Bioanodes/biocathodes formed at optimal potentials enhance subsequent pentachlorophenol degradation and power generation from microbial fuel cells

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A B S T R A C T
Bioanodes formed at an optimal potential of 200 mV vs. SHE and biocathodes developed at −300 mV vs. SHE in bioelectrochemical cells (BECs) enhanced the subsequent performances of microbial fuel cells (MFCs) compared to the un-treated controls. While the startup times were reduced to 320 h (bioanodes) and 420–440 h (biocathodes), PCP degradation rates were improved by 28.5% (bioanodes) and 21.5% (biocathodes), and power production by 41.7% (bioanodes) and 44% (biocathodes). Accordingly, there were less accumulated products of PCP de-chlorination in the biocathodes whereas PCP in the bioanodes was more efficiently de-chlorinated, resulting in the formation of a new product of 3,4,5-trichlorophenol (24.3 ± 2.2 μM at 96 h). Charges were diverted to more generation of electricity in the bioanodes at 200 mV while oxygen in the biocathodes at –300 mV acted as a primary electron acceptor. Dominant bacteria known as recalcitrant organic degraders and/or exoelectrogens/electrotrophs included Desulfovibrionon carbinoliphilus and Dechlorospirillum sp. on the bioanodes at 200 mV, and Desulfovibrio marinaeaechensis, Comamonas testosteroni and Comamonas sp. on the biocathodes at −300 mV. These results demonstrated that an optimal potential was a feasible approach for developing both bioanodes and biocathodes for efficient PCP degradation and power generation from MFCs.

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1. Introduction

A microbial fuel cell (MFC) is a device that uses microbes to convert the chemical energy stored in organic and inorganic compounds into electricity, providing a low-cost and low-maintenance reactor as well as a process that produces very little sludge [1]. While a wide range of easily degradable organics can be utilized in an MFC [2], there is now great interest in using the process for bioremediation of aquatic sediments and groundwater recalcitrant organics [3–5]. Although recalcitrant pentachlorophenol (PCP) has been illustrated to be degraded in both the bioanodes and the biocathodes of MFCs [6–8], the startup time was very long and PCP degradation was still slow. Exploring efficient strategies for faster startup and more quick PCP degradation is in great need.

Setting the anode/cathode potential to a fixed voltage using a potentiostat, known as bioelectrochemical cells (BECs), can exert effects on system performance, bacterial growth and/or composition of microbial consortia [9–20]. Bioanodes formed at a more positive potential than −0.265 V (versus standard hydrogen electrode, vs. SHE) can overcome the subsequent power overshoot due to the sufficient electron transfer components to shuttle electrons at rates needed for these more positive potentials [14]. Applying a potential of 0.04 V (vs. SHE) to an established bioanode developed at open circuit conditions can make the biofilms thinner and more heterogeneous [13]. The positive effects of setting the anode potential can be additionally reflected by pure electroactive organisms, which exhibited increasing growth with the increase of the applied anode potentials from 0.095 V to 0.595 V (vs. SHE) (Shewanella putrefaciens) [12], −0.16 V to 0 V (vs. SHE) (Geobacter sulfurreducens) [10], and 0.27 V to 0.32 V (vs. SHE) (Pseudomonas putida F1) [20]. In the case of biocathode, oxygen reducing biofilms formed under −0.305 V (vs. SHE) revealed better electrochemical characteristics than those developed at open circuit conditions [16]. A set potential of −0.005 V (vs. SHE) can also efficiently develop oxygen reducing biocathode with much higher current generation than the pure isolates extracted inside [15]. In view of long-term operation, however, a potential of 0.301 V instead of 0.441 V and 0.141 V (vs. SHE) was regarded optimal for the performance of oxygen reducing biofilms [19]. While the use of set potential for efficient performance of bioanodes and biocathodes has received a great deal of attention [9–20], only a limited number of reports have been published about the application of set potential to improve the degradation of...
recalcitrant organics [5,17,20]. In linkage with PCP degradation in the bioanodes [6] and biocathodes [8], in which there was still long biofilms acclimation time, slow PCP degradation and low power generation, optimal set potentials are expected to form efficient bioanodes and biocathodes with enhanced system performances for PCP degradation in MFCs.

In this study, several different potentials (−200, 0, 200, and 300 mV vs. SHE) were chosen to improve start-up time of the bioanodes. In the biocathode MFCs, potentials of −500, −300, −100 and 100 mV (vs. SHE) were used to develop the electrotransgenic biofilms. The bioanodes and biocathodes formed in BEC modes were then switched to MFC modes and compared to the controls which were operated at an external resistance of 500 Ω (bioanode) and 200 Ω (biocathode). System performances were evaluated in terms of PCP degradation rate and degradation pathway power, production, bacterial morphology, biocatalytic activity, and composition of microbial consortia. These results provide for the first time an approach for improving the performance of both bioanodes and biocathodes for PCP degradation and power generation from MFCs.

