

BIOGENIC PALLADIUM NANOPARTICLES FOR IMPROVING BIOELECTRICITY GENERATION IN MICROBIAL FUEL CELLS

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ABSTRACT

Electroactive bacteria with in-situ biogenic palladium nanoparticles increased power density of a microbial fuel cell (MFC) by 75%. The palladium nanoparticles were biosynthesized through bioelectrochemical reduction by the bacteria and remained bound to the cell membrane, facilitating bacterial extracellular electron transfer at the cell-electrode interface. This work revolutionizes knowledge of how bacteria biosynthesize metallic nanoparticles during microbial metabolism and introduces a novel bottom-up approach to fabricate a microbial electrochemical device for renewable energy production in a more eco-friendly and cost-effective way.

KEYWORDS

Biogenic palladium nanoparticles; bioelectrochemical reduction; biosynthesis; microbial fuel cells

INTRODUCTION

Electromicrobiology is an emerging field of study that investigates microbial electron exchange with external electrodes and microbial electrochemical functionalities [1, 2]. Significant advances in electromicrobiology have occurred, led by impressive discoveries of “Electroactive bacteria,” which are microorganisms capable of direct electron transfer to electrodes [3]. By incorporating electroactive bacteria into a microbial fuel cell (MFC), the combined biotic-abiotic system offers a solution for environmental sustainability by generating renewable bioelectricity with organic waste while producing value-added chemicals/biofuels [4, 5]. Despite its vast potential, however, the technology’s promise has not yet been translated as application because of its low power generation [6, 7].

Bacterial metallic nanoparticles synthesized from bacterial metabolism are garnering considerable attention because of their high potential for facilitating microbial extracellular electron transfer [8-10]. In particular, palladium nanoparticles (Pd-NPs) have a great potential as an electrocatalyst with remarkable catalytic performance [11]. Several bacterial species such as *Desulfovibrio desulfuricans*, *Escherichia coli* and *Geobacter sulfurreducens* are capable of transforming soluble Pb(II) into Pd-NPs, relying mainly on their ability of extracellular electron transfer [8, 12]. However, the reported work about *in situ* formed Pd-NPs for improving electricity generation in MFCs is either unavailable or quite limited.

In this work, we demonstrated the effect of *in situ* formed conductive Pd-NPs on the power generation in a paper-based MFC. Pd-NPs were biosynthesized directly on the surface of *Shewanella oneidensis* MR-1 through their electrochemical reduction of $\text{Na}_2(\text{PdCl}_4)$ solution [13]. Then the bacterial cells with Pd-NPs were applied to produce bioelectricity in the MFC (Fig. 1). The paper-

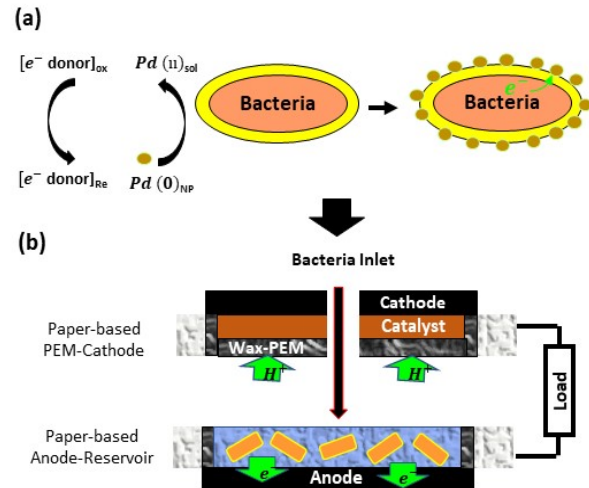


Figure 1: (a) General reaction schemes of the biosynthesis of Pd-NPs via bacterial metabolism. (b) Schematic diagram of the paper-based MFC

based MFC can be especially beneficial for single-use applications because it offers the transformative potential of low-cost, simple, and disposable electronics for use in resource-limited environments.

MATERIALS AND METHODS

Materials

Sodium tetrachloropalladate ($\text{Na}_2(\text{PdCl}_4)$), sodium formate (HCOONa), tryptone, yeast extract, sodium chloride (NaCl), dimethyl sulfoxide (DMSO), glutaraldehyde solution, phosphate buffer saline (PBS) and 3-glycidyloxypropyltrimethoxysilane were purchased from Sigma-Aldrich. Poly(3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT:PSS) (Clevios PH1000) was purchased from Heraeus. Conductive graphite ink (#E34561000G) was purchased from Fisher Scientific Company, LLC. Whatman™ Grade 3MM chromatography paper and Ag_2O (AA11407-14) were obtained from VWR International, LLC.

