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A High Performance Xylose Microbial Fuel Cell Enabled by *Ochrobactrum sp.* 575 cells

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A new strain *Ochrobactrum sp*.575 is applied as an anodic biocatalyst in a xylose MFC. After evolution under electrochemical tension in MFCs, *Ochrobactrum sp*.575 can deliver a maximum power density of 2625 mW m^{-3}, which is 20 times higher than the reported similar MFCs. The pH slightly increases with the operation progress of *Ochrobactrum sp.* 575 MFC indicates protons involvement in the anode electrochemical reaction. For the first time, fumaric acid, an important intermediate in succinate oxidation respiratory chain of gram-negative strains is discovered in the anodic supernatant of the MFC. It possibly indicates that the process of xylose digestion with *Ochrobactrum sp.* 575 depends on the succinate oxidation respiratory chain, which is quite different with the traditional NADH oxidation respiratory chain in other electroactive bacterial strains. The significant improvement of the MFC power density is very likely to be attributed to fumaric acid generated by the bacteria cells as an electron mediator to facilitate the electron transfer during the discharging process.

Introduction

Microbial fuel cells (MFCs) are devices that use microbes to convert the chemical energy stored in organic compounds including organic wastes into electricity [1]. It has been more than 100 years since the first report on bacterium-produced electricity [2] but only in recent years MFCs have been developed to be a promising green energy source. MFCs possess a number of prominent merits like degrading organic matters, mild operation conditions and low cost, thus becoming very attractive [3, 4]. MFCs also are very promising to simultaneously enable waste treatment and electricity generation. [3, 5, 6]. A wide range of soluble or dissolved complex organic wastes and renewable biomass can be used as MFC fuels. The major substrates can be various kinds of artificial and real wastewaters as well as lignocellulosic biomass [7]. In recent years, the production of fuel and energy from lignocellulosic biomass such as agricultural residues and woody biomass have drawn great attention because of the abundance, ready availability and renewable nature [8, 9].

As the most representative pentose, xylose is one of the main compositions of lignocellulosic hydrolysates, which comprises up to 13–23% of the organic matters in aqueous hydrolysates from lignocellulosic biomass [10, 11]. Utilization of xylose in MFCs provides a new approach for generation of renewable energy from biomass. Up to date most of the xylose MFCs utilize mixed bacteria culture as the biocatalysts [1, 12, 13]. In such a complicated system, it is hard to understand the detailed mechanism of the energy conversion and electron transfer. In this case, there is no clue to improving the cell design for enhancing the performance. An obvious solution is to choose a single strain with high catalytic activity as the catalyst for xylose MFCs.

In this work, we use a new strain -- *Ochrobactrum sp.* 575 that can use xylose as a sole carbon source in MFCs. The power generation performance and electrocatalytic activity of this strain are investigated. To understand the direct electron transfer process in the xylose MFC, electrochemical characterization combined with spectrum analysis of supernatant are investigated. A succinate oxidation respiratory chain involved mechanism is proposed to explain the phenomenon.

Experimental

MFC construction

The dual-chamber MFC used in this work was constructed with two bottles (100 mL capacity) separated by a proton exchange membrane. The chambers were sealed by rubber stoppers that punched a hole in the middle to make titanium wire to get through. Proton exchange membrane was boiled in 0.5% H_2O_2; deionized water; 1% H_2SO_4; deionized water 5 min successively. The carbon fiber brush electrodes (10mm diameter ×20mm length) made from carbon fibers and titanium wire[14] were used as the anode and cathode after cleaning with deionized water, drying at 100 ºC and UV-sterilizing for 3 h in a biological safety cabinet. The anolyte is the xylose medium and the catholyte was 50 mM $K_3[Fe(CN)_6]$ in 0.1M PBS. An external load (1.8 kΩ) was used to evaluate the long term discharge performance. The polarization and power output curves were measured by varying the output load resistor from 0.2 kΩ to 8 kΩ to monitor the steady-state current.

