Environmental Science & Technology Letters

Letter

pubs.acs.org/journal/estlcu

Protection of Electroactive Biofilm from Extreme Acid Shock by Polydopamine Encapsulation

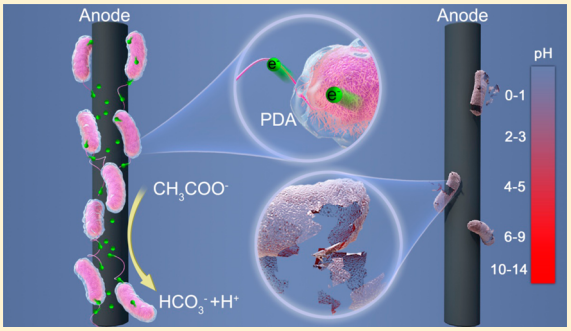
Qing Du,[†] Tian Li,[†] Nan Li,[‡] and Xin Wang*[,†]

[†]MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, No. 38 Tongyan Road, Jinnan District, Tianjin 300350, China

[‡]School of Environmental Science and Engineering, Tianjin University, No. 92 Weijin Road, Nankai District, Tianjin 300072, China

Supporting Information

ABSTRACT: Electroactive biofilm has a low tolerance for accidental shocks, such as extreme acid shock, which is a potential limitation for the application of bioelectrochemical systems (BESs), especially as a sensor for water quality monitoring. In this work, we encapsulated electroactive biofilms with biocompatible polydopamine (PDA) to protect against extreme acid shock. The bacterial cells were completely encapsulated in ∼50 nm films formed by PDA spheres, which protected their viability and current recoverability even after pH 0.5 and 1.5 shocks. The limiting current density of the PDA-encapsulated anode was 0.20 ± 0.05 A/m^2, which was 1900% higher than that of the unprotected control (0.01 ± 0.01 A/m^2) after strong acid shock (pH 0.5, 30 min). Without PDA encapsulation, the biofilm partly disintegrated with a thickness decreased by 68% from 72 to 23 μm, where 92% of the cells were dead. Our findings reported a novel and effective method for protecting electroactive biofilm under extreme conditions, which will greatly extend the use of BESs in the future.



INTRODUCTION

A bioelectrochemical system (BES), a green technology directly converting waste into electricity, hydrogen, or methane, has great potential to be used in wastewater treatment systems.[1−3] More interestingly, on the basis of the direct feedback of bacterial activity with a change in current, it has been demonstrated as a sensitive online device for biochemical oxygen demand (BOD) monitoring[4] or water quality early warning (biotoxicity sensors[5,6]). Before a BES can be widely applied to wastewater treatment systems or environmental monitoring, one of the greatest challenges is its stability, which means answering the the questions of how to stabilize its power output after long-term operation and how this system survives after accidental shocks. Much research has already done on the long-term stability of BESs,[7,8] but no research on the protection of this system from shocks has been reported. This is even more important to BES-based sensors because currently they are one-off after short-term exposure to an extreme environment or toxic substances.[9] The reacclimation of an electroactive biofilm that was killed by shocks normally takes an additional 5−60 days,[10] which limited the application of BESs.

Here we take extreme acid shock as an example. It may happen when acidic industrial wastewater interfuses into drainage. Because most cathodes of BESs use chemical oxygen reduction catalysts (such as Pt and activated carbon),[11] the protection of the bacterial anode is primary. Bacteria have evolved to retain their species and genetic information under harsh external environments by developing hard shells. Robust shells are considered beneficial to the inner cells. Sporelike structures,[12] for example, have been investigated by coating living cells with inorganic chemicals, including silica,[13] calcium carbonate,[14] and multilayers of polyelectrolytes,[15] to preserve the long-term stability of bioreactors and microfluidics. Nevertheless, they are all limited by the biological toxicity of the process of formation of inorganic shells or electrostatic layer-by-layer self-assembly of polyelectrolytes. Polydopamine (PDA) is a natural pigment with outstanding biocompatibility, rich functional groups, excellent adhesive force, and negligible cytotoxicity.[16] In a study by Yang et al.,[12] PDA was used in encapsulation of individual living yeast cells to create stronger resistance against lyticase. Park and co-workers[17] modified a poly(dimethysiloxane) substrate with PDA for patterning of cell lines, and the PDA adhesion layer remained stable even under strongly acidic conditions. However, it has not yet been used on a living biofilm. In our recent study, PDA was mixed in activated carbon as an anode to improve the power of microbial fuel cells, which partly showed that the PDA may not be toxic to electroactive bacteria.[18]

