RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

RSC Advances

Paper

Hydrophilicity Boosted Extracellular Electron Transfer in *Shewanella loihica* PV-4

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chen Zhao,[a] Chunmei Ding,*[b] Meiling Lv,[a] Yuan Wang,[a] Lei Jiang [a] and Huan Liu*[a]

Taking advantage of the abundant redox active C-type cytochromes on their outer membrane (OMCs), genus Shewanella enables extracellular electron transfer (EET), which is applicable in various bioelectrical devices. For the practical applications, high efficient EET is always desirable. Here, we revealed that tailoring the surface wettability of electrodes can drastically alter the EET activity of microbial Shewanella loihica PV-4: the EET current on superhydrophilic electrode is over 3 and 10 times higher than that on normal hydrophilic and hydrophobic electrode, respectively. Worthnoting is that the cell suspension with rather small initial cell density was particularly used, which can exclude the influence from other unfavorable factors in such a dynamic and flexible living system. It is proposed that the hydrophilic electrode favors reduced state of OMCs, and consequently both the EET activity and cell proliferation are highly facilitated.

Introduction

Members of the genus *Shewanella* that are widely distributed in nature have a unique property of being able to transfer metabolically generated electrons to external solid-state electron receptors (i.e. minerals and electrodes) under the anaerobic condition.[1] The process, denoted as extracellular electron transfer (EET), is a crucial process with respect to its extensive applications in bioelectricity, bioremediation and biogeochemical circulation of minerals in an environmental-friendly way.[2] No doubly, it has attracted broad interests from fundamental research to practical applications in the last decade.[3] The genus *Shewanella loihica* (*S. loihica*) PV-4, a typical exoelectrogenic bacterium, has been extensively studied for the understanding of EET since the abundant redox-active proteins of multiheme c-type cytochromes located on the outer membrane (OMCs), particularly OmcA and MtrC.[4]

Currently, the very low EET activity remains a bottleneck for its practical applications in bioelectrical devices, for example, microbial fuel cells (MFCs).[5] So far, extensive efforts have been made to enhance the microbial EET current. Two approaches were generally employed based on anode concerning, i.e., fabricating high surface area[6] and forming three-dimensional network architecture via self-assembly with nanoparticles.[7] As a typical interfacial process, the surface physicochemical properties of both microbe and electrode are rather important factors that can significantly affect the EET process,[8] but are usually ignored. Focusing on inherent property of OMCs, we notice that the surrounding local polar environment largely affects the electrochemical properties of purified redox-active OMCs.[9] More importantly, as a central redox-active ion in OMCs, ferric (oxidized) /ferrous (reduced) irons prefer diverse wettability.[10] Very recently, our group has reported that surface wettability of the electrodes strongly influences the microbial EET activity by exploring the electrochemical property of densely packed biofilm where the cell number was rather high and kept constant during the whole process of electrochemical culture.[11] However, in natural environment and bioelectric devices (MFC or MEC), bacterial biofilm formation, a crucial process that determines the device performance, has been always accompanied by the cell number increasing.[12] It means, the situation for systems where cells keep proliferating largely differ from that of the system with the constant cell number. Here, by continuous nutrition feeding, we revealed that the surface hydrophilicity of the electrode can largely boost bacterial EET current as a cooperative effect of the varied redox state of OMCs and the enhanced cell proliferation behavior on hydrophilic electrode.

In this work, EET activity was evaluated by the current generation in an electrochemical cell (EC) containing yeast extract for bacteria proliferation. The substantial improvement (over 10 fold) of EET current was achieved on superhydrophilic electrode compared with that on the normal hydrophilic and hydrophobic electrodes. We propose that the relatively weak interaction between microbes and polar electrode facilitated the electron transfer process by the flavin-cofactor pathway, as a result of the reduced OMCs. Moreover, the corresponding decreased oxidative pressure for microbes can benefit

[a] Key Laboratory of Bio-Inspired Smart Interfacial Science and Technology of Ministry of Education, School of Chemistry and Environment, Beihang University, Beijing 100191 (P. R. China). E-mail: liuh@buaa.edu.cn
[b] College of Polymer Science and Engineering, Sichuan University, Chengdu 610065 (P.R. China). E-mail: dingcm@scu.edu.cn

Electronic Supplementary Information (ESI) available: (1) Typical CVs of potassium ferricyanide on various electrodes; (2) Statistic current values after 24 h bacteria cultivation; (3) Current versus time curve in the presence of exogenously added riboflavins; (4) The detail calculating process for current density. See DOI: 10.1039/x0xx00000x

microbial proliferation, further highlighting the advantages of polar circumstance. We expect that our findings will be useful for the deep understanding of microbial electron transfer, and may shed new light on the design of high-efficient bioelectricity devices.

