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Encapsulation of living bioelectrode by hydrogel for bioelectrochemical systems in alkaline media

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The electroactive biofilms in the bioelectrodes of traditional bioelectrochemical systems (BES) are in direct interact with the aqueous solution and usually require a mild aqueous solution environment (e.g. 20 mM acetate and pH=7.0) to exert their optimum bioelectrocatalytic activity. In this communication, we present a concept of encapsulation of bioelectrode by hydrogel for BES in alkaline solution environment. A hydrogel-bioelectrode (*HBE*) was prepared by encapsulating living electroactive biofilm pre-grown in the bioelectrode with a poly(vinyl alcohol) hydrogel through a freezing/thawing process. Under the protection of the hydrogel, the *HBE* could keep a high bioelectrocatalytic activity in an alkaline feeding solution with acetate concentration over 80 mM and pH value of 11.0. Moreover it was very stable and tolerated low-frequency ultrasonic vibration. These results imply the extended applications of BES in the area of high-strength wastewater treatment, portable and implantable devices.

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Bioelectrochemical systems (BES) is one of recently developed technologies that use bacteria to catalyze different electrochemical reactions for diverse purposes.[1, 2] The key members of the bacteria mainly are electroactive bacteria such as *Geobacter* or *Shewanella* that are often called electricigens or exoelectrogens.[3-5] These bacteria usually grow on electrodes as form of biofilms (named as bioelectrode) to donate electrons. The BES and their-related technologies are promising approaches for capturing the energy from wastes [2, 6, 7] and have been proved to show potential applications in biosensors,[8] wastewater treatment,[9] bioremediation,[10, 11] desalination,[12] hydrogen production,[13] biosynthesis[14] etc. Generally, in order to make the electroactive biofilms keep a fast metabolism and thus exhibit a good bioelectrocatalytic activity, the BES usually operate in mild aqueous solution conditions, such as neutral pH and low concentration of substrate (e.g. 20 mM acetate). Due to the good fluidity and ion mobility of the aqueous solution, the change of substrate concentration and pH value, hydraulic disturbances and external vibration show a great impact on the bioelectrocatalytic activity of the bioelectrode.[15, 16] Over-high concentration of substrate, too high or low pH values etc., would greatly inhibit the metabolism of the electroactive biofilms;[17, 18] the hydraulic perturbation from the variation of feeding model and rate, external vibration and shake etc., would break the as-formed biofilm structure,[19] change or cut off the pathways of electron and mass transfer inside the biofilms, thus would greatly reduce the bioelectrocatalytic activity of the bioelectrodes. Therefore, the practical applications of the BES were limited in the mild environment. Alleviation or removal of these impacts on the bioelectrode are of great significance for the further development of the BES, such as high-strength wastewater[17, 20], portable and implantable devices.

Hydrogel is a highly absorbent network of natural or synthetic polymer chains, e.g. poly(vinyl alcohol) (PVA) [21] and Chitosan.[22] It possess a degree of flexibility very similar to natural tissue, due to their significant water content. Herein, we present a concept of encapsulation of bioelectrode by hydrogel for BES in alkaline solution environment. A hydrogel-bioelectrode (denoted as *HBE*) was fabricated by encapsulating living electroactive biofilms, which are pre-grown on the bioelectrode,

with a PVA hydrogel through a freezing/thawing process. Under the protection the hydrogel, the *HBE* could keep a high bioelectrocatalytic activity in an alkaline aqueous solution with acetate concentration over 80 mM and pH value of 11.0, and tolerate low-frequency ultrasonic vibration.