Bacterial cultivation

Ochrobactrum sp. 575 (preserved by China Center for Type Culture Collection, CCTCC M2013549) was isolated from root zoon soil and has been proved using xylose as sole carbon source. The bacteria cells from a single colony on lysogeny broth (LB) agar plate was inoculated in 5 mL LB medium for overnight culturing at 37 ºC, 180 rpm. The overnight culture was then inoculated in the xylose medium, which contained 1 g $NaHCO_3$, 0.85 g NaH_2PO_4, 0.5 g yeast extract, and 1 g xylose per 100 mL[15]. After the cell culture reached a steady state (OD_{600} = 1.2), the bacteria cells were harvested by centrifugation at 4ºC, 6000 rpm, 5 min, resuspended in xylose medium, and then transferred into the anodic chamber of the MFC. After the output voltage dropped down, the bacteria cells in anolyte was inoculated on fresh LB agar plate to obtain the single clone of the evolved bacteria cells. All the chambers, rubber stoppers and medium were autoclaved at 121ºC and xylose solution was filtered through 0.22 μm membrane to remove the microorganism. Before test, the suspension was purged by nitrogen for 30 min to remove oxygen.

Observation of biofilm anodes

Scanning electron microscopy (SEM, JSM-6510LV, Japan) was used to examine the morphology of bacteria adhered anodes. For sample preparation, a piece of carbon brush was cut from the anode after discharge and immersed in 2.5% glutaraldehyde in PBS buffer for 2 hours to fix the morphology of bacteria cells. After a series dehydration with 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% ethanol (20 min each rinse), the samples were dried in vacuum oven at room temperature for SEM observations.[16]

Electrochemical characterization

Electrochemical experiments were conducted in a three-electrode cell with carbon brushes as the working and counter electrodes and Ag/AgCl (saturated KCl) as the reference electrode. A potentiostat (CHI660E, Shanghai Chenhua, China) was used for all cyclic voltammetry (CV) measurements that conducted at scan rate of 30 mV s^{-1} or 1 mV s^{-1} with the potential range from -0.8 V to -0.1 V [17].

Spectrum analysis of anolyte supernatant

After two discharge cycles, the anolyte was centrifuged and stored at 4 ºC prior to analysis. Fourier transform infrared (FT-IR) spectra of the supernatant were examined ranging from 4000 cm^{-1} to 400 cm^{-1} in transmission mode. The UV-Vis spectra were measured from 200 nm to 700nm using a quartz cell with a 1 cm path length. The pH of anodic chamber was measured by a pH meter. All the tests were conducted at room temperature.

The supernatant of anolyte was also subjected to HPLC-MS measurement. Preparative HPLC was performed using ELITE P-230 pumps, and the optical rotations were measured on a JASCO P-1030 digital polarimeter. High-resolution (HR) MS measurements were performed on an Apex III (7.0 Tesla) FT-ICR mass spectrometer (Bruker, Billerica, MA, USA) using CF3COONa as an external calibration standard.

Results and discussion

Power generation of *Ochrobactrum sp.* 575 MFC

When the original *Ochrobactrum sp.* 575 was applied as an anodic catalyst for the MFC, the open circuit voltage was only 180 mV and the power density was about 85 mW m^{-3} at a constant load of 1.8k ohms (Table S1). To enhance the electrocatalytic performance of the strain, the cell cultures was inoculated from the anode chamber after a long term discharge (about 140 hours) and the obtained single clone (noted as Generation I) was inoculated in the anode of another MFC. The MFC catalyzed by Generation I cells delivered an open circuit voltage of 330mV and power density of 205 mW/m^3. The same treatment was repeated two times to obtain Generation II, Generation III cells respectively. Finally, the Generation III cells catalyzed MFCs achieved an open circuit voltage of 680 mV and a power density of 1473 mW m^{-3}, which is around 17 times higher than the original cells. To explain the reason of great improved performance of Generation III cells, the morphology of anodes was observed after 140h discharge. Figure 1 shows that for the original bacteria catalyzed anode, only a few bacteria cells adhered on the carbon fiber surface (Fig. 1a, b). While for the Generation III cells catalyzed anode, a high density of cells attached on the carbon fiber and formed a biofilm (Fig. 1c, d). This phenomenon suggests that after evolution the cells are willing to adhere on anode surface, which will facilitate the interfacial electron transfer between the cells and the anode.

