A novel mediatorless microbial fuel cell based on direct biocatalysis of *Escherichia coli*†

Tian Zhang,[a] Changzheng Cui,[b] Shengli Chen,*[a] Xinping Ai,[a] Hanxi Yang,[a] Ping Shen[b] and Zhenrong Peng[b]

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A mediatorless microbial fuel cell based on the direct biocatalysis of *Escherichia coli* shows significantly enhanced performance by using bacteria electrochemically-evolved in fuel cell environments through a natural selection process and a carbon/PTFE composite anode with an optimized PTFE content.

Microbial fuel cells (MFCs) utilize bacterial catalysis to directly generate electric power from carbohydrates, which can be found in a diverse range of sources such as crops, industrial and agricultural waste, and domestic waste water.[1] Although MFCs have the advantages of clean power generation and simultaneous waste utilization, their commercialization has been halted due to their low power output. In MFCs, the current is generated by diverting the catabolic electrons to the anode. The low power capability of a MFC is mainly due to the sluggish kinetics of the electron transfer between the bacterial cells and the fuel cell anode. To solve this problem, redox mediators such as methylene blue,[2] thionine,[3,4] neutral red[5,6] or quinone[7] are generally used. However, these synthetic redox mediators are mostly expensive and toxic to microorganisms, making mediator-type fuel cells difficult for practical applications. It has been recently shown that mediatorless MFCs may be constructed by using the Geobacteraceae family, like *Rhodoferax ferrireducens*[8] and *Geobacter sulfurreducens*,[9] since these bacteria possess membrane-bound redox proteins, like cytochromes, that can mediate electron transfer between the catabolic electron carriers and the electrodes. Although a coulombic efficiency as high as 80% was reported, the power density of these MFCs remains low (*e.g.*, $\sim$8 mW m^{-2}),[8] since such a high coulombic efficiency was obtained under very high overpotentials for glucose oxidation. Furthermore, the isolation and cultivation of these microorganisms involve rather complicated procedures.

Escherichia coli (*E. coli*) is one of the readily available and easily grown bacterial strains. In general, *E. coli* has to be used in combination with suitable electron mediators in MFCs, or else the production of electricity will be rather low. For instance, Park and Zeikus[10] have reported recently that *E. coli*-catalyzed MFCs, with neutral red or Mn^{4+} ions as anode mediators and Fe^{3+} ions as cathode mediators, exhibit maximum power densities of around 100 mW m^{-2}, which was considered significant progress in MFC development.[10] Almost at the same time, Schröder *et al.* showed a maximum power output of about 6000 mW m^{-2}, achievable with an *E. coli*-based two-chamber MFC by using polyaniline-modified platinum as the anode and ferricyanide as the cathode reactant.[11] This represents a marvellous achievement in MFC development, but it should be noted that Pt itself is a very efficient catalyst towards the electrochemical oxidation of organic molecules, and the observed current output may be mainly due to the electrocatalytic oxidation of glucose by Pt rather than by the bacteria.

Herein, we show that an *E. coli*-catalyzed MFC with a carbon-based anode exhibits a maximum power density of greater than 600 mW m^{-2} in the absence of any artificial electron mediators, which is much higher than the previously reported value of $\sim$100 mW m^{-2} achieved in an *E. coli*-based MFC with efficient mediators used in both the anode and the cathode.[10] Most importantly, we demonstrate that repetitive fuel cell discharge and cultivation cycling are essential to produce *E. coli* with enhanced catalytic activity in fuel cell environments. A mechanism in terms of Darwinian natural selection is proposed to explain such an activation phenomenon in bacterial biocatalysts. It is also shown that the anode composition and structure have an important impact on the performance of MFCs.

The *E. coli K12* was initially cultivated aerobically at 37 °C. It was then inoculated into a 250 mL conical flask containing a 200 mL standard medium‡ and cultivated at 33 °C for 18–20 h under anaerobic conditions by sealing the flask. The *E. coli* thus obtained is referred to as "original bacteria" and was subjected to a fuel cell discharge process by pumping the bacteria culture through the anode chamber of the fuel cell until the cell voltage was below 0.05 V. The bacteria suspension that underwent fuel cell discharge was inoculated into a fresh medium and cultivated under the same conditions as the original bacteria. The resulting *E. coli* is referred to as "electrochemical bacteria of generation I", which was again subjected to fuel cell discharge until the cell voltage was below 0.05 V. Repeating the inoculation, cultivation and discharge cycles produced electrochemical bacteria of generation II, III, and so on. A single chamber fuel cell (Scheme 1)§ with a carbon/PTFE composite film anode and a conventional air cathode was used to test the catalytic properties of the bacteria of each generation.