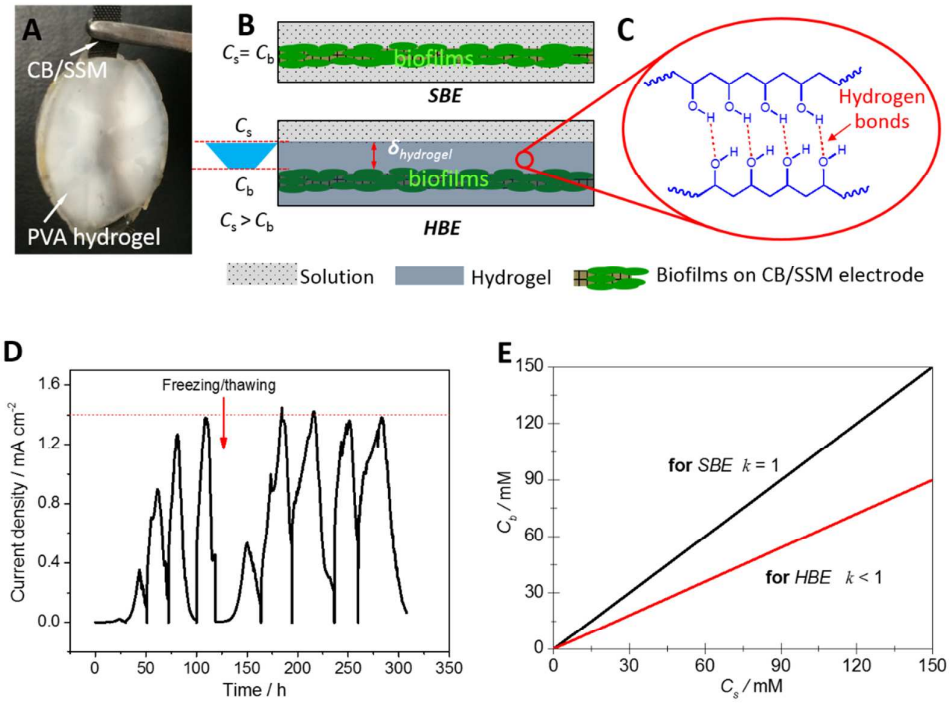

Fig. 1. (A) Digital picture of a hydrogel-bioelectrode (*HBE*). (B) Schematic diagrams of the solution-bioelectrode (*SBE*) and *HBE*. $\boldsymbol{C}_s$ and $\boldsymbol{C}_b$, are the concentration of acetate (or hydroxyl ion) at the solution/hydrogel interface and biofilm surface, respectively. (C) Macromolecular formula of PVA hydrogel cross-linked by freezing/thawing method. (D) Comparison of electrocatalytic activity of electroactive biofilms grown on a CB/SSM anode before and after freezing/thawing process, the medium is 100 mM PBS with 20 mM acetate and pH=7.0. (E) Curves demonstrating the relationships between the $\boldsymbol{C}_s$ and $\boldsymbol{C}_b$ in the *SBE* and *HBE*, respectively.

The digital picture of a *HBE* was shown in Fig. 1A, its schematic diagram was shown in Fig. 1B, and the fabricating process of the *HBE* was illustrated in Fig. S1. The key to the fabrication of the *HBE* is the selection of hydrogel material and crosslinking method, which must at least meet the following two requirements: (a) the hydrogel is biocompatible and its preparation process is harmless to bacteria; (b) the hydrogel has high ion mobility to ensure a high rate of ion diffusion and transfer. Due

to its good biocompatibility,[23] poly(vinyl alcohol) (PVA) was chosen as the hydrogel material to make the *HBE*. The PVA hydrogels prepared by chemical and irradiative cross-linking are not suitable for the *HBE*, because the cross-linking agents (e.g. dialdehyde) and the irradiation (e.g., γ-irradiation) respectively are usually harmful to the bacteria. Physical cross-linking method, freezing/thawing process, is a good way to prepare the PVA hydrogel for the *HBE*, because it does not need any cross-linking agents and irradiation, thus is non-toxic to the bacteria. A carbon black/stainless steel mesh (CB/SSM) electrode, reported in one of our recent works,[24] was used as the electrode to grow electroactive biofilms. Fig. S2 showed that thick biofilm with thickness of over 10 μm could be grown in the CB/SSM electrode. Electrochemical test results in Fig. 1D revealed that the freezing/thawing process showed almost no negative influence on the electrocatalytic activity of the electroactive biofilms. This result demonstrated that the freezing/thawing method is well suitable for the fabrication of the *HBE*. The crosslinking of PVA through the freezing/thawing method was due to the formation of PVA crystallite under the assistance of hydrogen bonds (as shown in Fig. 1C).[21, 25] For this reason, PVA with a high degree of alcohlysis (e.g. >98%) is usually applied to obtain high-density hydrogen bonds and a stable PVA hydrogel. The PVA (Aladdin, Mw=80,000, degree of alcohlysis >98%) hydrogel prepared by freezing/thawing a 10wt% PVA solution had a high swelling capacity of 500% in distilled water, demonstrating that the PVA have a high ion mobility. The diffusion coefficient of acetate, proton and hydroxide ion in PVA hydrogel prepared from 10% PVA aqueous solution are 2.53×10^{-6}, 6.75×10^{-6}, and 3.51×10^{-6} cm^2/s (table S2.) , respectively, compared to those in aqueous solution in the level of 2×10^{-5} cm^2/s.