Here we encapsulated a living electroactive biofilm with artificial PDA. The effect of a PDA coating on the current density of BESs was evaluated. We then compared the

Received: June 19, 2017
Accepted: June 30, 2017
Published: June 30, 2017

DOI: 10.1021/acs.estlett.7b00242
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

performance of a PDA-encapsulated electroactive biofilm with that of the control without treatment after extreme acid shocks.

MATERIALS AND METHODS

BES Fabrication and Inoculation. The three-electrode single-chamber BESs were constructed using cylindrical glass reactors (AiDa, Tianjin, China) with a net volume of 100 mL, a diameter of 5 cm, and a height of 5 cm.[19] A graphite rod electrode was used as the working electrode with an exposed area of 13.35 cm^2. A Ag/AgCl (3.5 M KCl) reference electrode was inserted close to the working electrode. A plain platinum plate (1 cm × 1 cm) was used as the counter electrode. All electrodes were operated in batch mode by connecting to a multichannel potentiostat (CHI 1000C, CH Instrument, Shanghai, China) at 25 ± 1 °C. Prior to being used, all graphite rod anodes were mechanically polished with 600-mesh abrasive paper and then dipped into distilled water for a thorough cleaning with an ultrasonic washer for 2 h.

All BESs were inoculated with effluent from the mixture of single- and double-chambered microbial fuel cells in our laboratory. The electrolyte contains a 50 mM phosphate buffer solution (PBS; pK_a = 7.2; pH 7.1; conductivity of ~7.65 mS/cm; containing 4.58 g/L Na_2HPO_4, 2.13 g/L NaH_2PO_4, 0.31 g/L NH_4Cl, and 0.13 g/L KCl), a trace mineral solution, a vitamin solution, and 1 g/L acetate. Before connecting to the BESs to circuits, we vigorously flushed the electrolyte with N_2/CO_2 gas [4:1 (v/v) N_2:CO_2] for 15 min to remove dissolved oxygen.

PDA Encapsulation and Extreme Acid Shock. A dopamine solution (2 mg/mL) was prepared by dissolving dopamine hydrochloride in Tris-HCl buffer (10 mmol/L, pH 8.5). When the electroactive biofilms on electrodes were mature (approximately five cycles), anodes were taken out and inserted into the 2 mg/mL dopamine solution for ~30 min to encapsulate the biofilm with PDA. Electrodes were then washed with distilled water to remove residual lye.

To avoid the co-effect of current change on biofilm, PDA-encapsulated anodes were inserted into a flask containing an acid electrolyte (pH 0.5 and 1.5, adjusted by adding 15% hydrochloric acid to the electrolyte used in the BESs) for 30 min as an acid shock. Anodes were then re-installed in the BESs for electrochemical tests. Two parallel reactors were run for each sample as duplicates. Electroactive biofilms without PDA were simultaneously operated as controls.

Electrochemical Tests. Chronoamperometry was used to record the current of the BESs every 100 s with a multichannel potentiostat (0 V vs Ag/AgCl, CHI 1000C, CH Instrument). For each BES, turnover cyclic voltammetry (CV) was conducted when the biofilm was mature, after PDA encapsulation and after extreme acid shock using a potentiostat (PGSTAT 302N, Metrohm). The scanning rate was 1 mV/s (stabilization period of 300 s) with a potential window ranging from −0.6 to 0.2 V according to previous studies.[20,21] The first-order derivative CVs (DCVs) derived from turnover CVs were calculated to identify the change in peak heights and midpoint potentials after each treatment. The potentials presented in all results here were referenced to the Ag/AgCl electrode (3.5 M KCl, 0.205 V vs the standard hydrogen electrode).

