



Self-mediated pH changes in culture medium affecting biosorption and biomineralization of Cd^{2+} by *Bacillus cereus* Cd01

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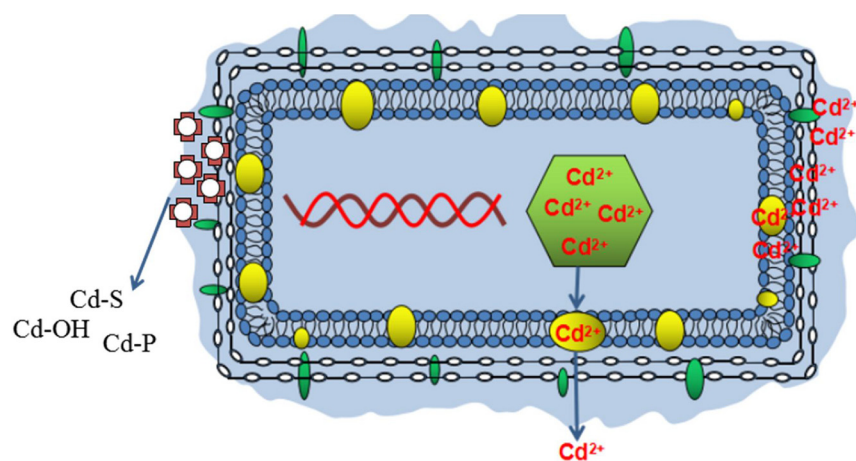
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GRAPHICAL ABSTRACT



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ABSTRACT

Biomineralization is an interesting naturally occurring process of forming minerals by microorganisms, which offers an efficient way to sequester heavy metal ions within relatively stable solid phases. In this study, *Bacillus cereus* Cd01 was selected to investigate effects of self-mediated pH on biosorption and biomineralization of Cd^{2+} in whole 72h cultivation period. Results revealed that strain Cd01-mediated pH decrease of the cultivation medium from 7.0 to 6.1 inhibited biosorption of Cd^{2+} on Cd01 cells at the initial cultivation period, while an increased pH from 6.1 to 7.4 facilitated biosorption of Cd^{2+} on Cd01 cells at the middle and late cultivation period. The reasons were mainly that self-mediated pH altered cell surface hydrophobicity and cell membrane fluidity of strain Cd01. Moreover, biosorption and bioaccumulation of Cd^{2+} on Cd01 cells in the period of increased pH promoted biomineralization of Cd^{2+} observed by the transmission and scanning electron microscopes. The analyses of energy dispersive spectroscopy, X-ray photoelectron spectroscopy and select area electron diffraction demonstrated that Cd^{2+} loaded on Cd01 cells was biomineralized into polycrystalline and/or

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amorphous cadmium sulfide and cadmium phosphate. These results suggest that strain Cd01 may play a potential role in biomineralization remediation of heavy metal contaminated soils.

1. Introduction

Soils contaminated by heavy metals (HMs) have been a widely concerned environment problem since HMs in soil is threatening and even deteriorating soil ecology, grain safety and human health due to their toxicity, non-biodegradability and bioaccumulation [1,2]. Overuse of phosphate fertilizers and discharge of pigments, electroplating, mining and metallurgical processes have been caused widespread contaminated soils by cadmium (Cd) [3]. As a result, Cd uptake in crops from Cd contaminated agricultural soils elevated and inevitably pose risks to human health via food chain since Cd, a non-essential nutrient element, is usually serious toxicity to organisms even in extremely low concentration range of 0.001–0.1 mg/L [4]. In China, 19.4% of the surveyed farmland across the Chinese mainland (approximately 26 million ha) was classified as HMs-contaminated soil according to the Chinese Investigation Communique of Soil Pollution. Chinese government thereby issued officially the national action plan on prevention and control of soil pollution on May 28, 2016, claiming that 90% of polluted farmland and industrial sites should be safety to use by 2020 (approximately 667,000 ha) and even 95% of all contaminated soils will be safety to use by 2030 [5–7]. Therefore, remediation of soil contaminated with HMs (especially by Cd) is becoming more urgent for China to guarantee food security and thrive soil ecology.

Many efforts have been attempted to explore the remediation techniques for heavy metal contaminated soils, including traditional physical and chemical approaches (e.g., soil replacement, turnover, pH adjustment, chemical fixation, leaching, and extraction), bioremediation (e.g., phytoremediation, microbial remediation and plant-microbe jointed bioremediation) and agricultural adjustment (e.g., agriculture rotation, low accumulators and inedible crop growing), etc [8–10]. However, some of the aforementioned methods are not efficient in terms of time, cost or environmental compatibility [5,8,11].