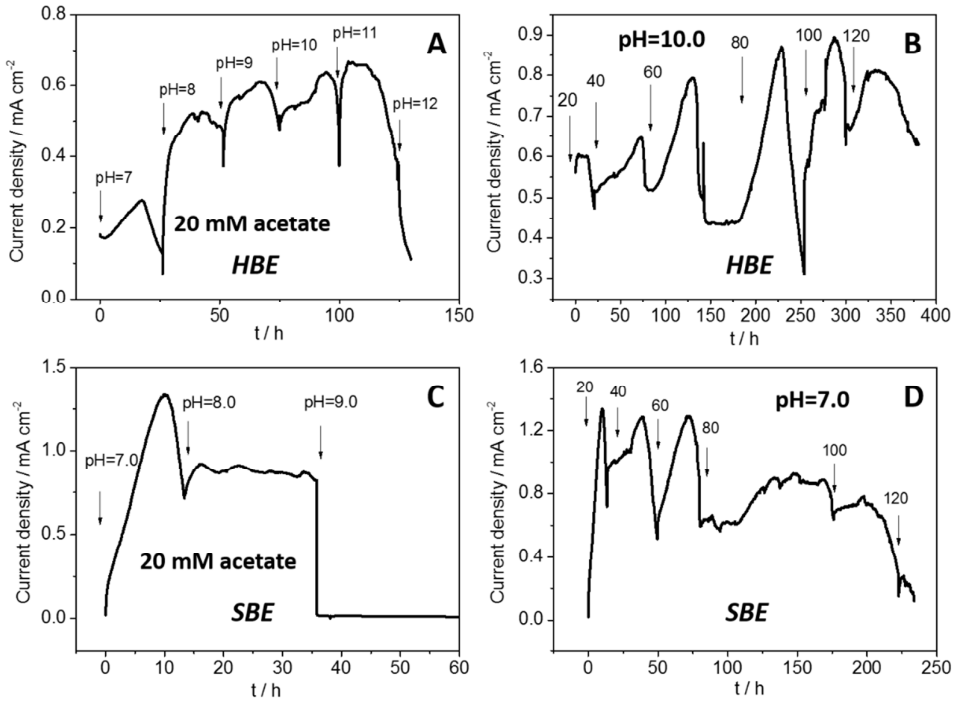

Fig. 2. Electrocatalytic activity of electroactive biofilms in *SBE* and *HBE*. (A) *HBE* in PBS with 20 mM acetate at different pH values; (B) *HBE* in PBS at pH = 10.0 with 20, 40, 60, 80, 100 and 120 mM acetate, respectively; (C) *SBE* in PBS with 20 mM acetate at different pH values; (D) *SBE* in PBS at pH = 7.0 with 20, 40, 60, 80, 100 and 120 mM acetate, respectively. The concentration of PBS is 100 mM. Arrows represent the time of refreshing the media with corresponding solution.

As shown in Fig. 1D, the *SBE* (bioelectrode without hydrogel), in which the biofilms directly contacted the aqueous solution, could generate a maximum current density of ~1.33 mA cm^{-2} in the commonly used mild aqueous solution condition (100 mM phosphate buffer solution (PBS) with 20 mM acetate at pH=7.0). The *HBE*, in which the biofilms were encapsulated by a layer of PVA hydrogel, only could deliver current density of 0.28 mA cm^{-2} in the similar solution condition (Fig. 2A). The lower current density in the *HBE* could be attributed to the relatively lower diffusion coefficient of the PVA hydrogel comparing to the solution, which resulted from the block of the cross-linked PVA macromolecular chains. A diffusional concentration gradient usually existed in the hydrogel layer of the *HBE* and resulted in lower concentration of acetate at the biofilm surface than at the solution/hydrogel interface,