Experimental section

Substrate modification

The method for the modification of substrates was referred to literature to achieve uniform monolayer coverage of molecules.[13] In brief, Smooth tin-doped In_2O_3 (ITO) electrodes were precleaned with detergent, acetone, ethanol and deionized water, then were modified chemically. For OH-ITO electrode: cleaned ITO electrodes were hydroxylated by the treatment of O_2 plasma, by which the ITO surface was coated uniformly by hydroxy groups. The SH-ITO and CH_3-ITO electrode were achieved by silanization procedure as reported in literature.[11] In brief, 5 mM (3-mercaptopropyl) trimethoxysilane or trichloro (propyl)silane was dissolved in hexane, which was followed by dipping hydroxylated ITO electrode into it for 50 min at room temperature. After that, the electrode was taken out and rinsed with chloroform and water for further use.

Microbe preparation

Microbes of *S. loihica* PV-4 were pre-cultivated aerobically in Marine Broth (MB, 20 g/L) at 30°C for 24 h. Subsequently, the pelleted cells were collected by centrifuging and cultivated aerobically at 30°C for 24 h in defined media with lactate as carbon source (abbreviated as DML: consisted of $NaHCO_3$ (2.5 g), $CaCl_2{\cdot}2H_2O$ (0.08 g), NH_4Cl (1.0 g), $MgCl_2{\cdot}6H_2O$ (0.2 g), NaCl (10 g) and HEPES (7.2 g) per liter, sodium lactate (10 mmol/L), pH 7.8.). Then the cells were collected and washed three times with DML prior to electrochemical process. To be notices, the DML media with yeast extract was particularly used for electrochemical culture of microbes, and the initial cell density in the electrochemical cell was rather low with the value of OD_{600} (the optical density of microbes at 600nm) to be set as 0.0001.

Characterization

A single-chamber, three-electrode system with lactate as carbon source and electron donor was used to monitor the electrochemical behaviour of bacteria. The chemically modified ITO electrodes (surface area: 3.14 cm^2) were mounted on the bottom of the reactor as working electrode, Pt wire and Ag/AgCl (KCl sat.) electrode were used as counter and reference electrode, respectively. DML containing yeast extract (yeast extract was microelement growth supplement), termed DML-Y, was used as electrolyte and de-aerated by bubbling N_2 gas (purity: 99.999 %) until a dissolved O_2 concentration 0.1 ppm was reached. The components of DML-Y were listed as follows: $NaHCO_3$ (2.5 g), $CaCl_2{\cdot}2H_2O$ (0.08 g), NH_4Cl (1.0 g), $MgCl_2{\cdot}6H_2O$ (0.2 g), NaCl (10 g), HEPES (7.2 g), yeast extract (0.5 g) per liter, sodium lactate (10 mmol/L). The electrochemical measurement was conducted at 30°C by utilizing electrochemical workstation CHI 1030B (CH Instruments, USA). Differential pulse voltammetry (DPV) measurement was conducted with an automatic polarization system (CHI 1030B, CH Instruments, USA), using 2 mV pulse increments, 50 mV pulse amplitude, 300 ms pulse width, and a 600 ms pulse period. The deconvolution of DPV curves was conducted as follows: the DPV curves were baseline-subtracted first by deducting the charging current by fitting the baseline from regions sufficiently far from the peak assuming continuation of a similar and smooth charging current throughout the peak region. After that, a peak-fit process was conducted on the obtained curves to distinguish E_p of different redox species using the software of Origin. Finally, the curves of each single peak were collected and normalized for comparison.

Water contact angles were characterized using a data-physics OCA20 contact angle system (Data-Physics, Germany). For scanning electronic microscope (SEM) observation, microbes located on electrodes were fixed with 2.5% glutaraldehyde at room temperature for 3 h, followed by rinsed with phosphate buffer for three times and dehydrated in increasing concentrations of ethanol 30%, 50%, 75%, 95% (vol%) for 10 min. After further rinsed in 100% n-butyl alcohol three times, the electrodes with microbes were freeze-dried, sputtered by Au, and imaged using field-emission scanning electronic microscope (SEM) (JEOL 6700F, Japan).