Microscope Analysis. One milliliter of BES effluent and 9 mL of Tris-HCl (pH 8.5) buffer were mixed with 20 mg of dopamine hydrochloride. The whole solution was incubated in a shaking incubator (160 rpm) for 30 min at 25 ± 1 °C and then ultrasonically agitated for 1 h to disassemble the bacterial clumps. Then, 10 μL of the solution was dropped on copper mesh for characterization with a transmission electron microscope (JEM100CXII, JEOL, Ltd.). Ten microliters of untreated BES effluent was used as the control. Scanning electron microscopy (SEM) (Shimadzu SS-550) was employed for observation of surface morphologies.

After extreme acid shock, anodes recovered in the BES under 0 V for 60 h. Then these working electrodes were taken out, and the biofilm-covered part was cut with a sterilized blade. Biofilm samples were stained with a LIVE/DEAD BacLight Bacterial Viability Kit (L13152, ThermoFisher Scientific Inc.).[19] Confocal laser scanning microscopy (CLSM) (TCS SP8, Leica) was used to image the spatial topography of a biofilm on anodes. The percentage of living cells (viability) and biofilm coverage were calculated by counting pixels. Layer-scanned images were stacked and analyzed using ImageJ.

RESULTS AND DISCUSSION

PDA Film and PDA-Encapsulated Bacteria. To observe the surface morphology of the PDA film, PDA was initially formed on half of the glassy carbon electrode [half coated and half untreated (Figure 1)]. There was an obvious boundary between the PDA-coated (right side) and untreated (left side) parts of the electrode, indicating that PDA completely covered the electrode surface. The thickness the PDA film was ~50 nm. However, the surface was not as flat as we thought (the convex was as high as 200 nm), which may be attributed to the short reaction time (30 min). It has been reported that a high dopamine concentration of 2 mg/mL with a longer reaction time (20 h) led to a thicker and flatter film.[22,23] Because of the semiconductivity of PDA, in this study, the reaction time was selected to ensure a complete covering of the electrode with a minimal effect on bacterial activity and biological electron transfer. Although the 200 nm convex was relatively large

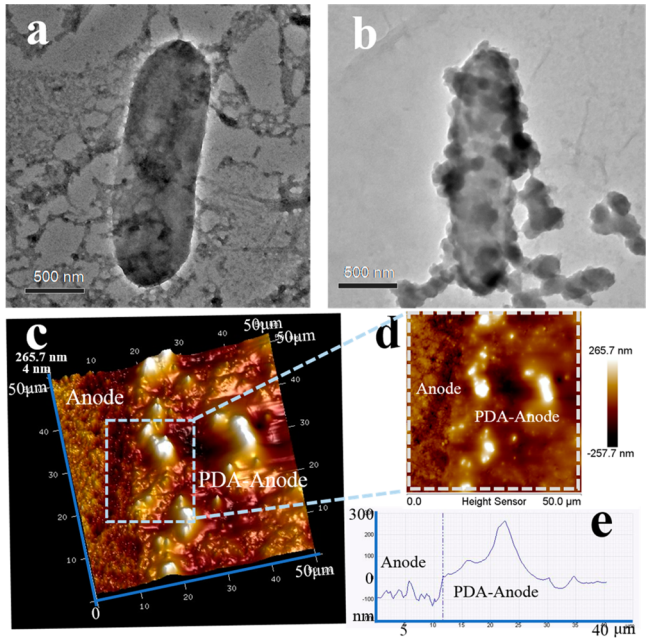


Figure 1. Transmission electron microscopy images of rodlike bacteria (a) with and (b) without PDA encapsulation. (c−e) Atomic force microscopy three-dimensional analysis of the inner surfaces of glassy carbon and PDA-modified glassy carbon. PDA was initially formed on half of the glassy carbon electrode (half coated and half untreated).

DOI: 10.1021/acs.estlett.7b00242
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

compared to the average thickness, this value is negligible with respect to the size of electroactive bacteria such as *Geobacter* (1−5 μm).

The bacterial cells were ∼3 μm in length. The cells after encapsulation were intact (Figure 1). A shell that was ∼50 nm thick, composed of closely linked spheres, was formed on the surface of the cells, indicating that the rodlike bacteria were successfully encapsulated by PDA. The whole process has no visible damage on cells. The biofilm grew well after PDA encapsulation, with a thickness that increased from ∼90 to ∼200 μm in 60 days (Figure S1). PDA on biofilm was stable. The PDA coating was resistant to ultrasonic agitation (Figure 1b) and continuous flow [5.0 mL/min (Figure S5)].