2. Material and methods

2.1. Reactor set up

Traditional H-type two-chamber reactors [6] were used here with set potentials on the bioanodes and hexacyanoferate (50 mM) in the cathodes. The same quantitative and porous graphite felt with a geometric surface area of 9600 m²/m³, PANEX33 160 K, ZOLTEK) and porous graphite felt (a geometric surface area of 292 cm² and an estimated specific surface area of 2000 m²/m³ (Sanye Co., Beijing, China)) [21] were used in each compartment. A cation exchange membrane (CEM) (CMI-7000 Membranes International, Glen Rock, NJ) (2.5 cm in diameter) was used to separate the electrodes [6]. Before installation, the material of both anode and cathode was repeatedly treated with HCl and NaOH to clean the surface [10]. The net working volume of each chamber was 100 mL. Four sets of bioanodes in the BECs were operated at potentials of −200 mV, 0 mV, 200 mV and 300 mV (vs. SHE) while one set of bioanodes in the MFCs were run at a fixed resistance of 500 Ω (bioanode controls) (triplicate reactors). After the bioanodes developed at various potentials in the BECs were finished, each of the bioanodes was shifted to MFC mode with the same resistance of 500 Ω for further assessment of system performance.

A tubular two-chamber reactor [8] was used here with set potentials on the biocathodes. Graphite fiber (a geometric surface area of 38 cm² and an estimated specific surface area of 9600 m²/m³, PANEX33 160 K, ZOLTEK) and porous graphite felt (a geometric surface area of 292 cm² and an estimated specific surface area of 2000 m²/m³, Sanye Co., Beijing, China) [21] were used as the anode and the cathode, respectively, producing a net working volume of 43 mL in the anode chamber and 85 mL in the cathode chamber. A CEM (4.0 cm in diameter and 4 cm in height) similar to that in an H-type reactor was employed to separate the anode and cathode of the tubular reactor [8]. These materials were also treated before installation [10]. Four sets of bioanodes in the BECs were operated at potentials of −500 mV, −300 mV, −100 mV and 100 mV (vs. SHE) while one set of bioanodes in the MFCs were operated at a fixed resistance of 200 Ω (biocathode controls) (triplicate reactors). When the biocathodes formed at various potentials in the BECs were finished, each of the biocathodes was shifted to MFC mode with the same resistance of 200 Ω for further assessment of system performance.

The cathode, anode and reference electrode (Ag/AgCl electrode, 195 mV vs. SHE) in both H-type and tubular reactors were connected to the three-electrode system of a potentiostat (Leici, Shanghai, China) which was used to set potentials for both the bioanode and the biocathode BECs, as well as simultaneously collect circuit current. All of the reactors were wrapped in aluminum foil to exclude light. All the anodes and cathodes were filled with NaH2PO4-Na2HPO4 buffer (pH 7.0) and operated at room temperature (22 ± 3 °C).

2.2. Inoculation and operation

Domestic wastewater with an optical density at the wavelength λ = 600 nm (OD600) of 0.5 ± 0.07 was collected from the primary sedimentation tank of the Lingshi Wastewater Treatment Plant in Dalian, China. This wastewater was used as bacterial inoculum to be combined with an equivalent volume of nutrient solution which is described in the Supplementary materials (SM). This mixed culture was then filled in the anodes and cathodes in tubular MFCs, as well as the anodes in H-type reactors. Anode and cathode chambers in tubular MFCs shared the same nutrient medium except for the addition of glucose (equivalent to 24.4 mM COD) in the anode and PCP (86 μM) in the cathode. In the case of H-type reactors, the same nutrient solution and glucose together with PCP (75 μM) was employed in the anode while hexacyanoferate (50 mM) was used as the catholyte. The anolyte in both the H-type and tubular reactors was sparged with ultrapure N2 gas for 15 min and the headspace was filled with ultrapure N2. In order to create an anoxic biocathode which was beneficial for PCP mineralization [7,8], the cathodic headspace was filled with mixed gases composed of N2 and O2 (60:40). In order to compare the performances of reactors set at potentials and MFC condition (no set potential), both H-type and tubular BECs started up at various potentials were switched to a fixed resistance (500 Ω for H-type reactors and 200 Ω for tubular reactors) at the end of the acclimation period and examined for PCP degradation, maximum power production by obtaining polarization curves, PCP degradation pathway, as well as charge distribution (CD%). The catholyte and anolyte in both H-type and tubular MFCs were refreshed once every cycle of 96 h. The reactors were sealed to avoid gas losses and operated in fed-batch mode. All of the inoculation and solution replacements were performed in an anaerobic glove box (YQX-II, Xinniao, Shanghai).