thus the *HBE* generated lower current density than the *SBE* did under the popularly used mild solution condition. However, under the protection of the PVA hydrogel, the current density of the *HBE* could greatly increase with increase of acetate concentration and pH value in the feeding solution. Fig. 2A showed that, in the 100 mM PBS with 20 mM acetate, the current density of the *HBE* increases from 0.28 to 0.67 mA cm^{-2} with increase of pH value from 7.0 to 11.0. Further increase of the pH value to 12.0 leads to the reduction of the current density to nearly zero, because the pH in the biofilms would also increase, which greatly slowed down the metabolism of the biofilms, even killed them. Similarly, as shown in Fig. 2B, control the pH value of the PBS at 10.0, the current densities of the *HBE* rise from 0.67 to 0.92 mA cm^{-2} with the increase of acetate concentration from 20 mM to 100 mM. While in the *SBE,* the current density decreases with increase of acetate concentration in the feeding solution. As shown in Fig. 2C and D, when the acetate concentration increases to 80 mM, the current density of the *SBE* decreases by 30% to about 0.92 mA cm^{-2}; when the pH increases to 9.0, the biofilms current density becomes zero. These results demonstrated that *HBE* could keep high bioelectrocatalytic activity at aqueous solutions with acetate concentration of 100 mM and pH value of 11.0.

When the HBE achieved a stable current generation, the rate of acetate and hydroxyl ion diffused through the hydrogel was equal to their rates consumed by the biofilms in the bioelectrode. An equation which described the relationship between the acetate (or hydroxyl ion) concentration in the solution ($\boldsymbol{C}_s$, mM) and at the biofilm surface ($\boldsymbol{C}_b$, mM) was deduced as (see the supplementary information for the detailed derivation),

$$C_b = \frac{D_d C_s}{D_d + \delta_{hydrogel} B} \quad (1)$$

where, $\delta_{hydrogel}$ is the thickness of hydrogel (mm), D_d (cm^2/s) is the diffusive coefficient of acetate (or hydroxyl ion) in the PVA hydrogel, and B is the consuming rate of acetate in the biofilm. B could be considered as a constant because the thickness of biofilms and the acetate consuming coefficient (D_c, cm^2/s) of the biofilms was invariable in the *HBE* under the setting solution condition. Eq. (1) obviously

revealed that C_b is linearly proportional to C_s, as shown in Fig. 1E. The slope (k) of the line was expressed as,

$$k = \frac{D_d}{D_d + \delta_{hydrogel} B} \quad (2)$$

The k was related to D_d and $\delta_{hydrogel}$; for the *SBE*, $k = 1$; while for the *HBE*, $k < 1$. Based on the experimental results, the *HBE* could keep the high bioelectrocatalytic activity at aqueous solution with high acetate concentration of 100 mM and high pH value of 11.0. The possible reasons are, in one aspect, according to the eq. (1), the C_b was lower than the C_s in the *HBE*, thus the pH and substrate concentration in the HBE could be higher than the *SBE*; in another aspect, the swelling capacity of the PVA hydrogel decreased with rising pH, as shown in table S3, and resulted in the decrease of diffusive coefficient D_d.

The improvement of current density of the HBE with increase of acetate concentration and pH value in the feeding solution under the protection of the PVA hydrogel could be explained as follow. A proper solution condition for the biofilms in SBE was 20 mM and pH=7.0, under which the biofilm could show high bioelectrocatalytic activity and generate a high current density (Fig. 1D). Eq.(1) showed that the concentration of substrate and hydroxyl ion at biofilms surface (C_b) is linearly with the concentration of substrate and hydroxyl ion in feeding solution (C_s). The slope k was smaller than 1 and depended on the hydrogel thickness, thus the C_b was smaller than the C_s and led lower biocatalytic activity due to the insufficient substrate supply and low migration of the protons. Increase of the acetate concentration and pH value in the feeding solution (C_s), the concentration of substrate and hydroxyl ion at biofilms surface (C_b) was increased correspondingly.

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (3)$$

According to the chemical reaction (metabolism) inside the bacteria in eq. (3), the metabolism of the bactera could speed up with increase of C_b, thus greatly improved their bioelectrocatalytic activity in the *HBE*. But, the final C_b at the biofilm surface must not be over the limitation that the living biofilms could bear.