Results and discussion

Here, a single-chamber, three-electrode system with lactate as carbon and electron source was used to investigate the EET behavior of *S. loihica* PV-4 on electrodes with different wettability.[14] Smooth tin-doped In_2O_3 (ITO) electrodes treated by oxygen plasma and silanes of three-carbon length containing different terminal groups were used as working electrode (see details in the Experimental Section). Specifically, the ITO electrode hydroxylated by the treatment of O_2 plasma (OH-ITO) showed superhydrophilicity with a water contact angle (WCA) approaching 0° (Fig. 1a). The ITO electrode coated with (3-mercaptopropyl) trimethoxysilane (SH-ITO) exhibited hydrophilicity with a WCA of 61.4° (Fig. 1b), while the ITO electrode modified with trichloro(propyl)silane (CH_3-ITO) was hydrophobic with a WCA of 101.3°(Fig. 1c). Three-carbon-length silanes largely avoided the electron transfer resistance and steric hindrance.[15] Thus the resulting electrodes exhibited identical electrochemical activity in the defined electrolyte (Fig. S1). Different from previous work,[11] this study was featured by the following two points: (1) the concentration of cell suspension in the EC was rather low with an initial OD_{600} (the optical density of microbes at 600 nm) of 10^{-3}, which enable the monolayer biofilm formation under the operation condition. The small OD_{600} value is advantageous as it excludes the possible effects of dynamic biofilm formation and is more favorable to elucidate the role of wettability in EET; (2) the electrochemical culture medium contains yeast extract as nutrition, which enables the microbial proliferation.[16] Therefore, wettability of electrode, i.e. local polarity environment at microbe/electrode interface, could be studied

as a crucial parameter for respiration-involved bacterial EET process.

The modified ITO electrodes with different wettability were used as working electrodes to characterize the electrochemical behavior of the microbe *S. loihica* PV-4. The representative current - time (I - t) curve at an applied potential of 0.2 V was shown in Fig. 1d. The EET current on OH-ITO electrode showed a steep increase after 5 h and reached ca. 31.6 mA/m^2 at approximately 17 h of electrochemical cultivation (Fig. 1d, curve 1). In contrast, the current reached a stable value of 7.6 mA/m^2 at 17 h on SH-ITO electrode (Fig. 1d, curve 2), and 2.5 mA/m^2 on CH_3-ITO electrode (Fig. 1d, curve 3). The result clearly showed that the EET current generated on superhydrophilic OH-ITO electrode was over 10 fold higher than that on CH_3-ITO electrode, and over 3 fold higher than that on normal hydrophilic SH-ITO electrode. The results have good reproducibility: the statistic current values with 5 trials were summarized in Fig. S2.

For the living *Shewanella*, EET can proceed either directly through the oxidized OMCs or via self-secreted flavins stabilized by the reduced OMCs, and the latter case is much more efficient than the former one.[17] To clarify the EET behavior of microbes on three electrodes, firstly, the cell suspension was subjected to cyclic voltammetry (CV) using a scan rate of 50 mV/s. CV with this fast scan rate revealed the rapid EET process directly via OMCs occurring at the microbe/electrode interface, where redox peaks of diffusive species was so low to be covered.[17b] As shown in Fig. 2a, clear redox peaks with a midpoint potential of ca. -220 mV, assignable to the OMCs, were observed for all the cases. Generally, the area of the redox peaks in CV curve provides the estimation of the surface coverage of redox species (OMCs) on electrode, i.e. the amount of electrically active OMCs that can directly interact with the electrode in our experiment. As shown in Fig. 2a, the amount of OMCs that can direct interact with electrode varied significantly on electrodes with different wettability according to the area of the redox peaks. With increasing the hydrophilicity of electrode, the amount of OMCs that can directly interact with the electrode decreases. It means that the EET via oxidized OMCs is not the dominant pathway in the system with a rather low initial OD_{600} value even at +0.2 V, which suggested a different EET pathway comparing with the system of high OD_{600} value.[17] Thus, it is reasonable to propose that the enhanced EET current on superhydrophilic electrode (OH-ITO) proceed mainly via flavins.

To further explore the role of flavins on the hydrophilicity

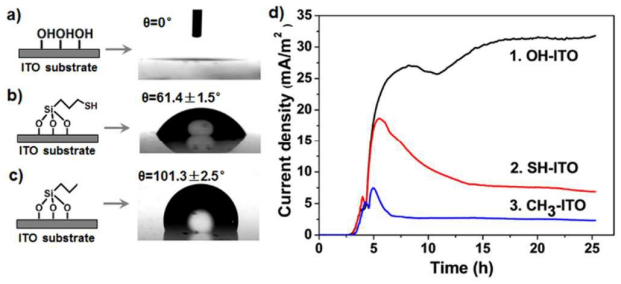

Fig. 1 Schematic illustration for the coated ITO electrodes and profiles of static water contact angle measurements of a) superhydrophilic ITO (OH-ITO), b) hydrophilic ITO (SH-ITO) and c) hydrophobic ITO (CH_3-ITO) electrode, respectively. d) Time courses of EET Current at an applied potential of 0.2 V for *S. loihica* PV-4 with initial OD_{600} of 10^{-3} on the OH-ITO (curve 1), SH-ITO (curve 2) and CH_3-ITO (curve 3), respectively.