Recently, microbial mineralization is attracting extensive interest from applied researchers and engineers as an effective and environmentally friendly bioremediation technology for the mild and moderated Cd contaminated agricultural soils [12,13]. Microbial mineralization is an interesting and universal nature phenomenon where soil microbes are paramount driving force for metal cycling and mineral formation in Earth surface environment [14,15]. For instance, some microbes, such as *Bacillus* sp. KK1 [16], *Bacillus cereus* 12-2 [17] and *Providencia alcalifaciens* [18], etc., can effectively mediate the biogeochemical cycling of heavy metal ions (e.g., Cd^{2+} , Pb^{2+} , Ni^{2+} and Hg^{2+} , etc.) and finally transform HMs from mobile species into very stable minerals. As a result, heavy metal ions were sequestered into mineral coop so that their bioavailability was reduced resulting in amelioration of toxicity and migration for HMs in soil [18]. For instance, Chen et al. found that *Bacillus cereus* 12-2 could biomineralize Pb(II) into nanosized rod-shaped $\text{Ca}_{2.5}\text{Pb}_{7.5}(\text{OH})_2(\text{PO}_4)_6$ crystal and thus effectively reduced bioavailability of Pb(II) in soil [17]. Besides, they also noted that biosorption of Pb(II) on strain 12-2 cells played an important role in Pb(II) biomineralization. Furthermore, Reith et al. [14] and Kang et al. [19] elaborated the detailed mechanism about reduction coupled with biomineralization for polyvalent metal ions (e.g., here referring to Au from trivalence to zero valence), which enriched understanding about biomineral bioremediation of soil contaminated with HMs.

Nevertheless, the interactions between HMs and microbe play an important role in biomineralization process of heavy metal ions, where the toxicities of HMs generally decreased microbial metabolic activities while microbial metabolic activities, such as pH alteration of the

adjacent environment, also conversely affected biosorption of HMs inside and outside microbial cells and even bioavailability of HMs [20]. However, little attention has been paid to the dynamic process about effects of microbe-mediated pH alteration on biosorption and subsequent biomineralization of heavy metal ions on microbial cells during whole microbial growth period.

In this study, a versatile *Bacillus cereus* Cd01 was isolated from Cd contaminated soil in Hunan, China, which can not only resist 5 mmol/L of Cd^{2+} but also alter pH of culture medium during whole growth period as well as mineralize Cd^{2+} into insoluble biomineral particles. Therefore, strain Cd01 was selected to investigate effects of self-mediated pH changes on biosorption and bioaccumulation of Cd^{2+} outside and inside Cd01 cells and further on biomineralization of Cd^{2+} during strain Cd01 grew in the culture medium containing different concentrations of Cd^{2+} for whole 72 h cultivation time. Additionally, some adaptive shifts of Cd01 cells under the stress of different Cd^{2+} concentrations, including cell surface hydrophobicity (CSH) and cell membrane fluidity, were also studied. These results would be expected to provide some valuable insights into microbial mineralization of HMs and boost microbial biomineralization application in future practical bioremediation for soils contaminated with HMs in the nature system.

2. Materials and methods

2.1. Chemicals

$3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ (ultrapure grade) and other inorganic reagents (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai. 1,6-diphenyl-1,3,5-hexatriene (DPH) and dodecyl sodium (pure > 98%) were obtained from Aladdin Reagent Co., Ltd., Shanghai. Protease K (20 mg/L) was purchased from Dongsheng Biotech Co., Ltd., Guangzhou. The certified reference material for composition analysis of loess soil (GBW07408) and biological component analysis of spinach (GBW10015) was separately purchased from the National Research Center for Certified Reference Materials and the Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, China. Ultrapure water was used for all tests.

2.2. Bacterium and cultivation conditions

Bacillus cereus Cd01 (strain Cd01) used in this study is a gram-positive facultative aerobic bacterium based on its sequence homology analysis of 16S rRNA gene (Fig. S1). Strain Cd01 was isolated from the Cd-contaminated soil in Hunan, China. The 16S rRNA gene sequence has been deposited in GenBank (accession No. MF351763), and strain Cd01 has also been deposited in China Center for Type Culture Collection (accession No. CCTCC M 2017358) as a patent strain. Strain Cd01 could resist 5.0 mmol/L Cd^{2+} in the preliminary experiment (Fig. S2). In order to maintain its strong resistance to Cd^{2+} , strain Cd01 was cultured in Luria Bertani (LB) solid agar plate (g/L, peptone 10.0, NaCl 10.0, yeast extract 5.0 and agar 2.0) containing Cd (mmol/L, 1.0) at 30 °C for 24 h. Strain Cd01 on the above plate was inoculated into fresh LB liquid medium (without Cd^{2+} and agar, pH 7.0; °C for 24 h. Strain Cd01 on the above plate was inoculated into fresh LB liquid medium (without Cd^{2+} and agar, pH 7.0). Then, strain Cd01 was cultivated at 30 °C on a gyratory shaker (150 rpm) to achieve the exponential phase before centrifugation (12,000g, 5 min). After washed twice with 50 mmol/L phosphate buffer saline (PBS, 50 mmol/L, pH 7.0), Cd01 cell suspension was adjusted to 1.2 at the optical density at 600 nm (OD_{600})

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