Preparation of paper-based MFCs

MFC devices were fabricated using a wax printing technique (Fig. 1b). Specific wax patterns were designed using AutoCAD software and printed out onto the chromatography paper using Xerox Phaser printer (ColorQube 8570). Then, the printed paper was placed in an oven set at 150 °C for 30s to melt the hydrophobic wax into the paper substrate, defining device boundaries, forming proton exchange membranes, and strengthening the paper while retaining its flexibility. The conductive anodic reservoirs were formed by injecting a 20 μL mixture

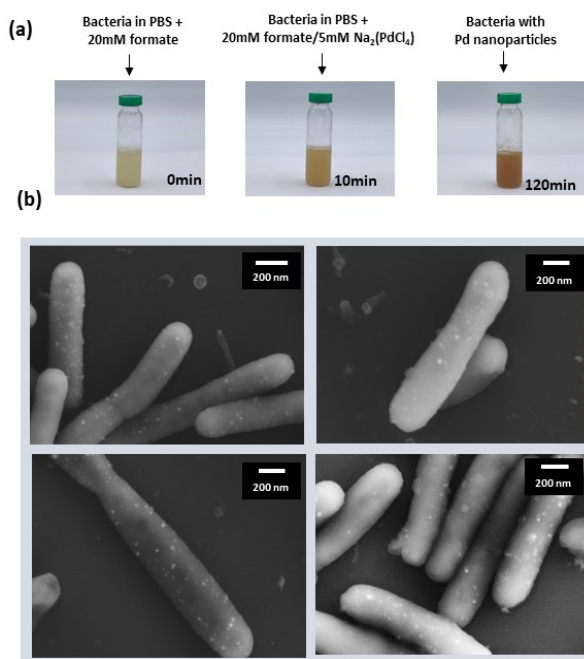


Figure 2: (a) Biological synthesis of Pd nanoparticles, and (b) SEM images of *Shewanella* Sp. with synthesized Pd nanoparticles.

of 1 wt% PEDOT:PSS and 5 wt% DMSO into the defined anodic reservoir [14]. To further increase anode reservoir hydrophilicity, 20 μ L of 2 wt% 3-glycidoxypolytrimethoxysilane was added to the reservoir and air-dried. The solid-state cathode was prepared by mixing 500 mg of Ag₂O in the 10mL of PEDOT:PSS solution. Finally, the graphite ink was screen-printed on top of the anodic reservoirs and cathodic wells through the paper-based masks.

Bacterial Inoculum and In Situ Reduction of Pd (II)

Shewanella oneidensis MR-1 were grown from -80°C glycerol stock cultures by inoculating in 20mL of L-broth medium with gentle shaking in air for 24h at 35°C. The L-broth media consisted of 10.0g tryptone, 5.0g yeast extract and 5.0g NaCl per liter. Broth cultures were then centrifuged at 5,000rpm for 5min to remove the supernatant. To biosynthesize the Pd-NPs, the bacterial cells were washed three times with PBS (Phosphate-buffered saline) solution and then were resuspended in 6 ml of deoxygenated PBS to be adjusted to OD₆₀₀ of 2 (Optical density at 600nm). The Pd (II) in the form of Na₂PdCl₄ at 5mM were added to the bacterial culture. Then, 20 mM sodium formate was introduced to the solution as an electron donor. Finally, the solution was sealed and incubated at 30 °C for 2h (Fig. 2a).

Electrical measurement setup

We measured the potential difference between the anodes and the cathodes with a data acquisition system (National Instruments, USB-6212), and recorded the readings every 1 min. via a customized LabView interface. The current through an external load was calculated via

Ohm's law and the output power was calculated via $P=V \times I$. Power and current densities were normalized to the anode area. MFC polarization curves and power outputs were derived and calculated based on the current value at a given external resistance (1 M Ω , 0.5 M Ω , 248 k Ω , 68 k Ω , 47 k Ω , 22 k Ω , 10 k Ω , 5k Ω , 3.3 k Ω , 2.2 k Ω , 1k Ω , 0.5 k Ω , 330 Ω , and 220 Ω).

Bacterial fixation and SEM imaging

The paper-based MFCs were disassembled and washed gently with 0.1 M PBS and adherent bacteria on the anode were immediately fixed using 4% glutaraldehyde solution overnight at 4 °C. Then, samples were washed with PBS and dehydrated by 5-min serial transfers through 50, 70, 80, 90, 95, and 100% ethanol. The prepared samples were placed in hexamethyldisilazane (HMDS) for 10 min, followed by air drying overnight. The fixed samples were examined using a FESEM (Field Emission SEM) (Supra 55 VP, Zeiss).