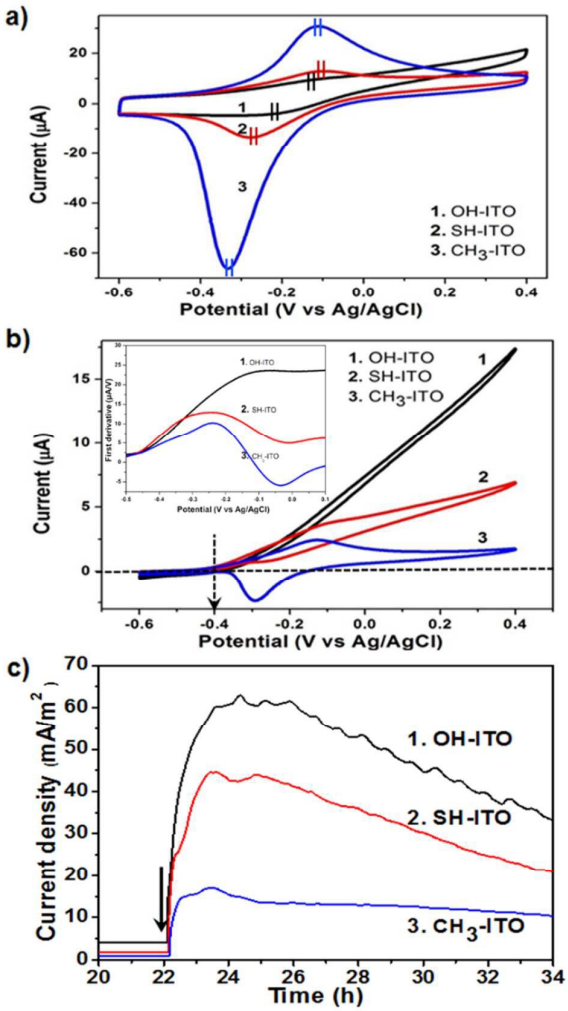

Fig. 2 a) Whole-cell cyclic voltammograms for cell suspension with a scan rate of 50 mV/s taken at after 22.5 h microbial electrical culture on superhydrophilic OH-ITO (curve 1), hydropholic SH-ITO (curve 2) and hydrophobic CH_3-ITO (curve 3) electrode. b) Whole-cell cyclic voltammograms of microbial suspension with a scan rate of 1 mV/s cultured electrochemically at +0.2 V on electrodes with different wettability, the inset shows the first derivative of the according voltammetric curves. c) Current versus time curve in the presence of exogenously added riboflavins at a constant potential of +0.2 V vs. Ag/AgCl (sat. KCl), and the riboflavin (the final concentration of 5.0 μM) was added in the moment indicated by the arrow.

boosted EET activity, CV measurement with a scan rate of 1 mV/s was conducted. which gives the diffusion molecules in the electrolyte enough time to react with the electrode. Thus the CV with such a slow scan rate reflects the flavin-associated slow diffusion process at the microbe/electrode interface.[11,17] As shown in Fig. 2b, the onset potential located at -0.4 V for anodic current on all electrodes reflects the nature of the terminal molecules of flavins.[18] The slopes of CV curves near the onset potential implies the EET activity via flavin-associated process, which tells more efficient electron transfer on the OH-ITO electrode than that on the normal hydrophilic and hydrophobic one. The difference was more clearly visualized by the first derivative of the anodic CV curves (inset of Fig. 2b), where the curve for OH-ITO showed the highest value. When the riboflavins were exogenously added into the system, the EET currents on three electrodes showed immediate increase, but gave different peak values of ca. 63.0 (OH-ITO), 42.0 (SH-ITO) and 16.0 (CH_3-TIO) mA/m^2 (Fig. 2c). It clearly suggested that flavins more actively participate the electron transfer process at microbe/polar-electrode interface, namely on a superhydrophilic electrode, resulting in a more efficient EET process. Control experiments without microbes were conducted, where no current was generated although with the addition of flavins (Fig. S3). This result clearly demonstrated that self-secreted flavins play an important role in EET process for the system with a rather low initial OD_{600} value at an applied potential of 0.2 V, differing from cases where high OD value was used.[17]