As shown in Fig. 1, the discharge curves of the fuel cell are significantly different when electrochemical bacteria of different generations are used. For the original bacteria of *E. coli*, the cell voltage is initially 0.4 V under close circuit conditions, decreasing to 0.1 V within a few hours. When the electrochemical bacteria of generation I are used, the initial cell voltage increases to 0.47 V and the voltage decline is significantly slowed down. If the electrochemical bacteria of generation II are used, the initial cell voltage

[a]*Department of Chemistry, Wuhan University, Wuhan 430072, P. R. China. E-mail: slchen@whu.edu.cn; Fax: +86 27 68754067; Tel: +86 27 68754693*
[b]*College of Life Science, Wuhan University, Wuhan 430072, P. R. China*
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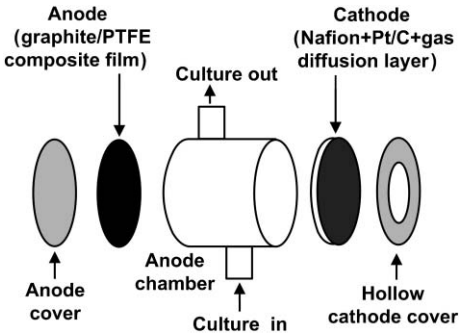

Scheme 1 The block diagram of the fuel cell components.

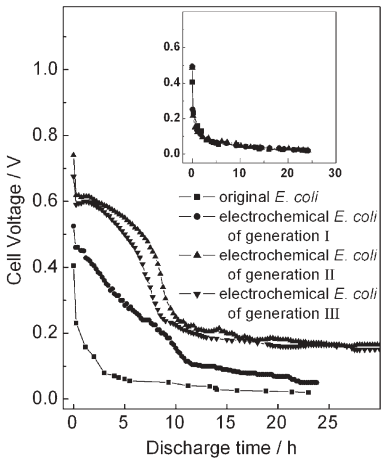

Fig. 1 The discharge curves of MFC using different generations of *E. coli*. The inset gives the discharge curves for the non-electrochemical *E. coli* of generations I, II and III.

increases to 0.6 V. Instead of continuously falling, the voltage stays above 0.55 V in the initial 5–7 h, forming an approximate discharge plateau. It then decreases to 0.25 V, forming another plateau that is sustained for about 50 h. The electrochemical bacteria greater than generation II show very similar fuel cell discharging behaviors, all with maximum cell voltages around 0.6 V and two voltage plateaus of above 0.55 and 0.2 V, respectively.

The significantly improved fuel cell performances for the electrochemical bacteria of generation II and those subsequent imply that an acclimation and selection process is necessary for bacteria to be efficient in fuel cell catalysis. We may call such an acclimation and selection process an "electrochemical activation of bacteria". In experiments, we found that all the bacteria showed an electrochemical activation process, which usually took three cycles of fuel cell discharge and cultivation. As long as the bacteria were electrochemically activated, the fuel cells gave a very reproducible and greatly improved discharge performance. In comparison, different generations of non-electrochemical bacteria were cultivated and their fuel cell performances were characterized. To obtain non-electrochemical bacteria, the inocula for cultivating the next generation of *E. coli* were extracted from the culture of *E. coli* before the fuel cell discharge process. That is, the non-electrochemical bacteria are cultivated with inocula that haven't experienced any electrochemical discharge. It can be seen that the non-electrochemical bacteria of different generations show an almost identical discharge behavior, with cell voltages decreasing rapidly with time (Fig. 1, inset), suggesting that the improved fuel cell performances are due to the electrochemical activation of the bacteria.

The electrochemical activation of bacteria for better fuel cell performance is understandable when considering the correlation between current production and bacteria growth during the fuel cell discharge process. In a MFC, the anode serves as the final electron acceptor on the bacterial catabolic chain. Electron transfer from the bacteria cell to the anode has a two-fold function. On the one hand, the anode harvests the electrons from the bacteria cells to produce current. On the other hand, electron transfer to the anode is necessary for complete bacterial respiration. In the absence of any other electron acceptors, the growth of a bacterium cell will be inhibited if the catabolic electrons cannot transfer to the anode successfully. It can be inferred that the bacterial cells which remain active after fuel cell processes should be those which can adapt to the anodic environment and easily transfer their catabolic electrons to the electrode. Inoculation with these bacteria should produce a strain with similar properties. Such an electrochemical activation mechanism in a bacteria strain is essentially similar to the Darwinian natural selection theory and would be very useful for optimizing and cultivating MFC biocatalysts.

