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Adjustable bidirectional extracellular electron transfer between *Comamonas testosteroni* biofilms and electrode via distinct electron mediators

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ABSTRACT

Bidirectional extracellular electron transfer of strain *Comamonas testosteroni* I2 was for the first time investigated with electrochemical active biofilms developed under different conditions. The electrochemical active biofilm developed under microbial fuel cell conditions was capable of anodic electron transfer via attached redox species with standard potential of 0.04 V (vs. SCE). Meanwhile the above redox species lost its catalytic capability when the biofilm was developed under a constant potential (−0.4 V vs. SCE). Instead, the microbe adjusted its electron transfer strategy to a soluble shuttle (standard potential −0.20 V vs. SCE) and enabled a cathodic current. Air exposure experiment verified that the soluble shuttle at negative potential had a positive response to the oxygen; meanwhile the anodic electron transfer via the attached species was rarely influenced.

1. Introduction

Bioelectrochemical (BEC) technologies have attracted vast interests in the past decade because of their potential applications in energy generation, bioremediation and chemical synthesis [1–4]. They stand out from other chemical and biological processes for their distinctive use of electrochemically active microorganisms as the "live catalysts" to simultaneously achieve chemical transformation and extracellular electron transfer (EET). Few electrochemically active bacteria (EAB) belong to *Geobacter* species and *Shewanella* species have been extensively studied in terms of EET mechanisms. However, the development of BEC platforms requires a wide range of EAB for selection based on electrochemical characteristics and specific applications. Thus, it is of great significance to identify new EAB and characterize their electrochemical activity [5–7].

Comamonas testosteroni (*C. testosteroni*) is a Gram-negative bacterium that was found to be enriched in the anode community in microbial fuel cells (MFCs) inoculated with domestic wastewater or anaerobic sludge [8–13]. The "outward" EET capability, i.e., flow of electrons toward the electrode, of *C. testosteroni* has been implied by previous studies in which the bacterium was used as a pure culture inoculum in MFC anode [14]. In addition, *C. testosteroni* has recently been found to dominate in MFC biocathode, suggesting that this microorganism could potentially mediate "inward" EET, i.e., consuming electrons from the electrode at an appropriate potential [15]. However, the EET mechanisms of *C. testosteroni* biofilms interacting with electrodes are largely unexplored, especially how the "outward" and "inward" EET differs under distinctive electrochemical conditions.

In this study, the bidirectional EET mechanisms of *C. testosteroni* were for the first time investigated through electrochemical and biochemical analyses with electrochemical active biofilms developed under different conditions. The "outward" EET was examined using MFCs with *C. testosteroni* biofilms on the anode , while the "inward" EET was elucidated using biofilms developed on the electrode poised at −0.4 V (vs. SCE) in a three-electrode system.

2. Experimental

2.1. Strains and culture conditions

Cultures of *C. testosteroni* I2 (LMG19554 from BCCM/LMG) were prepared in LB broth at 30 °C and 200 rpm. Cells were harvested by centrifugation at 4000 rpm for 10 min and then resuspended in M9

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salt medium [16], with 10 mM acetate (CH_3COONa) as carbon source. The cell suspension was inoculated into chambers with a ratio of 1:1 (v/v).

2.2. Electrochemical active biofilm development

A three-electrode single chamber system was adopted to develop the electrochemical active biofilm (biofilm A) under controlled potential (−0.4 V vs. SCE), in which 20 mM sodium nitrate was added as electron acceptor. Meanwhile, two-chamber MFC was set up and operated following our previous studies to achieve an electrochemical active biofilm developed under microbial fuel cells (biofilm B), in which the ferricyanide in cathode chamber acted as the final electron acceptor [17]. For both conditions, carbon cloth (GasHub, Singapore) with dimension of 3 cm × 4 cm was used as the working electrode. Twisted platinum wire electrode (Metrohm, Singapore) and saturated calomel electrode (SCE, +0.243 V vs. SHE) were used as counter and reference electrodes in three-electrode system. Biofilm C as control was developed in the same set-up conditions (chamber, electrode, inoculum and medium) as the three-electrode system for biofilm A but no potential was poised. Scheme 1A and B are the illustration of how biofilm A and B were developed.

2.3. Electrochemical and biochemical analysis

After the electrochemical active biofilms were successfully developed, biofilm A and B were conducted electrochemical analyses in their original chamber with the following sequence: cyclic voltammetry (CV) analysis with scanning rates of 50 mV/s and 1 mV/s, followed by short term chronoamperometry analysis at −0.4 V and +0.2 V.

To examine the influence of oxygen, the MFC anode was bubbled with air for 10 min. Then the chamber was sealed and scanned at 50 mV/s for half an hour, thus achieving air-exposed biofilm B. CV at 1 mV/s and short term chronoamperometry were conducted as described above. All of the voltages in this manuscript are referred to SCE, unless otherwise indicated and all the electrochemical analyses were conducted with Autolab PGSTAT 128N system.