To elucidate the flavin-associated EET processes on electrodes with different wettability, differential pulse voltammetry (DPV) measurement was conducted to better monitor the electrochemical activity of redox species in biological system at nanomolar level.[19] As shown in Fig. 3a, DPV curves show two anodic peaks: the more positive one is assignable to the OmcA-MtrCAB complex (a hypothetical protein complex to transport intracellular electrons to outher acceptors)[19] and the negative one is flavins-related peak[11,19]. Baseline-subtracted DPVs for two peaks were analyzed to clarify the electron transfer kinetics between microbes and electrodes. As shown in Fig. 3b, the redox potential (E_p) of flavin-related peak on OH-ITO and SH-ITO electrode are respective -320 mV and -344 mV, by 70 mV and 46 mV positive shift comparing with that on hydrophobic CH_3-ITO electrode (ca. 390 mV). As reported, heme center for flavin binding site is -120 mV (vs. Ag/AgCl),[20] thus, the larger the gap between heme center and flavin, the higher the resistance will take for electron transfer, which will be discussed in the following calculation process. Moreover, the half-width potential ($\Delta E_{p/2}$) was enlarged with enhancing the hydrophilicity of electrode, exhibiting a value of 157 mV on superhydrophilic OH-ITO, 130 mV on hydrophilic SH-ITO and 122 mV on hydrophobic CH_3-ITO. The $\Delta E_{p/2}$ of about 130 mV on SH-ITO electrode indicates that an one-electron transfer reaction process happened for flavins where an intermediate product of semiquinone was formed (oxidized flavin + $H^+ + e^- \rightarrow$ semiquinone).[19] Here, $\Delta E_{p/2}$ was enlarged to 157 mV by increasing the hydrophilicity and deduced to 122 mV by decreasing the hydrophilicity, which indicated that the electron transfer kinetics enlarged and deduced respectively. As reported, reduced OMCs are beneficial to stabilize semiquinones to form flavin-OMC co-factors, leading to a high efficient electron transfer.[11,19] Here, the E_p of OmcA–MtrCAB complex located at different positions on CH_3-ITO, SH-ITO and OH-ITO (Fig. 3c), indicating different ability to stabilize the reduced OMCs.[11] The superhydrophilic OH-ITO electrode gave a comparatively positive value of -164 mV, indicating that the reduced OMCs is more liable to be stabilized on OH-ITO, comparing with that on SH-ITO (-174 mV) and CH_3-ITO (-204 mV). Here, the ability to stabilize the reduced OMCs gets higher on more hydrophilic electrodes, lying in the order of superhydrophilic OH-ITO > hydrophilic SH-ITO > hydrophobic CH_3-ITO electrode, as proved by the positive shift of E_p of OmcA–MtrCAB complex on more hydrophilic substrate.[11] Taken together, it was proposed that the highly boosted EET activity on superhydrophilic OH-ITO electrode is attributable to the existence of more reduced OMCs, which favors the EET via falvin.

Here we examined the electron transfer kinetics and

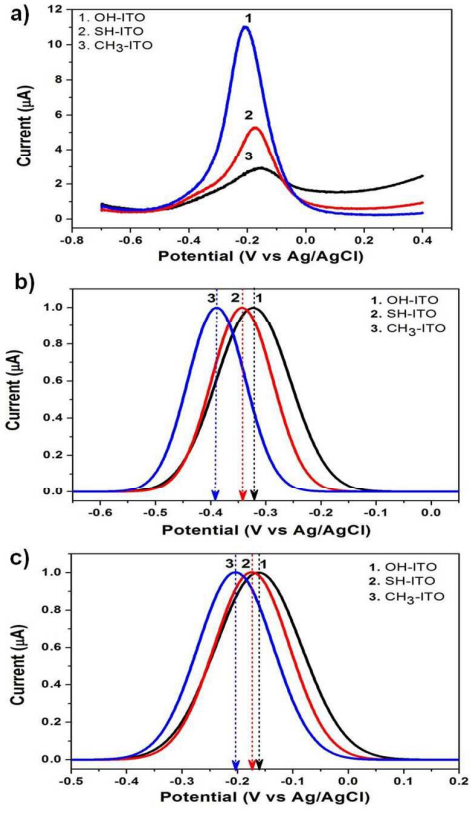

Fig. 3 a) Differential pulse voltammetry for microbes on the surface of OH-ITO (curve 1), SH-ITO (curve 2) and CH_3-ITO (curve 3) electrodes respectively; Baseline-subtracted DPV for b) OMC-flavins cofactors and c) OmcA-MtrCAB complex on OH-ITO (line 1), SH-ITO (line 2) and CH_3-ITO (line 3) electrodes respectively.