The xylose MFC catalyzed by Generation III cells was running at room temperature for four cycles and the current generation profile was recorded. (Fig. 2a) In the first 20 h of first cycle, the current increases slowly and reaches the maximum value (around 2700 mA m^{-3}) at about 24 h and maintained in the next 24 h, then begins to decrease slowly. When the current drops to half of the maximum value, the anolyte was replaced with fresh medium to start the next cycle. It is noted that there is no lag time for the increase of current in the following three cycles. The voltage reaches the maximum plateau in several hours. The reason might be that the biofilm form on the anode could possess a fast oxidation of xylose and pass the electrons to the anode. The four cycles of voltage output indicated the MFC could discharge steadily and repeatedly for a long time. Fig. 2b shows the polarization curve and power curve of the MFC. The maximum power density reaches 2625 mW m^{-3} at the current density of 8985 mA m^{-3}, which is 22.2 –fold higher than the reported xylose MFC[13]. Since the Generation III cells could deliver greater performance in xylose MFCs, they were used for following electrochemical and spectral analysis.

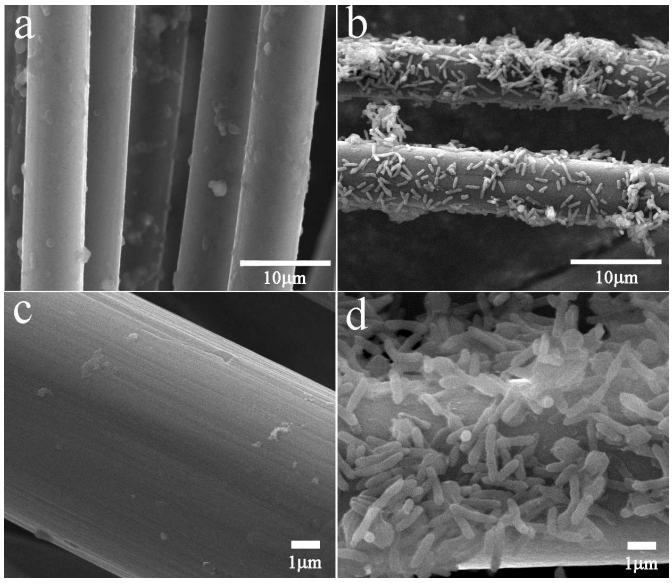

Fig. 1 SEM micrographs of original *Ochrobactrum sp.575* (a, b) and Generation III *Ochrobactrum sp.575* cells (c, d) adhered carbon fiber anodes.