Biofilm after Extreme Acid Shock. The control and the PDA-encapsulated biofilms were subjected to pH 0.5 and 1.5 shocks. When biofilms were scraped from graphite rod electrodes, the PDA-encapsulated biofilms were pink in color, while the controls without protection lost their color and became gray (Figure S2), indicating that the extreme acid shock may cause damage to cell structures. After a biofilm had been subjected to PDA coating and extreme acid shock and then continuous flow conditions, the PDA film was still stably modified on the biofilm (Figure S5). As shown in Figure 2, the biomass on the anode of the control after pH 0.5 shock (control-0.5) was significantly reduced, with only sporadic spotted parts remaining. The thickness of this biofilm was only 23 μm, approximately one-third of the thickness of PDA-encapsulated biofilm (PDA-0.5) (72 μm). It was shown that biofilm coverage on PDA-0.5 was 4 times higher than that of the control of the anode (green and red in Figure 2), which harbored a 3.5-fold higher proportion of living cells (green in Figure 2) that evenly covered the surface of the anode.

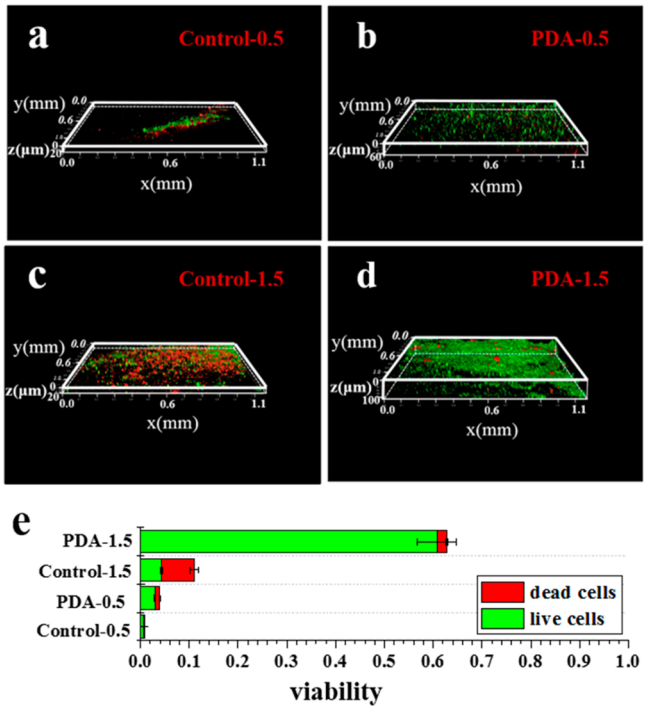


Figure 2. Stacked CLSM images of control biofilms after (a) pH 0.5 and (c) pH 1.5 shocks and PDA-capsulated biofilms after (b) pH 0.5 and (d) pH 1.5 shocks. The biofilm at 0 μm means the biofilm−electrode interface (bottom). (e) Viability is calculated by counting pixels.

Although the biofilm coverage of the control-1.5 at pH 1.5 was 10 times higher than that of control-0.5, 92% of these cells were dead (red in color). The increase in pH from 0.5 to 1.5 had no obvious effect on the thickness of the biofilm, with a similar value of 28 μm. However, with the protection of PDA, the biofilm (PDA-1.5) reached a 112 μm thickness, with a value 4.5-fold higher than that of control-1.5. Moreover, living bacterial cells were 17 times thicker than control-1.5 according to pixel counting.

Electroactive bacteria are generally vulnerable to extremely acidic conditions. According to Bearson et al.,[24] the lowest pH that electrogens can tolerate is ∼3. A high concentration of H^+ binds with the peptide bond of the proteins in the bacterial surface, leading to lysis of the cells. Here we found the biofilm was severely destroyed at pH 1.5 and 0.5, but PDA encapsulation protected the biofilms from destruction by acid. This is probably because the good biocompatibility and the rich functional groups of the PDA make it closely bind to the bacterial cells to form hard shells on the surface, which is resistant to extreme shocks. In addition, PDA is highly stable and cannot be destroyed by strong acid or degraded by bacteria, forming solid protection for the cells inside. These features lead to the resistance of PDA-encapsulated bacteria to strong acid.