compared the generated current density (*I*) between microbes and electrodes with different wettability based on Fig. 3. In our experiment, EET proceed via mediator flavins based on the following processes at microbe-electron interface:[11,19a] (1) reduction of oxidized flavin to semiquinone by OMCs (Oxidized Flavin + H^+ + e^- from OMCs → Semiquinone), by which process the microbial respiratory electron was injected into extracellular oxidized flavins via OMCs; (2) electron transfer from semiquinone to electrode (Semiquinone → Oxidized Flavin + e^- to electrode), which enables electron flow into electrode. In our system, applied potential for electrode is 0.2 V (vs. Ag/AgCl), and the E_p of semiquinone is much lower than that, ca. -0.2 V ~ -0.15 V. Thus, electron flow from semiquinone to electrode is an exergonic reaction. As a consequence, the former process is the rate-determining step. According to arrhenius equation and considering consecutive electrons were injected into heme, so we could only consider the forward ($k_{\vec{i}}$) reaction as shown in equation:

$$k \approx k_{\vec{i}} = k_0 \exp[-\frac{\alpha n F}{RT} \Delta E]$$

Here k_0 is the standard rate constant, n is number of electrons, α represents electron transfer coefficient, R is ideal gas constant, F is faradic constant and ΔE stands for potential discrepancy between flavin and heme. Thus, the ratio of *k* on different electrodes was obtained drawn from E_p for different electrodes (Fig. 3). As mentioned before, heme center for flavin binding site is -120 mV.[20] Here, we assume one-electron transfer process happened on three electrodes, then n=1. For OH-ITO, SH-ITO and CH_3-ITO, E_p of flavin is -320 mV, -344 mV and -390 mV respectively. If $k_{CH3\text{-}ITO}$ is made 1, based on above, $k_{OH\text{-}ITO}$ and $k_{SH\text{-}ITO}$ is obtained. Further, according to the Butler-Volmer equation, Assuming one-electron transfer process happened and *I* generated on CH_3-ITO electrode is 1, that obtained on SH-ITO and OH-ITO is calculated to be about 11.0-18.1 and 3.6-5.1 respectively, in accordance with the result in Fig. 1d (see the detail in supporting information 4).

Considering that the EC system here enables the microbial proliferation, the surface morphology of different electrodes after 28 h of electrochemical cultivation was examined using a field emission scanning electron microscope. As shown in Fig. 4, microbes with uniform size were distributed homogenously on all the electrodes. The Statistic cell density on three electrodes were summarized in Figure S4. As can be seen, cell density per 1000 μm^2 was 292 ± 10, 172 ± 24, 54 ± 16 on OH-ITO, SH-ITO and CH_3-ITO respectively. To be noted, the number of microbes on superhydrophilic OH-ITO was much bigger than that on SH-ITO and CH_3-ITO electrodes. This phenomenon can be explained by the interaction between electrode and microbes. When the microbes are highly connected to the electrode by OMCs, the intracellular redox atmosphere is expected to be oxidative, which is unfavorable for microbial respiration due to their safety protection against oxidative stress.[21] Specific to this work, *S. loihica* PV-4 is highly electrically connected to CH_3-ITO electrode and weakly interacted with superhydrophilic OH-ITO surface (Fig. 2a). In such conditions, polar circumstance of OH- ITO electrode provides beneficial conditions for microbial respiration and proliferation, which further enlarges the wettability-biased effect of *S. loihica* PV-4.

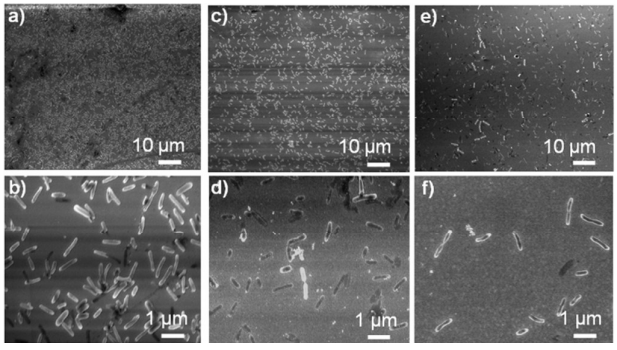

Fig. 4 SEM images of bacterial cells on a,b) superhydrophilic OH-ITO, c,d) hydrophilic SH-ITO and e,f) hydrophobic CH_3-ITO electrodes after 28 h electrochemical culture at +0.2 V. (b, d, f) shows the magnified SEM pictures.