2.3. Measurements

PCP and glucose metabolized products (minor organic acids) were analyzed using a high performance liquid chromatography (HPLC Agilent 1100). PCP intermediates, formed as necessary stages in PCP degradation pathways [6–8], were quantitatively determined by HPLC after a qualitative analysis using an atmospheric-pressure chemical ionization (API) (−) ion trap mass spectrometer coupled to an LC (Agilent HPLC-MS/MS6410) [6]. Hydrogen and oxygen in the headspace, and dissolved oxygen in the biocathodes were analyzed as described in the SM. Biomass and glucose in the biocathodes were determined as described [22]. Bacterial morphologies were observed by scanning electron microscope (Hitachi, S570, Japan). Bacterial community was analyzed using a polymerase chain reaction and denaturing gradient gel electrophoresis technology as previously described [5,7].

The circuit current (I/mA) in the BECs was obtained from the three-electrode system of a potentiostat (Leici, Shanghai, China). The voltage (U/V) in MFC mode was monitored using a multimeter, which was managed by a computer board card (Leici, Shanghai, China) and controlled by a program in Visual Basic. Current (I/A) was calculated as voltage (U/V) divided by the external resistance (R/Ω) whereas power (P/W) was obtained through voltage (U/V) multiplied by current (I/A). Power volume density (P/vol/W m⁻³) was defined as power (P/W) divided by the working volume (Vm³) of the anode (H-type) or cathode (tubular reactor) whereas current volume density (I/vol/A m⁻³) was calculated as current (I/A) divided by the working volume (Vm³) of the anode (H-type) or cathode (tubular reactor). Power and current area densities were also often normalized by the surface area of electrode or membrane (separator) in the case of two chamber systems. In the reactors used here, the surface area of both the anode and the cathode is greatly in excess of that of the separator (400 times larger in H-type and 34 times larger in tubular reactors), and the separator is known to
limit power production in two chamber MFCs in general [1,23,24]. Therefore, power area density (P/W m^2) and current area density (I/A m^-2) were defined as power (P/W) and current (I/A) divided by the area (S/m^2) of separator in the anode (H-type) or cathode (tubular reactor), respectively [1,23,24].

CD, one useful indicator reflecting the fate of the electrons in both the bioanode and the biocathode MFCs, was similarly calculated as previously reported [6–8]. Briefly, the initial total charges (Q_T/C) in the bioanodes are expressed as Eq. (1):

\[ Q_T = n_b x F \]  

(1)

where \( n_b \) is the number of moles of initial co-substrate of glucose, \( x_b \) is the moles of electrons per mole of glucose, and \( F \) is Faraday’s constant. As glucose is degraded, the electrons can be distributed to electricity (\( Q_e \)), intermediates (\( Q_i \)), biomass (\( Q_b \)), PCP de-chlorination products (\( Q_p \)), remaining substrate (\( Q_s \)) and other unknown processes (\( Q_l \)) [6]. Since a complete de-chlorination of 20 mg L^-1 PCP in 100 mL anodic solution theoretically only consumes 7.2 charges, which is much less than the theoretical initial 941.3 charges provided by the glucose (780 mg L^-1 as COD), \( Q_p \) is thus neglected and \( Q_T \) is simply expressed as Eq. (2):

\[ Q_T = Q_i + Q_e + Q_b + Q_s + Q_l. \]  

(2)

CDs for intermediates, electrons, biomass, remaining glucose, and the lost and unknown processes are then calculated as the ratio of the corresponding total charge recoveries relative to the initial charges content (\( Q_T \)).

In the case of biocathodes, the total charges transferred from the anode electrode (\( Q_T \)) are calculated using Eq. (3):

\[ Q_T = \int_0^t I dt. \]  

(3)
where current I (A) is the ratio of voltage output (U/V) at operational time t(s), and the external resistance of R (Ω). During PCP degradation, these electrons can be distributed to PCP de-chlorination (Q_paper), oxygen reduction (Q_o) and the lost and unknown processes (Q_L) \[8\]. Therefore, \( Q_T = Q_P + Q_O + Q_L \). CDs for PCP de-chlorination (this excludes contribution from conventional biological processes (open circuit conditions)), oxygen reduction and the unknown processes are then calculated as the ratio of the corresponding charge recoveries relative to the total charges.

PCP degradation rate (\( \frac{\gamma}{\mu\text{mol L}^{-1} \text{ h}^{-1}} \)) was PCP concentration (\( \mu\text{mol L}^{-1} \)) divided by the corresponding operation time of \( t \) (h). In order to clearly compare PCP degradation in BECs at various potentials,

Fig. 3. (A and B) Voltage output (U/V) and (C and D) power volume density (Pvd/W m\(^{-3}\)) are presented as a function of current volume density (jvd/A m\(^{-3}\)) in (A and C) the bioanodes and (B and D) the biocathodes after the switch from various potentials to MFC mode.