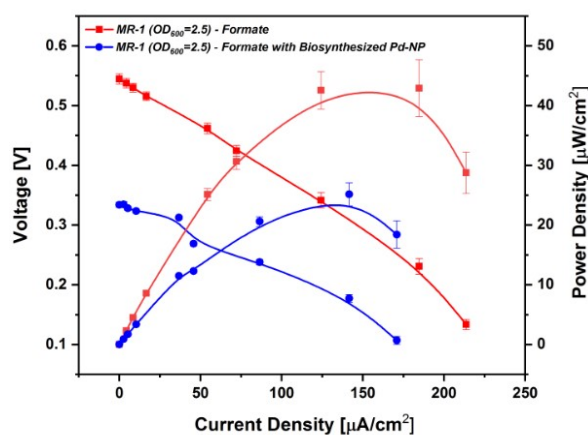


Figure 3: Power outputs and polarization curves of the MFCs powered by the bacteria with and without Pd-NPs.

RESULTS AND DISCUSSION

Biosynthesis of Pd-NPs

The biosynthesis of Pd-NPs by *S. oneidensis* MR-1 is shown in Fig. 2a. The reduction of Pd (II) to Pd-NPs was confirmed by observing the change in color from light yellow to dark brown. Figure 2b demonstrates that the biosynthesized Pd-NPs remained bound to the bacterial cell membrane.

Effect of Pd-NPs on MFC power generation

The effect of biosynthesized Pd-NPs on the electricity generation in *S. oneidensis* MR-1 was investigated by measuring power/current densities from paper-based MFCs. Previously, our group developed many paper-based MFCs for portable power sources, as well as for studies of the bacterial electrogenicity [15, 16]. The paper substrates allowed for rapid adsorption of bacterial samples, leading to fast electricity generation [17, 18]. Here, two-layer paper MFCs were used for the experiments [19].

The maximum power density of the MFCs without Pd-NPs was 25.12 μ W/cm² $\pm$ 1.93, while the device with *in*

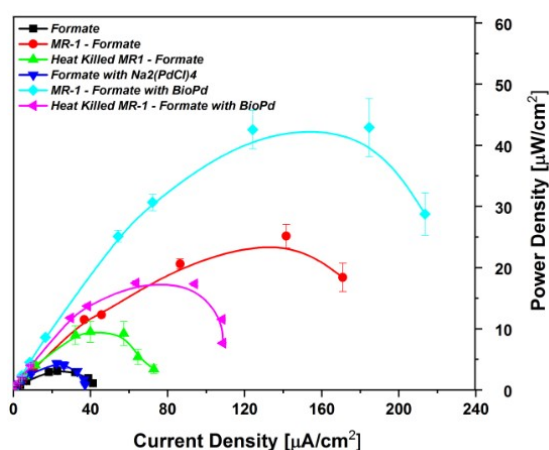


Figure 4: Power outputs and polarization curves of the MFCs powered by various samples.

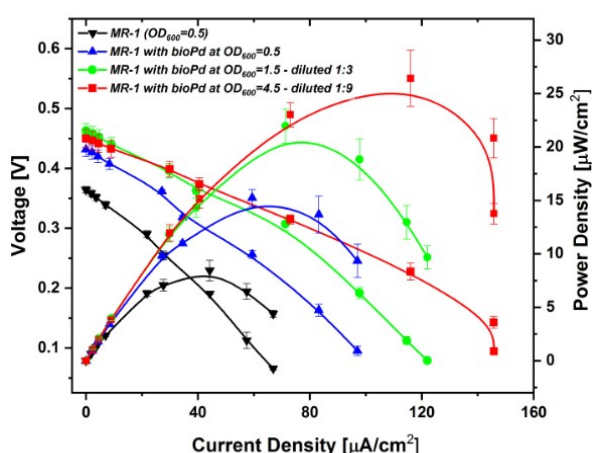


Figure 5: Power outputs and polarization curves of the MFCs powered by the bacteria with Pd-NPs biosynthesized with different bacteria concentrations

situ formed Pd-NPs generated $42.90 \mu\text{W}/\text{cm}^2 \pm 4.74$ (Fig. 3). The results indicate that the presence of the Pd-NPs on the surface of the cell membrane increases the conductivity and electron transfer rate of the bacteria.

Figure 4 shows the power and current densities from the MFCs with various samples including sodium formate with or without bacteria, dead bacteria in formate with or without Pd-NPs, sodium formate with $\text{Na}_2(\text{PdCl}_4)$, and bacteria with Pd-NPs in formate. The bacteria with Pd-NPs in an aqueous formate solution as an electron donor significantly increased the power generation. Furthermore, the results show that Pd-NPs on the dead bacterial cells generates certain amount of power due to their electrochemical reactions with formate but its value was much lower than that of live bacteria. This indicates that the power generation with nanoparticles can be boosted through bacterial metabolism. Furthermore, Figure 4 demonstrates that sodium formate and $\text{Na}_2(\text{PdCl}_4)$ without bacteria are unable to harvest energy.