Conclusions

In summary, we have demonstrated that surface wettability largely affects EET process of genus *Shewanella* in growth system. Here cell suspension with rather small initial cell density was utilized to exclude the unknown parameters from complex and dynamic biofilm, and the yeast extract supporting the proliferation of microbes could enlarge the wettability-biased effect of microbes. Specifically, the superhydrophilic electrode allows a larger ratio of reduced OMCs to be stabilized even at 0.2 V comparing with that on common hydrophilic and hydrophobic electrode, which is beneficial to not only highly efficient electron transfer via flavin-OMCs, but also microbial respiration and proliferation. We hope this finding could deepen the understanding of microbe-electrode interface and provide guidance for the design and fabrication of other bioelectrical devices.

Acknowledgements

The authors thank the financial support by National Research Fund for Fundamental Key Projects (2013CB933000), the National Natural Science Foundation of China (21574005, 51503126, 61227902), Program for New Century Excellent Talents in University (NCET-13-0024) and the Fundamental Research Funds for the Central Universities.

Notes and references

1 a) D. R. Lovley, *Energy Environ. Sci.* 2011, **4**, 4896; b) R. Nakamura, K. Ishii, K. Hashimoto, *Angew. Chem. Int. Ed.* 2009, **48**, 1606; c) K. H. Nealson, C. R. Myers, *Appl. Environ. Microbiol.* 1992, **58**, 439; d) M. Epifanio, S. Inguva, M. Kitching, J. P. Mosnier, E. Marsili, *Bioelectrochemistry,* 2015**,** **106**, 186.

2 a) B. E. Logan, *Nat. Rev. Microbiol.* 2009, **7**, 375; b) J. K. Fredrick son, M. F. Romine, A. S. Beliaev, J. M. Auchtung, M. E. Dris-coll, T. S. Gardner, K. H. Nealson, A. L. Osterman, G.

Pinchuk, J. L. Reed, D. A. Rodionov, J. L. M. Rodrigues, D. A. Saffarini, M. H-. Serres, A. M. Spormann, I. B. Zhulin, J. M. Tie-dje, *Nat. Rev. Microbiol.* 2008, **6**, 592; c) D. R. Bond, D. E. Holmes, L. M. Tender, D. R. Lovley, *Science* 2002, **295**, 483.

3 a) C. M. Ding, H. Liu, Y. Zhu, M. X. Wan, L. Jiang, *Energy Environ. Sci.* 2012, **5**, 8517;.b) M. Rosen-baum, F. Zhao, U. Schrçder, F. Scholz, *Angew. Chem. Int. Ed.* 2006, **45**, 6658; *Angew. Chem.* 2006, **118**, 6810.

4 a) M. Breuer, K. M. Rosso, J. Blumberger, *Proc. Natl. Acad. Sci. USA*, 2014, **111**, 611; b) L. Shi, T. C. Squier, J. M. Zachara, J. K. Fredrickson, *Mol. Microbiol.* 2007, **65**, 12; c) A. Okamoto, R. Nakamura, K. Ishii, K. Hashimoto, *ChemBioChem* 2009, **10**, 2329.

5 Y. Zhao, K.Watanabe, R.Nakamura, S. Mori, H. Liu, K. Ishii, K. Hashimoto, *Chem. Eur. J.* 2010, **16**, 4982.

6 a) C. M. Ding, H. Liu, M. L. Lv, T. Y. Zhao, Y. Zhu, L. Jiang, *Nanoscale*, 2014, **6**, 7866; b) Y. C. Yong, Y. Y. Yu, X. Zhang, H. Song, *Angew. Chem. Int. Ed.* 2014, **53**, 4480; c) X. Xie, G. Yu, N. Liu, Z. Bao, C. S. Criddle, Y. Cui, *Energy Environ. Sci.* 2012, **5**, 6862.

7 a) R. Nakamura, F. Kai, A. Okamoto, K. Hashimoto, *J. Mater. Chem. A* 2013, **1**, 5148; b) R. Nakamura, A. Okamoto, N. Tajima, G. J. Newton, F. Kai, T. Takashima, K. Hashimoto, *ChemBioChem* 2010, **11**, 643.

8 a) V. Flexer, J. Chen, B. C. Donose, P. Sherrell, G. G. Wallace, J. Keller, *Energy Environ. Sci.* 2013, **6**, 1291; b) A. Okamoto, K. Hashimoto, K. H. Nealson, *Angew. Chem. Int. Ed.* 2014, **53**, 10988; c) C. Santoro, S. Babanova, K. Artyushkova, J. A. Cornejo, L. Ista, O. Bretschger, E. Marsili, P. Atanassov, A. J. Schuler, *Bioelectrochemistry* 2015, **106**, 141.