Fig. 4. Bacterial morphologies on (A) the bioanodes formed at a potential of 200 mV (vs. SHE), (B) the biocathodes developed at a potential of −300 mV (vs. SHE), (C) the bioanode controls, and (D) the biocathode controls.
PCP degradation rate ($\eta/\%$) was specifically normalized as the percentage of PCP concentration ($\mu\text{mol L}^{-1}$) at $t$ (h) to the initial PCP concentration ($\mu\text{mol L}^{-1}$). Polarization curves and bioelectrochemical behaviors of the bioanode (H-type) and biocathode (tubular) MFCs were performed as described in the SM.

3. Results and discussion

3.1. Development of biofilms in BECs and system startup under various potentials

Different amounts of current were generated from BECs in response to the change of applied potentials, exhibiting a progressive increasing trend with the change of a potential from $-200$ mV to $200$ mV (bioanodes) (Fig. 1A), and $100$ mV to $-500$ mV (biocathodes) (Fig. 1B). This result indicated an adaptive evolution of the biofilms in the BECs that was facilitated by setting the right potential on both the bioanode and the biocathode systems [13,19,20]. For each fed-batch cycle and with the prolonged operation time, substrates in the bioanodes and the biocathodes were gradually consumed and used up, negatively affecting bacterial metabolism activity and electron transfer on the electrodes, and further resulting in the decline of circuit current (Fig. 1A and B) [1,25]. The ability of microorganisms to perform electron transfer in BECs is considered to be a fortuitous consequence of their capacity for other forms of extracellular electron transfer because electrodes are not natural electron acceptors or donors [1,25,26]. An optimal potential can provide an appropriate selective pressure for adaptation of microorganisms. This selective and evolutionary pressure can lead to the enhancement of the ability of microorganisms for electrochemical interaction with electrodes as well as improved current production, which is associated with clear differences in the properties of the outer surfaces of the cells [1,25]. A more negative potential of $-300$ mV on the bioanodes (Fig. 1A) and a more positive potential of $100$ mV (Fig. 1B) may have been detrimental to the bacterial ability for appropriate electrochemical interaction with electrodes and consequently led to the decrease of current production. Our observation was in agreement with what was reported, showing that there was a similar trend of change of set potential with circuit current in BECs [10,14,19].

PCP concentration in the bioanodes of BECs was also changed with the potential. A potential of $200$ mV was more beneficial for PCP removal than the others (Fig. 1C). After a 3-cycle acclimation (320 h) and at $200$ mV, there was no further appreciable decrease in PCP concentration compared to the previous operational cycle (Fig. 1C), implying the well-developed biofilms on the bioanodes and their stable capability for PCP removal [5,6,24]. This startup time of 320 h was shorter than the previous 475 h in the bioanode controls [6], demonstrating the benefit of $200$ mV to the development of bioanodes. In the biocathodes, a more negative set potential accelerated PCP decrease (Fig. 1D). After a 6-cycle acclimation (420–440 h), there was no apparent decrease in PCP concentration with the increase of cycle number at potentials of both $-300$ mV and $-500$ mV (Fig. 1D), indicating successful enrichment of electrotrophic biofilms as well as their stable capability for PCP degradation. In terms of PCP degradation, there was no appreciable difference between a potential of $-300$ mV (1.6% removal per hour, 7th cycle) and $-500$ mV (1.5% removal per hour, 7th cycle) (Fig. 1D), stressing similar effects of these two potentials on PCP degradation. In terms of PCP degradation, there was no appreciable difference between a potential of $-300$ mV (1.6% removal per hour, 7th cycle) and $-500$ mV (1.5% removal per hour, 7th cycle) (Fig. 1D), stressing similar effects of these two potentials on PCP degradation. This 420–440 h was appreciably shorter than the 600 h in the biocathode controls [8]. Taken together, set potentials of $200$ mV on the bioanodes, and $-300$ to $-500$ mV on the biocathodes benefited the development of biofilms for PCP degradation in the corresponding MFCs.

3.2. Steady system performance after switch from BEC to MFC mode

After the finish of acclimation of bioanodes and biocathodes in BECs at various potentials, the BECs were switched to MFC conditions (a fixed resistance of 500 $\Omega$ for H-type reactors and 200 $\Omega$ for tubular
The steady performance of bioanodes formed in BECs at a potential of 200 mV achieved the highest PCP degradation rate of 0.623 ± 0.039 μmol L⁻¹ h⁻¹ in MFC modes (Fig. 2A) whereas biocathodes developed in BECs at −300 mV achieved a PCP degradation rate of 0.932 ± 0.058 μmol L⁻¹ h⁻¹ in MFC conditions compared to 1.045 ± 0.053 μmol L⁻¹ h⁻¹ achieved in MFC modes pre poised in BECs at −500 mV (Fig. 2B). These PCP degradation rates were higher than 0.485 ± 0.027 μmol L⁻¹ h⁻¹ in the bioanode controls [6] (28.5% augment) (Fig. 2A) and 0.767 ± 0.048 μmol L⁻¹ h⁻¹ in the biocathode controls [8] (21.5% enhancement) (Fig. 2B). In contrast, a negative potential of −200 mV on the bioanodes (Fig. 2A) and a positive 100 mV on the biocathodes (Fig. 2B) abated PCP degradation. These results reflected the importance of an optimal set potential to PCP degradation in both the bioanode and biocathode MFCs.

