisms of how CNTs doped on GFs improve electron transfer from microbes to anodes, we performed pure-culture MFC experiments using *Psedudomonas aeruginosa* strain PAO, *Shewanella oneidensis* strain MR-1, and *Geobacter sulfurreducens* PCA. Strain PAO is known to solely employ the mediator pathway for transferring electrons to anodes (Rabaey, *et al.*, *Environ. Sci. Technol.*, 2005, **39**, 3401), strain MR-1 uses both the mediator and direct-contact pathways (Newton, *et al.*, *Appl. Environ. Microbiol.*, 2009, **75**, 7674), while strain PCA transfers electron directly to electrodes (Bond and Lovley, *Appl. Environ. Microbiol.*, 2003, **69**, 1548). Comparisons in effects of the CNT doping among MFCs inoculated with one of the three strains were considered to provide information as to which, the mediator or direct-contact pathway, was accelerated by the CNT doping.

Strain PAO was grown in a Luria-Bertani (LB) medium up to a late logarithmic growth phase (cultivation time of approximately 12 hours), and a resultant culture was introduced into a MFC reactor. Strain MR-1 was also grown in the LB medium, and, after cells were harvested by centrifugation, they were resuspended in a lactate

minimum medium (Newton, *et al*., *Appl. Environ. Microbiol.*, 2009, **75**, 7674), and introduced into a MFC reactor. PCA was grown in PSN medium (Bond and Lovley, *Appl. Environ. Microbiol.*, 2003, **69**, 1548), and MFC was inoculated with a resultant PCA culture. After these MFCs were operated for several days, their performances were evaluated as described in section "Experimental".

Fig. S2 shows IV and IP curves for PAO, MR-1, and PCA MFCs equipped with either the bare-GF or CNT-GF anodes. As shown in panel a, the doping of the GF anode with CNTs largely (over 5 folds in P_{max}) improved MFC performances, while the improvement was moderate in the MR-1 MFC (panel b), and there was only minor difference between bare-GF and CNT-GF in the PCA MFC. It is likely that the small improvement in the PCA MFC was ascribable to the electron-transfer mechanisms employed by strain PCA; namely, PCA uses the direct-contact pathways, and the CNT doping accelerates only the mediator pathway.

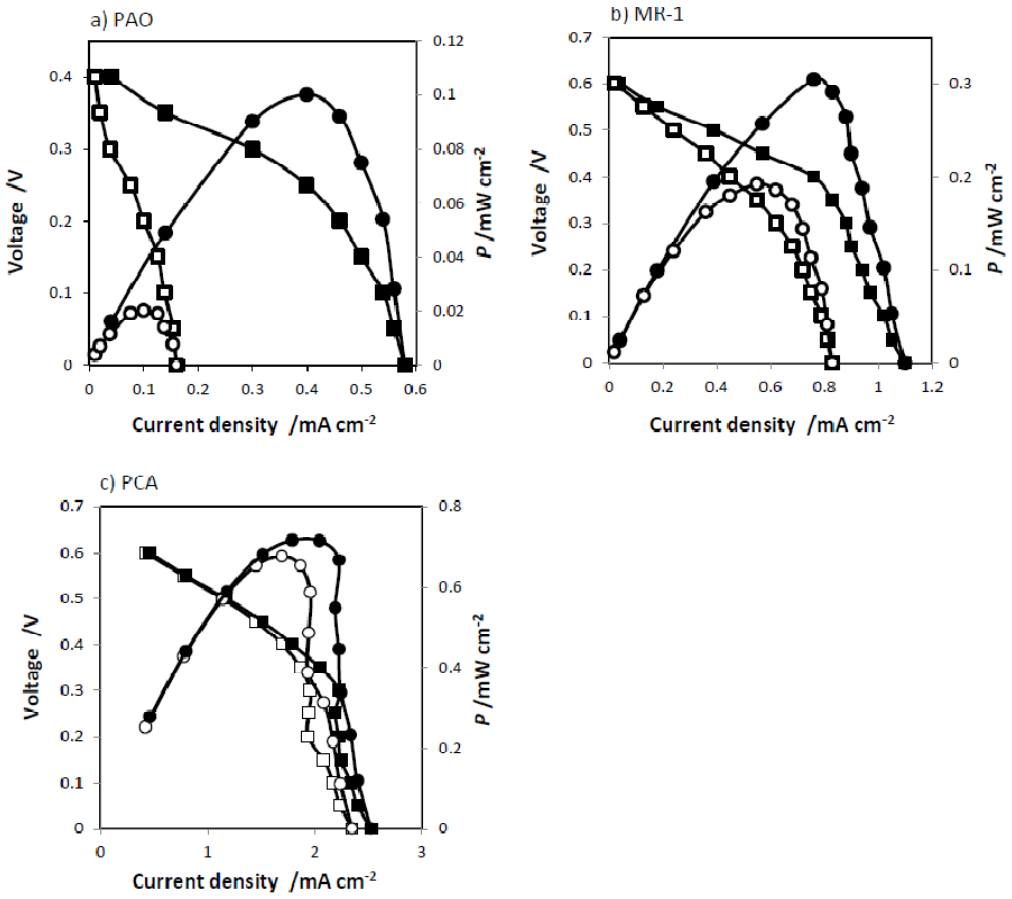

Fig. S3. IV (squares) and IP (circles) curves for MFCs inoculated with strain PAO (a), MR-1 (b), and PCA (c) that were equipped with the bare GF (open symbols) or CNT-GF (closed symbols).