Electrochemical Activity. Sigmoidal catalytic waves were displayed in all turnover CVs from overall reactors (Figure 3). Before biofilms were coated with PDA, all the anodes were parallel (Figure 3a). The limiting current density of PDA-encapsulated anodes decreased slightly by 16% to 5.74 ± 0.30 A/m^2 compared to that of the control (7.05 ± 0.42 A/m^2), and peak heights decreased by 20% in DCVs (Figure S3), probably because the encapsulation of PDA led to a slight loss of living microbes.[12] Extreme acid shock sharply decreased the current production level in all controls (Figure 3c). The limiting current densities of control-1.5 (0.08 ± 0.02 A/m^2) after acid shock (pH 1.5) was close to that of control-0.5 (0.01 ± 0.01 A/m^2), with values decreased by 74 and 95% compared to those of PDA-1.5 (0.29 ± 0.10 A/m^2) and PDA-0.5 (0.20 ± 0.05 A/m^2), respectively. DCVs showed a similar main pair of symmetrical peaks still centered at 0.39 ± 0.02 V (a value similar to the value of 0.39 ± 0.03 V reported previously in a *Geobacter* dominant system[25,26]) after acid shocks, indicating that the dominant redox pairs may not be affected during this process (Figure S3). Current densities of PDA-encapsulated anodes recovered soon after the acid shocks (Figure 3d), which further demonstrated that bacteria in the biofilm were protected by the PDA coating, so their metabolic activity recovered as soon as the extreme condition was removed. However, for the uncovered controls, no visible recovery of current was observed in 470 h. These results further confirmed that the PDA shell protected electroactive bacteria from being killed by the extremely acidic environment. The data in Figure S4 further confirm the findings described above.

In summary, we demonstrated that the activity of an electroactive biofilm can be protected by the easily synthesized PDA shell with a thickness of 50−200 nm. The shell is biocompatible and stable with respect to the environment and protected electroactive bacteria from being killed by pH 0.5 shock. This widely extends the use of BESs in a variable water environment and may change the one-off biosensors into self-repairable devices for biological toxicity detection and BOD monitoring in the future. Besides, PDA coating may protect

DOI: 10.1021/acs.estlett.7b00242
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

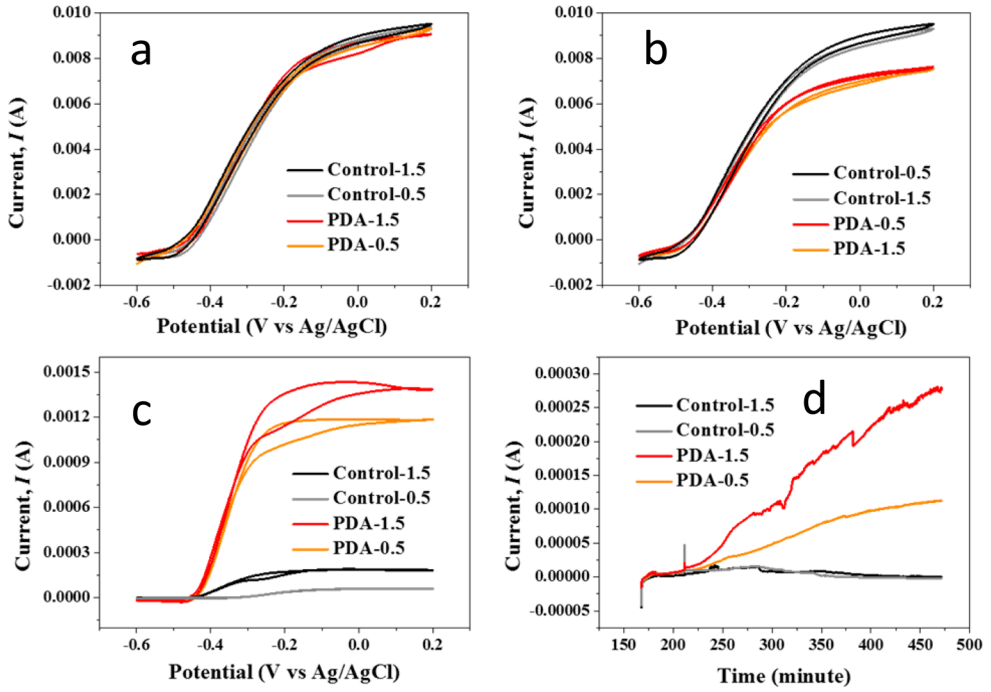


Figure 3. Turnover CVs of (a) mature biofilms, PDA-encapsulated biofilms (b) before and (c) after pH 1.5 and 0.5 shocks, and (d) current recovery (at 0 V vs Ag/AgCl) after acid shocks. The substrate was 1 g/L acetate.

bacteria from organic solvents, sonication, and heat treatment. Therefore, the PDA-coated electroactive bacteria can be used as the ink for patterning of new biosensors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.7b00242.