Both the bioanodes and the biocathodes developed in BECs at various potentials were also run in open circuit condition (OCC) to examine changes in PCP in the absence of circuit current. PCP degradation rates in open circuit mode only ranged from 0.098 ± 0.041 to 0.153 ± 0.039 μmol L⁻¹ h⁻¹ in the bioanodes (Fig. 2A), and 0.131 ± 0.038 to 0.224 ± 0.052 μmol L⁻¹ h⁻¹ in the biocathodes (Fig. 2B), apparently lower than those in closed circuit condition (CCC) (MFC mode), implicating the benefit of current generation to PCP degradation and the importance of exoelectrogenic/electrotrophic activities on PCP removal. These results were in agreement with other observations that pollutants including chloroethane and diesel were improved with current generation [3,27].

The maximum power obtained from polarization data clearly showed the changes in voltage output (Fig. 3A and B) and power production (Fig. 3C and D) in the bioanode (Fig. 3A and C) and biocathode reactors (Fig. 3B and D) with various set potentials. While the bioanodes formed in BECs at a set potential of 0–300 mV had similar open circuit potentials (OCP) (Fig. 3A), biofilms developed in BECs at 200 mV achieved the highest powers of 1.7 ± 0.1 W m⁻² and 352 ± 14 mW m⁻² in MFC modes (4.2 A m⁻² and 859 mA m⁻²), a 41.7% enhancement compared to the bioanode controls (Fig. 3C). Biocathodes formed in BECs at −300 mV achieved an OCP of 0.31 V (Fig. 3B) and a maximum power volume density of 3.6 W m⁻³ and a maximum power area density of 61 mW m⁻² (a current volume density of 23 A m⁻³ and a current area density of 382 mA m⁻²) in MFC conditions (Fig. 3D), 24% and 44% improvements, respectively than the bioanode controls (0.25 V and 2.5 W m⁻³ (41 mW m⁻²)). Compared to the biocathodes formed at −300 mV, biofilms developed at −500 mV exhibited higher powers of 4.5 W m⁻³ and 75 mW m⁻² (24 A m⁻³ and 398 mA m⁻²) (Fig. 3D). No hydrogen was found in the headspaces of both bioanodes and biocathodes at the tested set potentials, which cannot preclude the possibility of electron transfer here through the formation of trace amounts of hydrogen gas. However, around 1.0% hydrogen was determined in the headspace of the biocathodes formed at −500 mV, confirming the facilitation of electron transfer through the use of evolved hydrogen gas in the systems. Interspecies electron transfer via reduced molecules such as hydrogen and formate is an important and effective mechanism for interspecies electron exchange in multi-species aggregates [1,25,26]. The detected hydrogen in the biocathodes developed at −500 mV may have contributed to the higher power production than that at −300 mV, and further reflected the importance of hydrogen for electron transfer inside this microbial community.

3.3. Bacterial morphologies

Both the bioanodes at a potential of 200 mV (Fig. 4A) and the biocathodes at −300 mV (Fig. 4B) were covered with uniform bacteria compared to the bioanode (Fig. 4C) and biocathode (Fig. 4D) controls, which suffered from limited microbial adherence and lack of growth of the microorganisms. These uniform distributions of small colonies, instead of large colonies and aggregates may have benefited to the more efficient system performance (Fig. 3) [16]. In addition, bacteria on the bioanodes at 200 mV were surrounded by some icky-like matter (Fig. 4A), which was suspected to be extracellular polymer substances (EPS). We cannot exclude that this EPS was related to the ability of adherence of exoelectrogens on the anode, and thus favorable to electron transfer from exoelectrogens to the
anode. Electron transfers between electrode surface and bacteria is a rate limiting step in current production, and associated with interactions between electrode and exoelectrogens or electrotrophs, as well as interspecies cooperation [1,25,26]. More work thus needs to be done to clarify the role of these structures in these bioelectrochemical systems.

