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Cathodic Cr(VI) reduction by electrochemically active bacteria sensed by fluorescent probe

Hua Xue [a], Peng Zhou [b], Liping Huang [a,*], Xie Quan [a], Jinxiu Yuan [a]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering, Ministry of Education (MOE), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China
[b] College of Chemistry, Dalian University of Technology, Dalian 116024, China

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ABSTRACT

Biocathode microbial fuel cells (MFCs) have been shown to be useful in reductively changing Cr(VI) into Cr(III)-related precipitates on the surface of electrochemically active bacteria (EAB), but the occurrence of intracellular Cr(VI) reduction in the EAB are still unclear. Based on the four EAB isolated from Cr(VI)-reduced biocathode MFCs and tentatively assigned to *Stenotrophomonas* sp. YS1, *Stenotrophomonas maltophilia* YS2, *Serratia marcescens* YS3 and *Achromobacter xylosoxidans* YS8, a naphthalimide-rhodamine based Cr(III) fluorescent probe was used for the first time to sense the intracellular Cr(III) ions, inevitably and transiently formed from Cr(VI) reduction in these EAB. More Cr(VI) was reduced in the MFC biocathodes, resulting in intracellular accumulation of total chromium of 45.1 ± 1.3–60.5 ± 1.4% with a composite of Cr(III) ions (23.7 ± 2.6–27.3 ± 1.1%) and other forms of chromium-complex (18.7 ± 2.5–32.2 ± 0.8%). In the open circuit controls, intracellular total chromium only ranged 10.2 ± 1.5–11.7 ± 0.8% (Cr(III) ions: 8.2 ± 0.6–9.5 ± 0.9%; other forms: 0.2 ± 0.6–0.3 ± 0.9%). This study illustrates that cathode directed more intracellular Cr(VI) reduction in the EAB and gives an alternative approach for developing imaging and functionally sensing Cr(III) ions in the MFC biocathodes.

1. Introduction

Hexavalent chromium [Cr(VI)] is a priority toxic chemical present in wastewaters from electroplating, pigment, and lumber and wood product processes. Its contamination of the soil and groundwater is a significant environmental hazard, especially in developing countries [1]. The newly developed self-generating biocathode microbial fuel cells (MFCs) that function on the basis of electron transfer from cathode electrodes to electrochemically active bacteria (EAB), have received more attention for Cr(VI) removal due to their self-sustaining, low-cost, low-maintenance as well as very little sludge generation [2–8]. Although Cr(VI) is believed to be changed into Cr(III)-related precipitates of $Cr(OH)_3$ and Cr_2O_3 on the surface of cathodic EAB [2,4–7], there is still lack of illustration about the occurrence of intracellular Cr(VI) reduction in the EAB. Sensing the fate of Cr(VI) reduction in these EAB is beneficial for understanding the role of reductive cathode in Cr(VI) reduction and the subsequent clarification of relationships among cathode, EAB and final electron acceptor of Cr(VI) in the MFCs.

Multiple chemical or physical approaches can quantify Cr(III) precipitates produced from Cr(VI) reduction, including global kinetic analysis techniques, titrimetry, spectrophotometry, fluorimetry, chromatography, flame atomic absorption spectrophotometry, inductively coupled plasma atomic emission spectroscopy, X-ray absorption near edge structure, X-ray photoelectron spectroscopy and transmission electron microscope equipped with an energy-dispersive X-ray analyzer [9–11]. Confirmation of products of bacterial reduction for Cr(VI) is additionally based on physiological analysis of wild-type and mutant strains stressed by Cr(VI) as well as the response of global gene and protein expression of microbes by the transcriptomic and proteomic methods [1]. However, these techniques employed are not able to determine the exact phase of the Cr(III) ions and thus give little information about the fate of Cr(III) ions in the EAB. Approaches of efficiently and accurately sensing the Cr(III) ions in the EAB are thus in great need.

Metal fluorescent probes are highly selective and sensitive as a powerful tool for mapping heavy metals in living cells because

* Corresponding author.
E-mail address: lipinghuang@dlut.edu.cn (L. Huang).

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of excellent photophysical properties and good biocompatibility in addition to the operational simplicity, nondestructive methodology, high sampling frequency and low cost of equipment, as well as direct visual perception [12,13]. Rhodamine based Cr(III) fluorescent probes possess merits of fluorescence enhancement compared to others of fluorescence quenching [12,14–16]. These probes can bind specifically and selectively to Cr(III) ions in the presence of large excess of other competing ions with associated changes in its optical and fluorescence spectral behavior. Since these rhodamine based Cr(III) fluorescence probes have been used to sense Cr(III) ions in mammalian cells and organisms such as human cancer living cells and zebrafish organs [13–15], it would be expected to sense Cr(III) ions in the EAB from Cr(VI)-reduced MFCs, which to the authors' knowledge, has not been previously reported.

Following the sensitive recognition of fluorescent probe to Cr(III) ions, the EAB cells are necessarily sectioned to further confirm the distribution of Cr(III) ions in the EAB. Bacterial cellular locations of chromium can be divided into membrane-associated, and periplasmic and intracellular fractions, where Cr(VI) is reductively changed into other forms of Cr(III)-precipitates and chromium-organ via the transiently formed and hardly measured Cr(III) ions [1,9,10,17,18]. Cell membrane, and periplasmic and intracellular fractions are generally prepared through ultrasonic cell disruption and centrifugation [19,20]. In parallel, periplasmic fraction can be acquired through osmotic shock, which is an effective method with the lowest contamination to release periplasmic inclusions in Gram negative bacteria [21,22]. Since the solid cell membrane generally harbors chromium-complex instead of dissolved Cr(VI) and Cr(III) ions [10,17,18], chromium-complex associated with the bacterial cell membrane is reasonably equivalent to total chromium. For periplasmic and intracellular fractions, the chromium-complex can be obtained from the differences between total chromium, and dissolved Cr(VI) and Cr(III) ions. While total chromium and dissolved Cr(VI) associated with these cellular sections can be obtained through standard methods [23], quantifying the Cr(III) ions in the periplasmic and intracellular fractions of the EAB based on the sensitive Cr(III) fluorescence probes is expected to clarify cellular locations of these various valence states of chromium in the EAB.

