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Imaging and distribution of Cd(II) ions in electrotrophs and its response to current and electron transfer inhibitor in microbial electrolysis cells

Liping Huang[a,*], Hua Xue[a], Qian Zhou[a], Peng Zhou[b], Xie Quan[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 electrolysis cells (MECs) with electrotrophs on the cathodes are proved to be attractive for Cd(II) removal with simultaneous hydrogen production. However, the underlying roles of current and electron transfer inhibitor of 2,4-dinitrophenol (2,4-DNP) in the distribution of dissolved Cd(II) ions in the electrotrophs are largely unknown. Based on four indigenous electrotrophs (*Ochrobactrum* sp X1, *Pseudomonas* sp X3, *Pseudomonas delhiensis* X5 and *Ochrobactrum anthropi* X7) isolated from well developed Cd(II)-removal biocathodes of MECs, a quinoline-based Cd(II) fluorescent probe was used to imaginably and quantitatively map Cd(II) ions in electrotrophs, and its response to current and 2,4-DNP in MECs. Current directed the distribution of Cd(II) ions in the electrotrophs with the occurrence of an initial quicker import and the subsequent quicker export, both of which were somewhat inhibited by 2,4-DNP. In terms of total cadmium, current always directed less cadmium in catholyte and electrotrophs with independence of the presence of 2,4-DNP. This study gives an alternative approach for developing imaging and mapping Cd(II) ions in the electrotrophs, and thus benefits for clarifying the roles of current and 2,4-DNP in electrotrophic removal of Cd(II) ions from catholyte in MECs.

1. Introduction

Heavy metal of Cd(II) typically found in the wastewaters from electroplating, inorganic pigment, and battery manufacturing industries, is known to toxic and carcinogenic to living organisms when discharged to the environment. Conventional biological processes can be used either completely, or coupled with traditional physical-chemical processes for Cd(II) removal in contaminated sites [1,2]. Recently, biocathode microbial electrolysis cells (MECs) with self-generating and sustainable electrotrophs on the cathodes [3–7] are proved to be effective for Cd(II) removal with simultaneous hydrogen production [8,9]. However, the underlying role of current in the distribution of dissolved Cd(II) ions in the electrotrophs of the metallurgical biocathodes are largely unknown. In addition, bacteria are known to develop an active ATPase-consumed system for pumping Cd(II) ions from cytoplasm, through the periplasm and across the outer membrane to the external solution, which can be inhibited by an electron transfer inhibitor of 2,4-dinitrophenol (2,4-DNP) [1,10]. In terms of biological hydrogen production, 2,4-DNP is proved to inhibit the activity of hydrogenase and thus decreased hydrogen production by both purple non-sulfur bacterium *Rhodobacter sphaeroides* sp. MDC6522 and cyanobacteria *Cyanothece* sp. Miami BG 043511 [11,12]. Issues regarding responses of the electrotrophic inward dissolved Cd(II) ions and hydrogen production in these MECs to 2,4-DNP and current remain open to question, which would be of vital importance for understanding the role of electrotrophs in Cd(II) removal in MECs [13].

Different valence forms of cadmium in bacteria have not been well addressed, let alone those in the electrotrophs of MECs. Besides dissolved Cd(II) ions, other forms of cadmium located in bacterial cell membrane, periplasm and cytoplasm must be taken into consideration due to the always occurrence of complexation of cadmium with peptides (metallothioneins) in cytoplasm and periplasm, and the precipitation at the cell surfaces with bacterial release specific chemicals [10,14]. To date, in most cases, species of cadmium in bacterial cells is largely unclassified and cadmium in bacterial cells is generally determined by

* Corresponding authors.
E-mail addresses: lipinghuang@dlut.edu.cn (L. Huang), quanxie@dlut.edu.cn (X. Quan).

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atomic adsorption spectrometry after the samples were chemically treated by EDTA, lysozyme or HNO_3-$HClO_4$ [1,9,10,15]. In terms of other forms of cadmium, multiple approaches including electrospray ionization-mass spectrometry (EIS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), X-ray absorption near edge structure (XANES), X-ray photoelectron spectroscopy (XPS) and transmission electron microscope equipped with an energy-dispersive X-ray analyzer (TEM-EDX) can confirm cadmium-complex speciation [16,17]. However, these techniques employed fail in providing quantitative real-time tracking of the exact phases of dissolved Cd(II) ions and the subsequent other forms of cadmium, and thus give little information about the fate of different forms of cadmium in the electrotrophs and its response to current and 2,4-DNP in MECs.

Fluorescent probes is a unique approach for imaging and sensing targets in living cells due to their rapid response, high selectivity, excellent sensitivity and good biocompatibility in addition to the operational simplicity, nondestructive methodology, high sampling frequency, low cost of equipment and real-time detection [18]. The observation of cellular sectional part rather than outside surface through confocal laser scanning microscopy (CLSM) and ImageJ software analysis enables to confirm the successful penetration of probe into the cell inside [18,19]. Living bacteria such as *Escherichia coli*, *Mycobacterium tuberculosis* H37Rv, *Pseudomonas aeruginosa* PA01 and *Rhodobacter ferrooxidans* SW2 have been used for the imaging of different fluorescent probes [20–23], providing evidences of bacterial permeability for the corresponding fluorescent probes. In terms of electrotrophs, rhodamine based Cr(III) or Cu(II) fluorescent probes have been proved to permeate the associated electrotrophic cells and image the corresponding inward metal ions [24,25], providing good references for tracking target metal ions in electrotrophs. Considering the importance of electrotrophs for Cd(II) removal with simultaneous hydrogen production in the mixed culture biocathodes of MECs [8,9], much effort is necessarily needed to employ suitable Cd(II) fluorescent probes for sensing Cd(II) ions in electrotrophs in response to current and 2,4-DNP in order to clarify the role of electrotrophs in Cd(II) removal from catholyte in MECs.