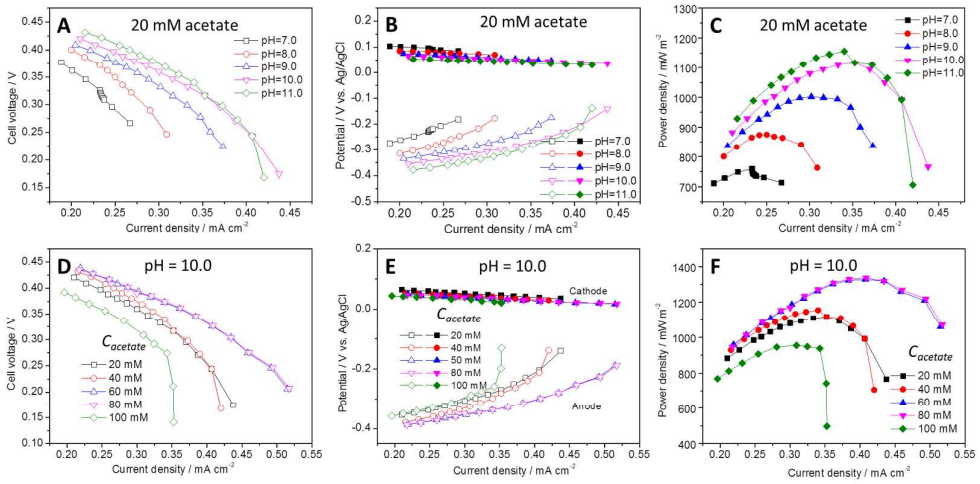

Fig. 3. (A) Cell polarization, (B) anode and cathode polarization, and (C) power density curves of the MFC equipped with *HBE* in 50 mM PBS with 20 mM acetate at different pH values. (D) Cell polarization, (E) anode and cathode polarization, and (F) power density curves of the MFCs equipped with *HBE* in 50 mM PBS with different acetate concentration at pH = 10.0.

The performance of MFC equipped with *HBE* anode under feeding solutions with higher pH value and higher acetate concentration were also studied (Fig. 3). The polarization curves in Fig. 3A and 3B revealed that the pH value change showed little effect on the cathode potential, hinting that the power density improvement of the MFC was mainly provided by the *HBE* anodes. As shown in Fig. **3**C, with acetate concentration at 20 mM and raising the pH value of solution from 7.0 to 11.0, the maximum power density of the MFCs increases from 759 to 1120 mW m^{-2}. Similarly, the polarization curves in Fig. 3D and 3E revealed that the effect of the substrate concentration change on the cathodic potential was also could be neglected. Fig. 3F showed that the maximum power density of the MFCs increases from 1116 to 1350 mW m^{-2} with substrate concentration rising from 20 mM to 80 mM at pH value of 11.0. However, further increase of the substrate concentration to 100 mM leads to the decrease of maximum power density to 955 mW m^{-2}. The maximum power density generated from the MFC with *HBE* anode in PBS medium with 80 mM acetate and pH =10.0 was higher than the MFC with *SBE* anode (1271 mW m^{-2}) in PBS medium with popularly used condition (with 20 mM acetate, pH=7.0) (Fig. S3).

The MFC performance demonstrated that the *HBE* could deliver high maximum power density under the alkaline solution with acetate concentration of 80 mM and pH values of 11.0, which were in accordance to the bioelectrochemical test results above.

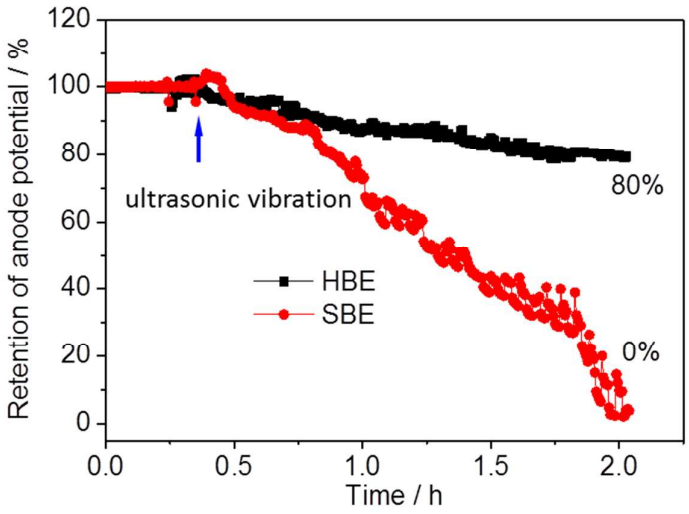

Fig. 4. Retention of potential at SBE and HBE under ultrasonic vibration. Feeding solution is 100 mM PBS with acetate concentration of 40 mM at pH = 10.0.