To characterize biofilms on the carbon cloth, the biofilms were stained using LIVE/DEAD BacLight Bacterial Viability Kit L7012 (Molecular Probes, Inc.) and visualized by Carl Zeiss Confocal Laser Scanning Microscopy (CLSM) LSM 780 [18]. Samples collected from chambers with biofilm A and C were filtered through 0.20 μm filters (Pall Corporation, Singapore) before acetate quantification using HPLC (Shimadzu, Singapore) as described in previous work [19,20].

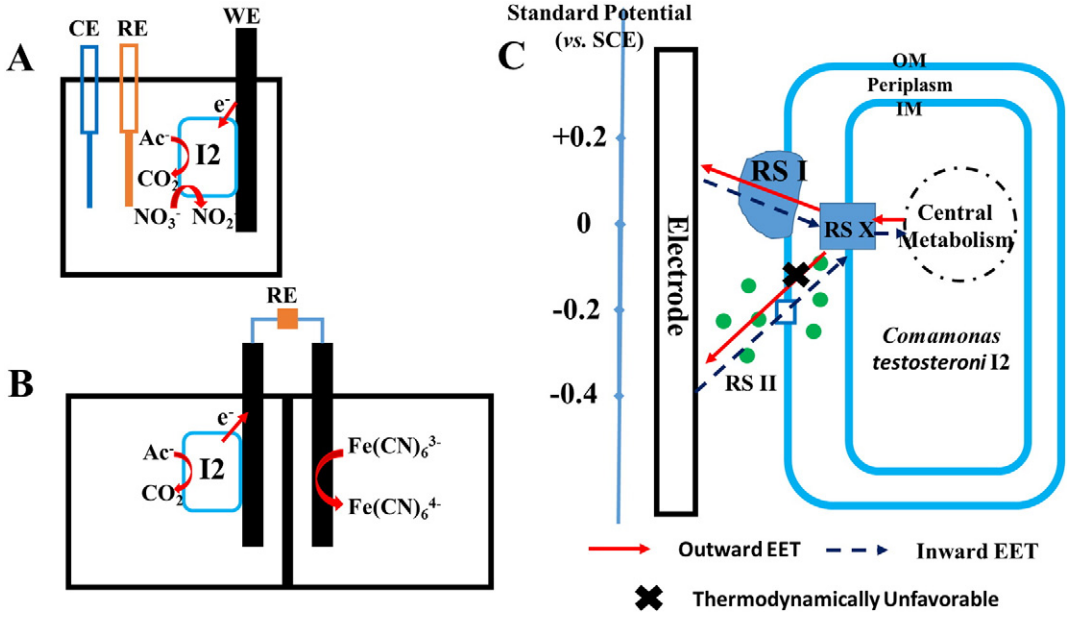
3. Results and discussion

3.1. C. testosteroni I2 capable of bidirectional electron transfer

The chronoamperometry result of *C. testosteroni* I2 in three-electrode system with a constant potential of −0.4 V is shown in Fig. 1A. Negative current was observed from the very beginning, implying that the microorganism consumed electrons through an "inward" EET pathway. Low cathodic current of −0.8 μA was observed for the first 20 h and then gradually increased and reached the maximum value of −32 μA. Bioelectricity was generated when *C. testosteroni* was used as the pure culture anode inoculum in MFC (Fig. 1B). Similar trend was observed as the absolute current was low in the first 20 h and then gradually increased to 11.4 μA after 80 h.

3.2. "Outward" and "inward" EET achieved via different redox species

CV analysis was conducted to study the electrochemical behavior with well-developed biofilm A and biofilm B. In the CV profile of biofilm A at a scanning rate of 1 mV/s, one peak (RS I) at 0.1 V in anodic curve can be identified, with small catalytic response in the cathodic curve below 0 V (Fig. 2A, blue profile). Strong catalytic current starting from −0.11 V was confirmed in the cathodic profile with a sigmoid shape, which was consistent with the chronoamperometry results at −0.4 V and implied the existence of another redox species (RS II). Fig. 2B (blue profile) shows the CV of clean carbon cloth electrode in three-electrode system anolyte after biofilm A development, from which one pair of reversible peaks centered at −0.20 V can be found (RS II). It evidenced that RS II is a soluble mediator secreted by *C. testosteroni* I2. The transformation from sigmoid shape (Fig. 2A) to reversible peaks (Fig. 2B) in the CV profile of RS II verified that the electron consumption process required the participation of biofilms on the



Scheme 1. (A) Three-electrode system for biofilm A development; (B) microbial fuel cell for biofilm B development; (C) proposed bidirectional EET pathways of *Comamonas testosteroni* I2.