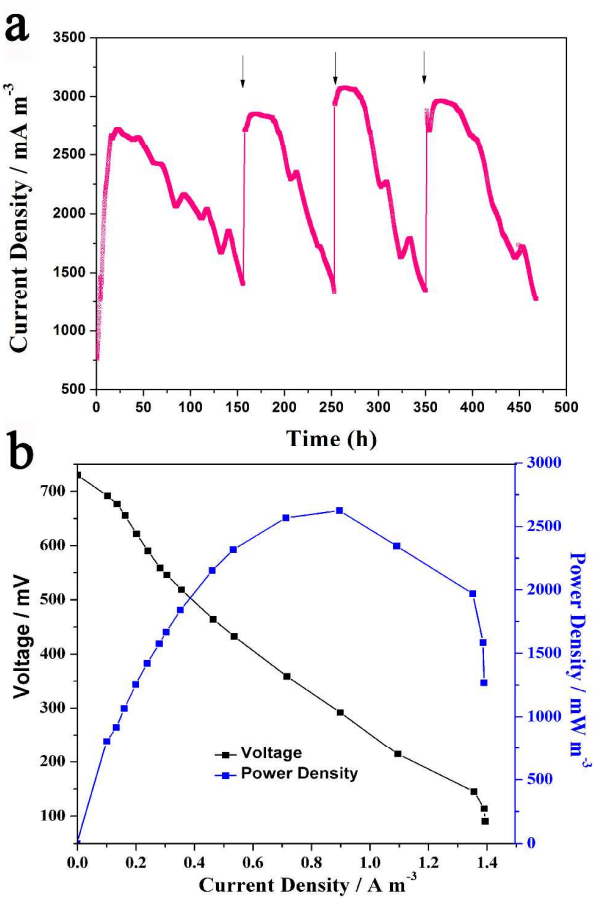

Fig. 2 a: Current generation profile of the Generation III *Ochrobactrum sp. 575* MFC with external resistance of 1.8kΩ. The arrows indicated the replacement of the anolyte. b: The polarization and power output curves of Generation III *Ochrobactrum sp. 575* MFC.

Electrocatalytic behavior of evolved *Ochrobactrum sp. 575* anode

In order to explore the electrocatalytic mechanism of *Ochrobactrum sp. 575,* its electrochemical behavior in MFC anode was investigated. Fig. 3a shows that two pair of redox peaks can be observed on the cyclic voltammogram of *Ochrobactrum sp*. 575 cell cultures with xylose medium. However, when the cells were suspended in phosphate buffer, no peak can be found, which indicating that the redox peaks could be due to the redox reaction of certain xylose metabolic products. The CV curve (Fig. S1) obtained at low scan rate (1 mV s^{-1}) clearly shows the catalytic current and the onset potential of xylose oxidation of ~-0.38V. With the discharging time increasing, the redox peaks shift to positive potential and the peak current of the more positive redox pair increases significantly (Fig. 3b), which could be due to new metabolic products generated or the redox potential changed from the pH variation during the discharging. To find the explanation, the cyclic voltammograms of anolyte at different pH were measured (Fig. 3c). The results show that when the pH value is 9, two pairs of redox peaks can be found and they shift to positive potential when the pH value decreases to 8. While when the pH value decreases to 7, the more positive redox pair increases a lot but the more negative peaks decreases. There is only one pair of peaks can be observed when the pH value is lower than 7 and this redox pair shift to positive as the decrease of pH. These results suggest that the proton is involved in the redox reaction of anode. Further, the relationship between the scan rate and

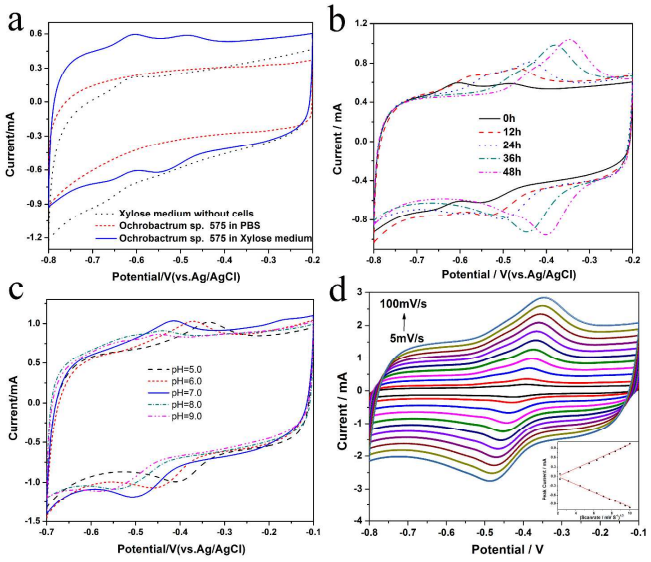

Fig. 3 a: Cyclic voltammograms of carbon fiber brush electrode in different electrolytes (scan rate: 30 mV s^{-1}). b: Cyclic voltammograms of anode in *Ochrobactrum sp. 575* suspension at different time (scan rate: 30 mV s^{-1}). c: Cyclic voltammograms of anolyte with different pH values (scan rate: 30 mV s^{-1}). d: Cyclic voltammograms of *Ochrobactrum sp. 575* anode in xylose medium at different scan rate. The inset is function of peak current *vs.* (scan rate) $^{1/2}$.