3.4. Electrochemical analysis using cyclic voltammetry

Cyclic voltammetry (CV) was used to identify the activities and redox potentials of the anodic and cathodic biofilms formed under various set potentials. One oxidation peak was observed and the value increased from −0.055 V at a potential of −200 mV to 0.30 V at a potential of 200 mV in accordance with the increase of peak current from 0.32 mA to 1.66 mA [Fig. 5A]. A more positive potential of 300 mV led to the decrease of oxidation peak to 0.14 V (0.62 mA) whereas the bioanode controls had an oxidation peak of 0.185 V (0.11 mA) [Fig. 5A]. These results suggested the higher electroactivity of biofilms developed at 200 mV, which was reflected in Fig. 3A and C. Compared to the change of oxidation peak, the reduction peak exhibited a narrow potential range, from −0.37 V to −0.35 V with the set potentials except −0.04 V at a potential of −200 mV [Fig. 5A]. Derivative CV showed the substantial differences in redox activity ranges [Fig. 5C], implying that the electron transfer components of the bioanodes incubated at various set potentials might be different [28].

In the case of biocathodes, reduction peaks increased from −0.15 V at a potential of −100 mV to −0.01 V at a potential of −500 mV whereas oxidation peaks slightly changed from 0.01 V to 0.05 V accordingly [Fig. 5B]. Biocathodes at −300 mV and −500 mV achieved a maximum current of 0.68–1.25 mA and a minimum of −1.38 to −1.01 mA [Fig. 5B]. The redox active potential ranges for the biocathodes developed at −500 mV and −300 mV were apparently broader than the others [Fig. 5D], explaining the more efficient electrochemical activities in Fig. 3B and C.

3.5. PCP degradation and charge distribution

PCP de-chlorination is a stepwise process, where ortho-chlorines are removed at the fastest rate, and hydroxylation of the para-chlorine is also commonly observed [29]. With the presence of an easily degradable substrate such as glucose or acetate, PCP is also co-metabolized based on reductive de-chlorination, in which PCP serves as an electron acceptor and the easily degradable substrate is used to reduce the toxicity and growth inhibition of PCP on microorganisms. In addition, the easily degradable substrate can act as an inducing agent for biodegradative enzymes as well as an electron donor for bacterial growth [29]. In the case of both the bioanodes [6] and the biocathodes [8] of MFCs without polarization, current generation is more beneficial for PCP de-chlorination than the open circuit conditions, leading to more release of PCP intermediates in the bioanodes [6] and PCP mineralization in the biocathodes [8].

PCP in the bioanodes developed at a potential of 200 mV gradually decreased, reaching 15.6 ± 1.9 μM at 96 h [Fig. 6A], compared to 29.2 ± 1.6 μM in the bioanode controls [Fig. 6B], demonstrating the positive effect of 200 mV on PCP degradation. 2,3,4,5-Tetrachlorophenol (2,3,4,5-TeCP) and tetrachlorohydroquinone (TeCHQ), two of the PCP dechlorination products, achieved 6.6 ± 1.1 μM (2,3,4,5-TeCP) and 4.3 ± 0.6 μM (TeCHQ) in the bioanodes formed at 200 mV [Fig. 6A], lower than 14.6 ± 1.2 μM (2,3,4,5-TeCP) and 6.6 ± 1.3 μM (TeCHQ) in the bioanode controls [Fig. 6B]. This result demonstrated that a set potential of 200 mV was beneficial for less accumulation of 2,3,4,5-TeCP and TeCHQ in the bioanodes. Interestingly, 3,4,5-trichlorophenol (3,4,5-TCP), de-chlorinated from 2,3,4,5-TeCP in conventional anaerobic processes and absent from the bioanode controls [Fig. 6B] [29], climbed to 24.3 ± 2.2 μM at 96 h in the bioanodes formed at 200 mV [Fig. 6A], implying the more efficient de-chlorination from PCP to 3,4,5-TCP via 2,3,4,5-TeCP. This suggested that either the capabilities of the anodic bacteria developed at 200 mV or the composition of the involved bacterial community changed. The possible PCP degradation pathways in the
bioanodes at 200 mV were thus proposed as outlined in the SM (SM Fig. S1).

In the case of biocathodes, a set potential of −300 mV efficiently degraded PCP, from an initial 89.8 μM to 0.31 μM at 96 h, a 99.7% removal (Fig. 6C) compared to 85.1% in the biocathode controls (Fig. 6D). Concurrent with PCP degradation, TeCHQ formed via hydroxylation on the ortho position in conventional biological processes [29], reached 2.0 ± 0.4 μM at 48 h in the biocathodes formed at −300 mV (Fig. 6C), much lower than 9.6 ± 0.5 μM in the biocathode controls (Fig. 6D). 2,3,6-trichloro-1,4-dihydroquinone (2,3,6-TCHQ), a downstream metabolite from TeCHQ de-chlorination, and 2,6-dichloro-1,4-dihydroquinone (2,6-DCHQ), subsequently released from 2,3,6-TCHQ, also exhibited the same lower trends in the biocathodes at −300 mV (Fig. 6C) compared to 85.1% in the biocathode controls (Fig. 6D). These results explained the efficiency of PCP degradation and reflected the beneficial −300 mV for less accumulation of PCP intermediates in the biocathodes. The possible PCP degradation pathways were outlined in SM Fig. S2.