Growth of a biofilm after PDA encapsulation (Figure S1), color of each biofilm after acid treatments (Figure S2), first-derivative CVs derived from Figure 3 (Figure S3), data duplicated from Figure 3 (Figure S4), and SEM images of a PDA-encapsulated biofilm after extreme acid and continuous flow treatments (Figure S5) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: xinwang1@nankai.edu.cn. Phone: (86)22-58890402. Fax: (86)22-23501117.

ORCID

Xin Wang: 0000-0002-3522-5627

Author Contributions

Q.D. and T.L. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (21577068) and the Fundamental Research Funds for the Central Universities.

■ REFERENCES

(1) Logan, B. E.; Wallack, M. J.; Kim, K. Y.; He, W.; Feng, Y.; Saikaly, P. E. Assessment of Microbial Fuel Cell Configurations and Power Densities. *Environ. Sci. Technol. Lett.* **2015**, *2*, 206−214.

(2) Liu, J.; Liu, J.; He, W.; Qu, Y.; Ren, N.; Feng, Y. Enhanced electricity generation for microbial fuel cell by using electrochemical oxidation to modify carbon cloth anode. *J. Power Sources* **2014**, *265*, 391−396.

(3) Guo, K.; Freguia, S.; Dennis, P. G.; Chen, X.; Donose, B. C.; Keller, J.; Gooding, J. J.; Rabaey, K. Effects of surface charge and hydrophobicity on anodic biofilm formation, community composition, and current generation in bioelectrochemical systems. *Environ. Sci. Technol.* **2013**, *47*, 7563−7570.

(4) Chang, I. S.; Jang, J. K.; Gil, G. C.; Kim, M.; Kim, H. J.; Cho, B. W.; Kim, B. H. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. *Biosens. Bioelectron.* **2004**, *19*, 607−613.

(5) Song, X. N.; Cheng, Y. Y.; Li, W. W.; Li, B. B.; Sheng, G. P.; Fang, C. Y.; Wang, Y. K.; Li, X. Y.; Yu, H. Q. Quorum quenching is responsible for the underestimated quorum sensing effects in biological wastewater treatment reactors. *Bioresour. Technol.* **2014**, *171*, 472−476.

(6) Liu, B.; Lei, Y.; Li, B. A batch-mode cube microbial fuel cell based "shock" biosensor for wastewater quality monitoring. *Biosens. Bioelectron.* **2014**, *62*, 308−314.

(7) Zhang, F.; Pant, D.; Logan, B. E. Long-term performance of activated carbon air cathodes with different diffusion layer porosities in microbial fuel cells. *Biosens. Bioelectron.* **2011**, *30*, 49−55.

(8) Oh, S. E.; Logan, B. E. Voltage reversal during microbial fuel cell stack operation. *J. Power Sources* **2007**, *167*, 11−17.

(9) Kang, K. H.; Jang, J. K.; Pham, T. H.; Moon, H.; Chang, I. S.; Kim, B. H. A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. *Biotechnol. Lett.* **2003**, *25*, 1357−1361.

(10) Li, T.; Zhou, L. A.; Qian, Y. W.; Wan, L. L.; Du, Q.; Li, N.; Wang, X. Gravity settling of planktonic bacteria to anodes enhances current production of microbial fuel cells. *Appl. Energy* **2017**, *198*, 261−266.