the peak current was investigated at pH 7. The redox peak current has a linear relationship with the square root of the scan rate (Fig. 3d), revealing a diffusion control process. According to the analysis of the electrochemical behavior, it could be concluded that the *Ochrobactrum sp. 575* cells digest xylose and generate some kind of metabolite that possesses redox activity. The redox reaction of this metabolite includes a dehydrogenation/hydrogenation process.

Analysis of the supernatant of the anolyte

The pH of anolyte was measured during a cycle of MFC discharge (Fig. 4). The pH declined greatly along with the rapid increasing of cell voltage in the first 20h, and then the pH decreased slowly during the voltage of MFC was stability and decrease. It suggests that a large amount of organic acids are generated during the start up period until the voltage reached the maximum. It is well in agreement with the electrocatalytic analysis results.

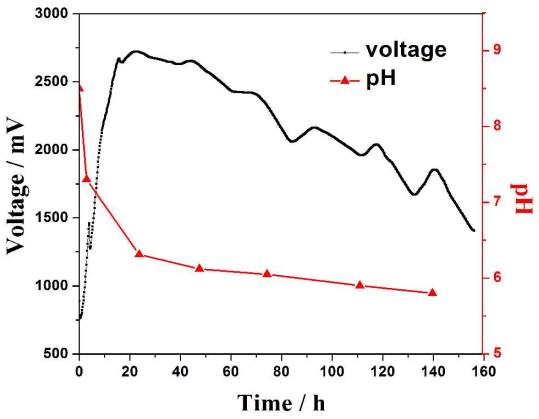

Fig. 4 The variation of pH value in anodic chamber and MFC output voltage in a cycle of discharge.

To clarify the organic acid generated from the xylose metabolism, the FTIR and UV-Vis spectra of the anolyte supernatant, xylose medium and the supernatant of bacteria culture in LB medium were examined. FTIR spectra of the supernatant present a strong and poignant peak at 2025cm^{-1}, which does not appear in xylose medium (Fig. 5a). The peak indicated olefin or alkyne existing in the supernatant that produced by metabolism of bacteria cells. In figure 5b, the supernatant of anolyte displays a strong absorption at 220 nm in UV-Vis spectrum, indicating the conjugated structure. Since the supernatant of bacteria cultured in LB does not have such profiles in FTIR spectra and UV-Vis absorption, it suggests that the *Ochrobactrum sp. 575* cells do not generate such kind of organic acid when feed with LB medium. Boily et al. reported that fumaric acid had a peak absorption at 220 nm in UV-Vis spectra[18]. According to the analysis above, we propose that the organic acid in the supernatant that possess olefin structure could be fumaric acid. For further verification, the supernatant was analyzed with HPLC-MS. As shown in Fig. S2, there is a 173.37 peak, which is in accordance with the molecular weight of fumaric acid. In addition, the cyclic voltammograms of fumaric acid in xylose medium is similar to supernatant of anolyte (Fig. S3). When the anolyte was replaced with the fresh xylose medium in the MFC, the discharging current dropped significantly (Fig. S4). The addition of fumaric acid in (1 mg mL^{-1}) results in a fast increase of the discharge current as well as the current plateau. The results indicate that fumaric acid is playing an important role in the catalytic process of this xylose MFC.