The charge distribution (CD) in the bioanodes at 200 mV exhibited 31.7% of total charges in electricity, 21.2% as acetate, 11.2% in lactate and 13.8% as biomass, with 22.1% lost to unknown processes (such as aerobic respiration and intermediates that were not measured or unknown extracellular polymeric substances) at a batch cycle time of 72 h (Fig. 7A). In the biocathode controls, a majority of charges (16.7%) were in lactate, 12.8% in biomass and 11.4% as electricity, resulting in 59.2% lost to unknown processes (Fig. 7B). Glucose in the bioanodes at 200 mV (Fig. 7A) was more slowly released with concomitant productions of less lactate, more acetate and higher electricity, and further resulted in less electrons to the unknown processes. In the open circuit controls, a majority of the charges in glucose (42.8%) were in lactate, 40.9% in biomass, producing 16.3% lost to unknown processes (Fig. 7C). In the biocathode controls, however, charges used for oxygen reduction decreased from 77.6% at 24 h to 62.3% at 96 h, leading to the large portion of charges used for unknown processes (Fig. 7D). A higher ratio of oxygen reduction at −300 mV here may indicate the role of −300 mV for creating beneficial conditions for microbial oxygen reduction with concomitant PCP de-chlorination.

3.6. Bacterial community

Overall, the bacteria predominately found on the bioanodes formed at 200 mV belonged to genera of Alphaproteobacteria (56%), Bacteria (31%) and Deltaproteobacteria (13%) (Table 1, SM Fig. S3). All of the identified bacteria including Desulfovibrio carbonilophilus and Dechlorosporillum sp. (Table 1) were not found on the bioanode controls previously reported using the same conventional reactors [6], stressing the importance of an optimal set potential to the selection of anodic bacterial community. Additionally, these bacteria shared a common character of degrading multiple recalcitrant organics including PCP, 2,3,4,6-tetrachlorophenol, bisphenol A, carbazole, diesel, benzyl alcohol, phenylethanol, naphthenic acids, pyridine, quinoline, petroleum, disulfodiphenylether carboxylates and polycyclic aromatic hydrocarbon [30–37]. The presence of the diverse bacteria capable of degrading multiple recalcitrant compounds in these systems and their substantial differences from those as the importance of circuit current on glucose metabolism pathways. It is thus presumably deduced that a potential of 200 mV may have created a selective condition, under which anodic biofilms with higher catalytic activities for power generation, PCP degradation and acetate metabolism pathways were selectively enriched.

In the case of biocathodes at −300 mV and in the first 24 h of operation period, PCP de-chlorination attributed to electricity generation consumed 12.9% of the total charges transferred from the cathode (Fig. 7C) whereas oxygen reduction utilized 84.4% of the total charges and 2.8% was lost to unknown processes (Fig. 7C). This trend slightly changed at 96 h, exhibiting 15.2% for PCP de-chlorination (this de-chlorination had excluded contribution from conventional biological processes), 83.2% for oxygen reduction, and 2.2% was lost to unknown processes (Fig. 7C). In the biocathode controls, however, charges used for oxygen reduction decreased from 77.6% at 24 h to 62.3% at 96 h, leading to the large portion of charges used for unknown processes (Fig. 7D). A higher ratio of oxygen reduction at −300 mV here may indicate the role of −300 mV for creating beneficial conditions for microbial oxygen reduction with concomitant PCP de-chlorination.

### Table 2

<table>
<thead>
<tr>
<th>Band</th>
<th>Accession no.</th>
<th>Class or genus</th>
<th>GenBank closest match</th>
<th>Identity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isolation source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>AY352452</td>
<td>Bacteroidetes</td>
<td>Uncultured Bacteroides sp. clone NEV4</td>
<td>97</td>
<td>Ethene-enriched consortium degrading vinyl chloride and chlorophenol; present on both the anode and cathode</td>
</tr>
<tr>
<td>2-2</td>
<td>JQ588341</td>
<td>Bacteria</td>
<td>uncultured bacterium clone MW-R25</td>
<td>99</td>
<td>Microbial communities in long-term water-flooded petroleum reservoirs</td>
</tr>
<tr>
<td>2-3</td>
<td>JN674090</td>
<td>Betaproteobacteria</td>
<td>Comamonas sp. p19</td>
<td>99</td>
<td>Degrading PCP, quinoline, and chlorinated aromatic hydrocarbons; present on both the anode and cathode</td>
</tr>
<tr>
<td>2-4</td>
<td>JF729307</td>
<td>Deltaproteobacteria</td>
<td>Comamonas testosterone sp. PA1</td>
<td>99</td>
<td>PCP-degrading and exoelectrogen</td>
</tr>
<tr>
<td>2-5</td>
<td>JF808781</td>
<td>Alphaproteobacteria</td>
<td>Uncultured alpha proteobacterium clone R7-28</td>
<td>95</td>
<td>Bacteria for antibiotics removal in an A/O-MBR process</td>
</tr>
<tr>
<td>2-6</td>
<td>NR042704</td>
<td>Deltaproteobacteria</td>
<td>Desulfovibrio maraisensis sp DSM 19337</td>
<td>97</td>
<td>A 1,4-tyrosol-oxidizing bacterium isolated from olive mill wastewater</td>
</tr>
</tbody>
</table>