The average power density associated with the two discharge plateaus for the electrochemical bacteria of generation II and those subsequent are about 600 and 80 mW m^{-2}, respectively; quite encouraging achievements from the viewpoint of mediatorless bioelectrocatalysis of the pure strain of *E. coli*. A power density of 600 mW m^{-2} is comparable to the maximum power density produced with a MFC using mixed bacterial consortia obtained from waste water (560 mW m^{-2}),[12] and much higher than that reported by Park and Zeikus for a *E. coli*-catalyzed MFC (152 mW m^{-2}) using immobilized electron mediators on both the anode and cathode.[10] This represents a significant progress and a great prospect for the development of high efficiency and low cost MFCs.

We found that the PTFE content of the composite anode can significantly alter the MFC's performance. Those shown in Fig. 1 are obtained with anode containing about 20% (w/w) PTFE. Deviation of the PTFE content in anode from this value results in a decrease in the cell voltage and the duration of the voltage plateaus of the discharge curves. The utilization of small graphite particles provides a large active area on the electrode, whereas the PTFE framework in the electrode forms a porous microstructure that renders most of the active graphite surface accessible to the bacteria. Also, the presence of PTFE may modify the wetting properties of the interior graphite surfaces, helpful for the adsorption and immobilization of the bacterial cells. The hydrophobic nature of PTFE may also prevent the bacteria from congregating inside the electrode to form a condensed biofilm that would increase the mass transport and electron transfer resistances.

It is also worthwhile mentioning that the performance shown above is achieved by a single chamber MFC with a conventional air cathode that works under natural convection and diffusion conditions (see Scheme. 1). Therefore, energy loss due to cathodic polarization is unavoidable. Fig. 2 gives the variation of the anode and cathode potentials during the discharge process of the electrochemical bacteria of generation II. No matter which generation of bacteria is used, the cathode potential remains

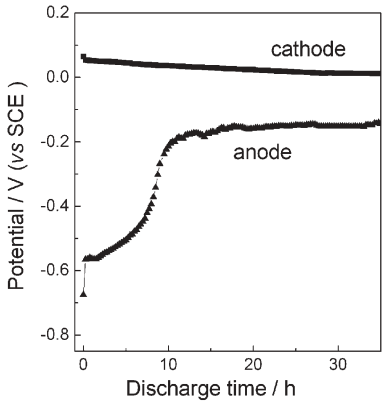

Fig. 2 The variation of the anode and cathode potentials during the fuel cell discharge.

below 0.1 V *vs.* SCE (saturated calomel electrode), which is much lower than the equilibrium potential of the O_2/H_2O system in a neutral medium, *i.e.*, 0.55 V (*vs.* SCE). This means that there remains much scope for cathode optimization. Therefore, the high performance of the MFC in this study is mainly due to the novel design of the anode, as well as the electrochemical activation of the bacterial strain.

It can be seen from Fig. 2 that the anode potential–time curve also shows two plateaus, which are around −0.55 and −0.15 V, respectively. Correspondingly, two oxidation peaks were observed in the linear voltammetric curves obtained on glass carbon-supported graphite/PTFE composite electrodes in the electrochemical bacteria culture of generation II and those subsequent (see ESI†). The first oxidation peak covers a potential region from −0.55 to −0.2 V and the second one starts at about −0.15 V. These voltammetric features suggest a two-step anodic process associated with the *E. coli*-catalyzed oxidation of glucose on the graphite/PTFE composite anode. At present, we cannot elucidate the underlying mechanism responsible for these two reaction processes due to lacking a detailed understanding of the complicated biocatalytic electrochemical reactions. Nevertheless, these results do suggest the possibility of constructing bacteria-catalyzed fuel cells without the need for mediators. Most importantly, if the first kinetic process can be enhanced to maintain the anodic potential at low polarization, a significant improvement will be expected in the MFC performance.

In conclusion, we have shown that the performances of a microbial fuel cell can be greatly enhanced *via* the optimization of the bacterial strain and the anode composition. The bacteria may undergo a Darwin-type of natural selection process in electrochemical environments. These results demonstrate a new approach for the improvement of MFC performance and a great prospect for practical applications.

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Notes and references

‡ 10 g glucose, 5 g yeast extract, 10 g $NaHCO_3$ and 8.5 g NaH_2PO_4 per litre, pH = 7. The inocula sizes are all 2.5% (v/v) in this study.
§ The anode (150 μm in thickness) was prepared with fine graphite particles (5 μm in diameter) and a PTFE emulsion using a roller press method. The air cathode (Pt loading: 0.2 mg cm^{-2}) was constructed using the hot pressing method. The anode chamber was a cylindrical cavity 3 cm long and 1.8 cm in diameter, made from an organic glass rod. The fuel cell was discharged at 33 °C under constant-load mode with a resistor (1.98 KΩ). Details about the electrode's preparation and fuel cell measurements are given in the ESI.†

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