Small chemical of quinoline-based Cd(II) fluorescent probe of Fig. S1 (molecule weight: 416) owns merits including high selectivity, being used at mild environment, and simply synthesized [26]. Considering the permeable bacterial cell membrane for other small chemicals even with molecule weight over 400 [23,27,28], the quinoline-based Cd(II) fluorescent probe is expected to be loaded into the electrotrophs and rapidly sense the inward dissolved Cd(II) ions, thus achieving the image of dissolved Cd(II) ions in the electrotrophs in response to current and 2,4-DNP in MECs, which to the authors' knowledge, has not been previously reported.

Following the imaging and quantity of the dissolved Cd(II) ions and the total cadmium in periplasm and cytoplasm of the electrotrophs, other forms of cadmium harboring in these sections can be quantitatively obtained from the differences between total cadmium and dissolved Cd(II) ions. Since solid cellular membrane can understandably retain other forms of cadmium instead of dissolved Cd(II) ions, the value of other forms of cadmium located in cellular membrane is reasonably equal to that of total cadmium. Quantifying the subcellular locations of the various forms of cadmium in the electrotrophs in response to current and 2,4-DNP is expected to be beneficial for understanding the role of electrotrophs in Cd(II) removal in MECs.

Driven by mentioned above, the quinoline-based fluorescent probe (Supplementary Material, SM, Fig. S1) [26] was evaluated for the direct sensing of dissolved Cd(II) ions and the subsequent other forms of cadmium in four electrotrophs in response to current and 2,4-DNP in MECs. This characterization was connected to the removal of Cd(II) ions from catholyte and the distribution of total cadmium in the cathode chambers of MECs. The results showed that the quinoline-based Cd(II) fluorescent probe effectively sensed dissolved Cd(II) ions in response to current and 2,4-DNP in MECs. This study not only provided a prospect for the use of this fluorescent probe for the mapping of dissolved Cd(II) ions and the subsequent other forms of cadmium in the living electrotrophs, but also benefits for clarifying the roles of current and 2,4-DNP in electrotrophic removal of Cd(II) ions from catholyte in MECs.

2. Materials and methods

2.1. Electrotroph isolation and identification

The electrotrophs were isolated from the biocathodes of MECs [8] by streaking cathode scrapings onto agar plates containing anaerobic medium amended with agar and Cd(II) (179 μM) under anoxic conditions in an anaerobic glove box. Anaerobic medium, and bacterial isolation and incubation were referred to previous reports [24,25].

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 referred to previous descriptions [24,25]. 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.

2.2. MEC operation with isolates

Duplicate cubic two-chamber reactors with graphite felt (1.0 × 1.0 × 0.5 cm, 8 pieces, Sanye Co., Beijing, China) as the anode and cathode electrodes were used in all experiments, and for each of the duplicate reactors three replicate experiments were performed, all of which guaranteed the reproducibility of the results [9,29]. An additional headspace of 12 mL in the cathode was purposely scheduled for hydrogen collection [9,29]. A reference electrode (Ag/AgCl, 195 mV vs. SHE) was installed in the cathode chamber to monitor cathode potential. A voltage of either 0.5 V or 1.0 V was added to the circuit using a power source (DC Power Supply PS-1502DD, Yihua, Guangzhou, China). A small resistor of 10 Ω was connected in series with power supply to allow calculation of the current. The currents created at voltages of 0.5 V and 1.0 V together with the corresponding hydrogen production, Cd(II) removal and electrotrophic imaging were compared to reflect the effect of current on system performance and imaginable Cd(II) ions in electrotrophs. All potentials were collected using a data acquisition (PISO-813, Hongge Co., Taiwan).

The anode was inoculated using the effluent of MECs well acclimated to acetate (12.2 mM). The composite of anolyte was similar to previously reported [30,31]. The cathode was slowly inoculated with the isolates (10^8 CFU/mL, 6 mL) and the catholyte, composed of Cd(II) (179 μM), NH_4HCO_3 (245 μM), $KHCO_3$ (75 μM), $MgSO_4{\cdot}7H_2O$ (8 μM), vitamins 0.6 mL/L and mineral 0.6 mL/L. Since the attachment of cells onto the electrode surface initially occurred via rapid physical adsorption, rather than via slower biofilm formation [32], the inoculated catholyte was intentionally released and slowly refilled (twice) to make the total of electrotrophs sufficiently attached on the electrode. Initial pH and solution conductivity in anolyte and catholyte was similarly 5.8 and 5.8 mS/cm, respectively, which was appropriate for electrotrophic activities and less affected system performance [24,25,33]. Control reactors under open circuit conditions (OCC), where the anodes and