Under the protection of hydrogel, *HBE* could work not only in solution with high concentration of acetate and pH value, but also under ultrasonic vibration environment. MFCs equipped with *SBE* and *HBE*, respectively, were put in a low-frequency ultrasonic instrument (KQ-100VDE, Ultrasonic Instrument Co. Ltd of Kunshan, China, 100W, 28.45 kHz). As shown in **Fig. 4**, after ultrasonic vibration for about 2 h, the electroactive biofilms in the *SBE* was destroyed, resulting in a nearly zero anode potential retention. While the *HBE* still had anode potential retention of 80% under the protection of the hydrogel layer. The physical junction of the crystal structure in the PVA hydrogel play the role of immobilization of the biofilm in the bioelectrode and attain a continuous electron transfer pass way between biofilms and electrode. Thus, the HBE could tolerate the low-frequency ultrasonic vibration. This result demonstrated that the encapsulation of the hydrogel in the *HBE* could protect the electroactive biofilms from being broken by external force, such as ultrasonic

vibration. It also could tolerate the hydraulic perturbation from the variation of feeding model and rate, external shake etc..

In summary, we have fabricated a *HBE* by encapsulating living electroactive biofilm pre-grown in a bioelectrode with a PVA hydrogel through a freezing/thawing method for BES in harsh environment. Under protection of the hydrogel layer, the *HBE* kept a high bioelectrocatalytic activity in alkaline solution with high acetate concentration (> 80 mM) and high pH value (=11.0), and good tolerance to low-frequency ultrasonic vibration. Our results imply the extended applications of BES in the areas of high-strength wastewater treatment, portable and implantable devices.

Materials and methods

Fabrication of hydrogel bioelectrode

PVA (Aladdin, Mw=80,000) with alcoholysis degree of over 98% was used to prepare the PVA aqueous solutions. In a typical process, 10 g PVA was dissolved in 50 mM PBS (pH=7.0) with 20 mM acetate, then heated to 80 ^{o}C under continuous mechanically stirred for 6 h to form a uniform and transparent solution. The process for the fabrication of *HBE* was shown in Fig.S1. To fabricate the *HBE*, a bioelectrode pre-grwoen with stable electroactive biofilms was required. Preselected mixture bacterial cultures based on primary electroactive biofilms were used. [26, 27] The bacterial source for the primary biofilm formation was primary wastewater from the local wastewater treatment plants QingShan (Nanchang, China). The biofilms growth process was controlled by a potentiostat (CHI660D) using three-electrode system in a cubic cell with cylinder chamber (φ=3 cm) (Fig. S4A). CB/SSM (size of 1×3 cm) was used as working electrode (WE), graphite plate with size of 2×2 cm^2 was used as the counter electrode (CE), Ag/AgCl (sat. KCl, +0.195 V vs. standard hydrogen electrode, Tianjin Aida Electronic Co., Ltd, China) served as reference electrode (RE). A potential of +0.2 V (vs. Ag/AgCl) was applied on the CB/SSM working electrode, and recorded the current generation. Aqueous solution containing 100 mM PBS, 20 mM

acetate substrate, trace metal and vitamin solution (see the supplementary information for the detailed composition) was used as the medium. The biofilm growth process was conducted at a temperature of 35 ^{o}C, the medium was refreshed every 24 hrs. Stable current generation was achieved after several circle, demonstrating that a stable bioelectrode was formed. Then, removed the medium, the CE and RE, and added the 10 wt% PVA solution to the chamber slowly. The cell was underwent a freezing/thawing process to form *HBE*. The freezing/thawing process was conducted by freezing the MFC at -20 ^{o}C for 24h and thawing at 25 ^{o}C. The thickness of the PVA solution onto the WE was controlled as 1 mm, which was also equal to the final thickness of the hydrogel on the WE. The bioelectrochemical tests of the *HBE* were also controlled by a potentiostat using three-electrode system in a cubic cell with cylinder chamber. The condition for the bioelectrochemical tests was same to the biofilm growth process experiments.

MFC performance tests

Air-cathode single-chamber MFC configuration was chosen to test the *HBE* in MFC. The air-cathode with activated carbon as oxygen reduction catalyst, polytetrafluoroethylene (PTFE) as binder, and CB as conducting additive, was assembly using rolling method following previous work [28], as described detailed in supplementary information. The distance between the anode and the cathode was set at 4 cm. A constant resistor with resistance of 1000 Ω was loaded between the anode and the cathode. The potentials of anode, cathode, and the voltage across the resistor were recorded. Anodic, cathodic, cell polarization and power density curves were measured by changing the external resistance from 100,000 to 50 Ω.

Acknowledgements

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