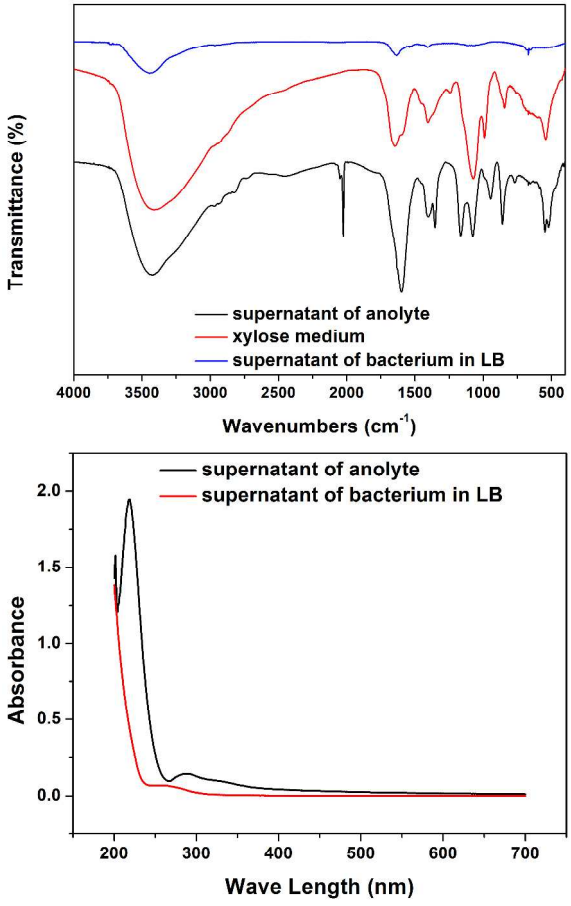

Fig. 5 a: FTIR spectra of the supernatant of the anolyte, xylose medium and the supernatant of bacteria cultures. b: UV-Vis absorption of anode supernatant and the supernatant of bacteria cultures.

Mechanism for high power generation performance of *Ochrobactrum sp. 575* MFC

According to the results and analysis the high power output of Generation III *Ochrobactrum sp. 575* MFC should be attributed to the fast digestion of xylose by the cells and fumaric acid produced from the evolved cells for a fast extracellular electron transfer. The accumulation of fumaric acid in the MFC anode suggests a succinate involved respiratory chain of *Ochrobactrum sp. 575* cells. In microorganism cells there are two types of respiratory chains. One involves NADH oxidation, which is found in most microorganisms, and the other one includes succinate oxidation (Fig. 6), of which the main difference is due to their different electron donors, NADH or succinate. When succinate is used as the electron donor, fumaric acid generates[19, 20]. It has been proved that fumaric acid is the metabolic intermediate of many strict anaerobe and facultative anaerobe [21]. The observed fumaric acid in the xylose MFC anode indicates that the succinic acid rather than NADH could be an important electron donor for the electron transport. The relationship between the electrocatalytic activity of the bacteria strain and the change of its electron transport profile is still under investigation.

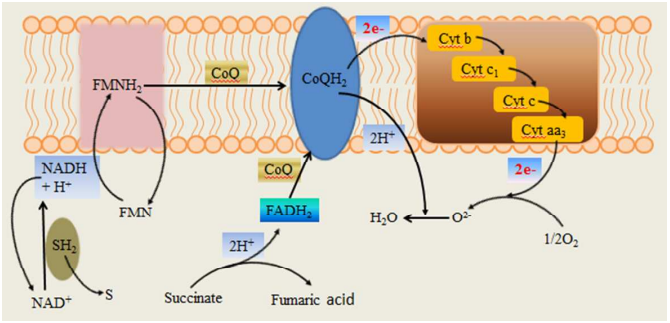

Fig. 6 Schematic diagram of two main respiratory chains.

Conclusions

In this work, electrocatalytic behaviors of *Ochrobactrum sp.* 575 for MFC anode was investigated and a power density of 2625 mW/m^3 was achieved. It is significant improvement for a single strain catalyzed MFC in comparison to the reported mixed culture catalyzed MFCs. Accumulation of fumaric acid in the anodic chamber is found during the progress of xylose oxidation by *Ochrobactrum sp.* 575 cells. This work provides a new bacteria strain for MFC to harvest electricity while resolving the xylose, and offers a new strain for fundamental studies of electron transfer-involved mechanisms in a bioelectrocatalytic process.

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Notes and references

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