<sup>a</sup> The values represent the similarities between the associated DGGE band sequence and the closest-match sequence from GenBank.
on the bioanode controls, can therefore explain the efficient PCP de-chlorination (Fig. 6A and B). While most of these bacterial exoelectrogenic activities are unknown, uncultured Nitro bacter sp., previously present on the biocathodes of MFCs [38], was observed here, demonstrating this bacterial nonspecific character to the electron-acceptor anode and electron-donor cathode [1,19,26]. These diverse bacteria as recalcitrant organic degraders on the bioanodes at 200 mV may be potentially relevant to electricity production and thus extend beyond the commonly studied Shewanella and Geobacter species [1,2,5,25,26].

The composition of bacterial community on the biocathodes developed at −300 mV (Table 2) was also completely different from those on the bioanode controls previously reported using the same condition reactors [8], reflecting the role of −300 mV on the development of this bacterial community. Microorganisms including Desulfobulbus marrackechnesis, Comamonas testosteroni and Comamonas sp., and degrading multiple recalcitrant organics of PCP, chlorophenol, quinoline, chlorinated aromatic hydrocarbons, vinyl chloride and 1,4-tyrosol [29,39–43], were observed on the bioanodes at −300 mV, partially explaining the preferable trends in PCP degradation pathways (SM Fig. S2). Electrotrrophic Comamonas sp. and uncultured Bacteroidetes sp. on the oxygen reducing biocathodes [19], and exoelectrogenic C. testosteroni and uncultured Bacteroidetes sp. [44–46] were present on the bioanodes at −300 mV, implying against the nonspecific characters of some electrotrophs and exoelectrogens to the anode and cathode [1,19,26], and the potential link between bacterial electrochemical activities and their abilities for multiple recalcitrant organics degradation. Activities of bacteria on both the anodes and the cathodes were significantly influenced by set potentials. Bioanodes formed at an optimal potential of 200 mV and biocathodes developed at −300 mV enhanced the subsequent performances of MFCs compared to the corresponding controls. The startup times were reduced to 320 h (bioanodes) and 420–440 h (biocathodes) whereas PCP degradation rates were improved by 28.5% (bioanode) and 21.5% (biocathode), and power production by 41.7% (bioanode) and 44% (biocathode). These results demonstrated that an optimal set potential was a feasible approach for developing both bioanodes and biocathodes for efficient PCP degradation and power generation from MFCs. While a set potential of 200 mV on the bioanodes led to PCP de-chlorination forward and produced a new product of 3,4,5-TCP, biocathodes formed at −300 mV achieved less accumulation of PCP degradation intermediates compared to the biocathode controls. Charges were diverted to more generation of electricity in the bioanodes at 200 mV whereas oxygen in the biocathodes at −300 mV acted as a primary electron acceptor. Dominant bacteria that are known recalcitrant organic degraders and/or exoelectrogens/electrotrophs included D. carbinoliphilus and Dchlorsporillum sp. on the bioanodes formed at 200 mV, and D. marrackechnesis, C. testosteroni and Comamonas sp. on the biocathodes developed at −300 mV.

It is difficult to make definitive conclusions regarding the function of one specific bacterium in PCP degradation and power generation taking place in these complex bioanode and biocathode systems. Interspecies cooperation cannot be excluded in order to forward the stepwise PCP degradation as well as power generation. The exoelectrogenic or/and electrotrrophic activities and their roles in chemoheterotrophic (anode) and chemolithotrophic (cathode) dechlorination processes remain unknown. In fact, not all the members of the community were responsible for anode or cathode respiration, since fermentation and other respiratory processes allowed different organisms to proliferate [1,19,25,26]. Considering the capability of biofilms to adapt to different potentials and the subsequent implementation of different electron transfer pathways [9,26,47], there may be some specific relations between electron transfer pathways and PCP degradation with the presence of these formed exoelectrogenic or/and electrotrrophic biofilms. Further investigations in these directions based on pure cultures [48] are warranted.

Acknowledgments

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Appendix A. Supplementary data

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References