In this study, we chose one naphthalimide-rhodamine based Cr(III) fluorescent probe [16] and evaluated its application for the direct sensing of Cr(III) ions and the subsequent mapping of various valence states of chromium in four EAB isolated from the Cr(VI)-reduced biocathodes of MFCs. Using single strains allow us to directly understand the EAB at the electrode surface for Cr(VI) reduction [3,8] whereas comparing the EAB behaviors is beneficial for understanding the distribution of various valence states of chromium in the EAB.

2. Materials and methods

2.1. Sampling and EAB isolation

Bacterial isolates were obtained from the biocathodes of MFCs fed with Cr(VI) [7] by streaking cathode scrapings onto agar plates containing anaerobic medium amended with agar, Cr(VI) (385 μM) and $NaHCO_3$ (119 μM) under anoxic conditions in an anaerobic glove box. Anaerobic medium contained NH_4Cl (5794 μM), KCl (1743 μM), $MgCl_2{\cdot}6H_2O$ (885 μM), $CaCl_2{\cdot}2H_2O$ (612 μM), KH_2PO_4 (11.0 mM), $K_2HPO_4{\cdot}3H_2O$ (12.7 mM) and 1 mL of trace elements [24]. Incubation of agar plates and bacterial isolation procedure were described in SM. After the finish of EAB isolation, EAB isolates were sub cultured on the same medium but without Cr(VI) and similarly incubated on the agar plates as described in SM and prepared for subsequent experiments.

2.2. Isolates identification

DNA was extracted using Ezup DNA kit (cat. No. SK8255, Sangon Biotech (Shanghai) Co. Ltd., China) based on the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal primers 518F (5′ CAGAGTTTGATCCTGGCT3′) and 1540R (5′AGGAGGTGATCCAGCCGCA3′). PCR solution components, amplification, purification, clone procedure and sequencing were described in SM. The 16S rRNA gene sequence data were compared with the GenBank database using the Blast server at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) to accurately identify the bacterial strains.

2.3. MFC operation with isolates

Identical two-chamber MFCs consisted of an anode and a cathode placed in a plastic (Plexiglas) cylindrical chamber of 2 cm in length and 3 cm in diameter were assembled and tested. Porous graphite felt (each piece: 1.0 × 1.0 × 0.5 cm, 8 pieces, Sanye Co., Beijing, China) was inserted with carbon rods and used for the cathodes and anodes. The anode and cathode chambers were separated by a cation exchange membrane (CEM) (CMI-7000 Membranes International, Glen Rock, NJ) with a projected surface area of 7.1 cm^2. An external resistance of 510 Ω was always used during MFC operation. The anodes were similarly inoculated using the effluent of MFCs well acclimated to acetate (12.2 mM). The cathodes were sterilized by autoclaving at 121 °C for 20 min, and then inoculated with the isolates (6 mL with 10^8 CFU/mL). These isolates were enriched as described in SM. Both the anodes and cathodes were fed with the anaerobic medium mentioned above except acetate was replaced by $NaHCO_3$ (119 μM) in the catholyte to provide an inorganic carbon source for the bacterial isolates, with Cr(VI) added to the cathodes at a concentration of 385 μM. Prior to adding the solutions into the electrode chambers, the anolyte and catholyte were sparged with N_2 gas for 15 min. A reference electrode (Ag/AgCl, 195 mV vs. SHE) was installed in the cathode chamber to measure cathode potential, with all potentials reported here vs SHE. All inoculation and solution replacements were performed in anaerobic glove box (YQX-II, Xinmiao, Shanghai). The initial pH in catholyte was adjusted to 5.8 using 2.4 M HCl in order to provide pH conditions that were not detrimental to bacterial growth as well as beneficial for Cr(VI) reduction [9]. The final pH at each fed batch cycle operation slightly increased to 6.0 ± 0.1, guaranteeing the reduction of Cr(VI) and Cr(III)-associated precipitates on the biocathodes [5–7]. Solution conductivities in both anolyte and catholyte were similarly adjusted to be 5.8 mS/cm using 0.1 M KCl. A higher solution conductivity decreases MFC internal resistance and thus improves system performance [25]. However, the physiology and growth of the EAB only require a certain level of solution conductivity [26]. This 5.8 mS/cm in the anode and cathode chambers was thus one balance, which not only was appropriate for respiration of EAB on the anodes and cathodes, but also less affected system performance. For each inoculation, data were collected at 3rd–5th batch cycle operation to exclude possible effects of initial adsorption and system deterioration due to Cr(VI) reduction products accumulation over time [27,28]. At the end of each cycle operation, catholyte was completely refreshed. Other operation conditions, control experiments and duplicate reactor analysis were described in SM.

2.4. Preparation of fluorescent probe and its specific selectivity to Cr(III)

The naphthalimide-rhodamine based fluorescent probe was exactly prepared as Mao et al's report [16]. For each experiment unless otherwise stated, a 6 μL liquid of probe (dissolved in 10 mM