The Pd-NPs were biosynthesized with three different bacterial concentrations: 0.5, 1.5 and 4.5 of OD_{600} . After biosynthesis, each solution was adjusted to have the same

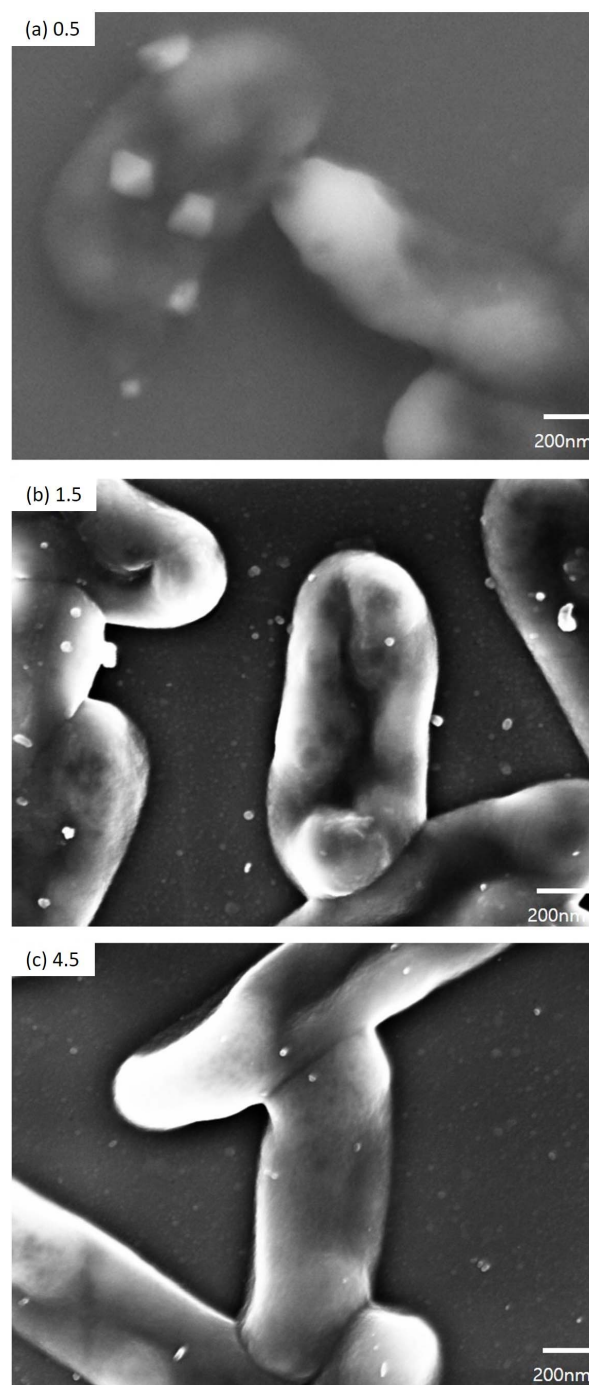


Figure 6: SEM images of bacterial biogenic nanoparticles biosynthesized at different bacterial concentration: (a) 0.5, (b) 1.5, and (c) 4.5 of OD_{600}

bacterial concentration for power comparison (Fig. 5). The MFC with 4.5 OD_{600} sample generated the highest power density of $26.42 \mu\text{W}/\text{cm}^2 \pm 2.62$, while the MFCs with 0.5 OD_{600} sample produced the lowest power density of $8.44 \mu\text{W}/\text{cm}^2 \pm 0.97$. The bacterial concentration during the biosynthesis significantly affected the MFC power performance mainly because of the size of Pd-NPs. Larger Pd-NPs were observed with higher bacterial concentrations used in biosynthesis (Fig. 6).

CONCLUSION

In this work, Pd-NPs were successfully in situ biosynthesized in *Shewanella oneidensis* MR-1 culture to improve electricity generation in a paper-based MFC. The power density of the MFC was increased by 75% after Pd-NPs remain bound to the cell membrane. After Na₂PdCl₄ introduction, the SEM image clearly showed the particles on the cells and the color of bacterial culture changed from light yellow to dark brown, indicating the formation of Pd-NPs. *S. oneidensis* showed the ability to produce Pd-NPs in different sizes, which significantly affected bacterial extracellular electron transfer at the cell-electrode interface. This work provides a simple but powerful strategy for improving the electricity generation of MFCs with *in situ* biogenic nanoparticle synthesis.

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