9 a) R. P. Swenson, G. D. Krey, *Biochemistry* 1994, **33**, 8505; b) Z. Zhou, R. P. Swenson, *Biochemistry* 1996, **35**, 15980; c) A. El Kasmi, J. M. Wallace, E. F. Bowden, S. M. Binet, R. J. Linderman, *J. Am. Chem. Soc.* 1998, **120**, 225; d) S. Song, R. A. Clark, E. F. Bowden, M. J. Tarlov, *J. Phys. Chem.* 1993, **97**, 6564.

10 a) X. Yuan, S. Sun, F. M. Hawkridge, J. F. Chlebowski, I. Taniguchi, *J. Am. Chem. Soc.* 1990, **112**, 5380; b) H. Liu, S. Matsuda, T. Kawai, K. Hashimoto, S. Nakanishi, *Chem. Commun.* 2011, **47**, 3870.

11 C. M. Ding, M. L. Lv, Y. Zhu, L. Jiang, H. Liu, *Angew. Chem. Int. Ed.* 2015, **54**, 1446.

12 a) D. Sun, S. Cheng, A. Wang, F. Li, B. E. Logan, K. Cen, *Environ. Sci. Technol.* 2015, **49**, 5227; b) K. Artyushkova, J. A. Cornejo, L. K. Ista, S. Babanova, C. Santoro, P. Atanassov, A. J. Schuler, *Biointerphases* 2015, **10**, 019013.

13 a) D. Huang, Z. Xiao, J. Gu, N. Huang, C. Yuan, *Thin Solid Films* 1997, **305**, 110; N. Balachander, C. N. Sukenik, *Langmuir*, 1990, **6**, 1621; Y. Wang, X. Wang, Y. Guo, C. Cui, Q. Lin, W. Yu, L. Liu, L. Xu, D. Zhang, B. Yang *Langmuir* 2004, **20**, 8952.

14 a) A. Okamoto, R. Nakamura, K. Hashimoto, *Electrochim. Acta* 2011, **56**, 5526; b) S. Matsuda, H. Liu, K. Hashimoto, S. Nakanishi, *Electrochemistry* 2012, **80**, 330.

15 M. D. Porter, T. B. Bright, D. L. Allara, C. E. D. Chidsey, *J. Am. Chem. Soc.* 1987, **109**, 3559.

16 S.L. Li, K. H. Nealson, *Front. Microbiol.* 2015, **6**, 111.

17 a) H. Liu, S. Matsuda, S. Kato, K. Hashimoto, S. Nakanishi, *ChemSusChem* 2010, **3**, 1253; b) H. Liu, G. J. Newton, R. Nakamura, K. Hashimoto, S. Nakanishi, *Angew. Chem. Int. Ed.* 2010, **49**, 6596.

18 E. Marsili, D. B. Baron, I. D. Shikhare, D. Coursolle, J. A. Gralnick, D. R. Bond, *Proc. Natl. Acad. Sci. U. S. A.* 2008, **105**, 3968.

19 a) A. Okamoto, K. Hashimoto, K. H. Nealson, R. Nakamura, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 7856; b) A. Okamoto, K. Saito, K. Inoue, K. H. Nealson, K. Hashimoto, R. Nakamura, *Energy Environ. Sci.* 2014, **7**, 1357.

20 M. Breuer, P. Zarzycki, J. Blumberger, K. M. Rosso, *J. Am. Chem. Soc.* 2012, **134**, 9868

21 a) S. Matsuda, H. Liu, A. Kouzuma, K. Watanabe, K. Hashimoto, S. Nakanishi, *PLoS ONE* 2013, **8**, e72901; b) A. Sucheta, B. A. C. Ackrell, B. Cochran, F. A. Armstrong, *Nature* 1992, **356**, 361; c) J. Hirst, A. Sucheta, B. A. C. Ackrell, F. A. Armstrong, *J. Am. Chem. Soc.* 1996, **118**, 5031.

By using system with a rather small initial OD and favoring for cell proliferation, we demonstrate that the superhydrophilic electrode enables the drastical boosted bacterial EET activity of *Shewanella loihica* PV-4: the EET current on superhydrophilic electrode is over 3 and 10 times higher than that on normal hydrophilic and hydrophobic electrode, respectively. It is proposed that the hydrophilic electrode favors reduced state of OMCs, and consequently both the EET activity and cell proliferation are highly facilitated.