DOI: 10.1021/acs.estlett.7b00242
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

(11) Zhang, F.; Cheng, S.; Pant, D.; Bogaert, G. V.; Logan, B. E. Power generation using an activated carbon and metal mesh cathode in a microbial fuel cell. *Electrochem. Commun.* **2009**, *11*, 2177−2179.
(12) Yang, S. H.; Kang, S. M.; Lee, K. B.; Chung, T. D.; Lee, H.; Choi, I. S. Mussel-inspired encapsulation and functionalization of individual yeast cells. *J. Am. Chem. Soc.* **2011**, *133*, 2795−2797.
(13) Yang, S. H.; Lee, K. B.; Kong, B.; Kim, J. H.; Kim, H. S.; Choi, I. S. Biomimetic encapsulation of individual cells with silica. *Angew. Chem., Int. Ed.* **2009**, *48*, 9160−9163.
(14) Fakhrullin, R. F.; Minullina, R. T. Hybrid cellular-inorganic core-shell microparticles: encapsulation of individual living cells in calcium carbonate microshells. *Langmuir* **2009**, *25*, 6617−6621.
(15) Zamaleeva, A. I.; Sharipova, I. R.; Porfireva, A. V.; Evtugyn, G. A.; Fakhrullin, R. F. Polyelectrolyte-mediated assembly of multiwalled carbon nanotubes on living yeast cells. *Langmuir* **2010**, *26*, 2671−2679.
(16) Liu, Y.; Ai, K.; Lu, L. Polydopamine and Its Derivative Materials: Synthesis and Promising Applications in Energy, Environmental, and Biomedical Fields. *Chem. Rev.* **2014**, *114*, 5057−5115.
(17) Ku, S. H.; Lee, J. S.; Park, C. B. Spatial control of cell adhesion and patterning through mussel-inspired surface modification by polydopamine. *Langmuir* **2010**, *26*, 15104−15108.
(18) Du, Q.; An, J. K.; Li, J. H.; Zhou, L. A.; Li, N.; Wang, X. Polydopamine as a new modification material to accelerate startup and promote anode performance in microbial fuel cells. *J. Power Sources* **2017**, *343*, 477−482.
(19) Wang, X.; Zhou, L. A.; Lu, L.; Lobo, F. L.; Li, N.; Wang, H.; Park, J.; Ren, Z. J. Alternating Current Influences Anaerobic Electroactive Biofilm Activity. *Environ. Sci. Technol.* **2016**, *50*, 9169−9176.
(20) Feng, C. J.; Liu, Y. N.; Li, Q.; Che, Y.; Li, N.; Wang, X. Quaternary Ammonium Compound in Anolyte without Functionalization Accelerates the Startup of Bioelectrochemical Systems using Real Wastewater. *Electrochim. Acta* **2016**, *188*, 801−808.
(21) Liu, Y.; Kim, H.; Franklin, R. R.; Bond, D. R. Linking spectral and electrochemical analysis to monitor c-type cytochrome redox status in living Geobacter sulfurreducens biofilms. *ChemPhysChem* **2011**, *12*, 2235−2241.
(22) Kang, S. M.; Hwang, N. S.; Yeom, J.; Park, S. Y.; Messersmith, P. B.; Choi, I. S.; Langer, R.; Anderson, D. G.; Lee, H. One-Step Multipurpose Surface Functionalization by Adhesive Catecholamine. *Adv. Funct. Mater.* **2012**, *22*, 2949−2955.
(23) Lu, Z.; Xiao, J.; Wang, Y.; Meng, M. In situ synthesis of silver nanoparticles uniformly distributed on polydopamine-coated silk fibers for antibacterial application. *J. Colloid Interface Sci.* **2015**, *452*, 8−14.
(24) Bearson, S.; Bearson, B.; Foster, J. W. Acid stress responses in enterobacteria. *FEMS Microbiol. Lett.* **1997**, *147*, 173−180.
(25) Inoue, K.; Qian, X.; Morgado, L.; Kim, B. C.; Mester, T.; Izallalen, M.; Salgueiro, C. A.; Lovley, D. R. Purification and characterization of OmcZ, an outer-surface, octaheme c-type cytochrome essential for optimal current production by Geobacter sulfurreducens. *Appl. Environ. Microbiol.* **2010**, *76*, 3999−4007.
(26) Lloyd, J. R.; Leang, C.; Myerson, A. L. H.; Coppi, M. V.; Cuifo, S.; Methe, B.; Sandler, S. J.; Lovley, D. R. Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in Geobacter sulfurreducens. *Biochem. J.* **2003**, *369*, 153−161.

DOI: 10.1021/acs.estlett.7b00242
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX