International Biodeterioration & Biodegradation 116 (2017) 10–16

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Self-assembly of complex hollow CuS nano/micro shell by an electrochemically active bacterium *Shewanella oneidensis* MR-1

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ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
19 September 2016
Accepted 19 September 2016

Keywords:
Hollow CuS microshell
Biofabrication
Shewanella oneidensis MR-1
Nanorod
Micro/nanomaterials

ABSTRACT

In the present study, *Shewanella oneidensis* MR-1 was successfully exploited to produce CuS nanocrystals not only as a biotemplate but also as a supplier of sulfur source. The biogenic H_2S produced *via* the thiosulfate reduction in periplasm grabbed Cu(II) ions bound with extracellular polymeric substance of *Shewanella* and precipitated as monodisperse CuS nanoparticles extracellularly. These nanoparticles aggregated in the extracellular matrix gradually and then formed CuS nanorods. Ultimately, a complex hollow CuS microshell self-assembled on the cell surface was observed for the first time. The biogenic CuS microshell could significantly enhance the adsorption ability of *S. oneidensis* MR-1 toward Cr(VI). This work may facilitate a better understanding about the biosynthesis mechanism of nanomaterials and contribute to the application in environmental remediation.

1. Introduction

Generally, sulfur species from contaminated sites, including sulfite, thiosulfate and even sulfate, can be biotransformed to H_2S under anaerobic conditions (Lens et al., 1998; Meulepas et al., 2009). The harmful H_2S may cause severe pollution and corrosion problems. Suppressing H_2S contaminant requires additional costs (Hubert et al., 2003). Thus, application of new strategy in the treatment of biogenic H_2S has attracted great interests in recent years. Among them, biosynthesis of sulfide nanomaterials can bring extra benefits coupled with the control of H_2S release.

Nanotechnology is one of the fastest moving areas of science and technology (Daniel and Astruc, 2004). Recently, many efforts have been put into the synthesis of chalcogenide semiconductors because of their useful properties and technological importance (Qin et al., 2005). As a *p*-type semiconductor, copper sulfide has potential application in gas sensors, cold cathode emitters, catalyst, switches, and solar cells (Sakamoto et al., 2003; Wan et al., 2004; Li et al., 2009). Various morphologies of copper sulfide, such as nanotubes, nanovesicles, nanodisks, nanoparticles, nanowires, and nanorods, have been synthesized successfully (Saunders et al., 2006; Zhang et al., 2006). Compared with physical and chemical approaches, biologically-assisted synthesis of nanomaterials is considered to be a better way due to its reliability and nontoxicity (Li et al., 2011). In such case, numerous microbial systems involving yeasts, bacteria, viruses and fungi, are employed as environmentally friendly nano-factories for production and assembly of different kinds of nanomaterials (Durán and Seabra, 2012). Among them, bacteria are the most potent for large scale production of nanomaterials because of their vigorous metabolism, strong tolerance, and wide distribution. Up to now, several sulfide semiconductors such as CdS (Sweeney et al., 2004), PbS (Bai and Zhang, 2009), ZnS (Labrenz et al., 2000) and FeS (Watson et al., 1999), have been successfully synthesized by various bacteria. However, biofabrication processes of sulfide nanomaterials has been neglected. To date, the mechanisms involved in the biosynthetic processes are largely unknown.

The electrochemically active bacteria *Shewanella* is widely dispersed in nature especially in aquatic environments (Vogel et al., 2000). Given its special electron transmembrane transport network (Clarke et al., 2011), *Shewanella* has been widely used for the bioreduction of various pollutants (Herrera and Videla, 2009; Hong

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http://dx.doi.org/10.1016/j.ibiod.2016.09.021
0964-8305/

and Gu, 2009; Wang et al., 2010; Fernando et al., 2012). Recently, *Shewanella* has been employed for the biofabrication of metal nanoparticles, such as Ag (Suresh et al., 2010), Au (Suresh et al., 2011), Pt (Konishi et al., 2007), Pd (Windt et al., 2005) and even alloy (De Corte et al., 2011), through extracellular reduction. Unlike the sulfate-reducing bacteria, *Shewanella* cannot reduce sulfate to synthesize sulfide nanomaterials. But *Shewanella* can utilize thiosulfate, sulfite, and even element sulfur as a source to produce S^{2-} (Burns and DiChristina, 2009; Shirodkar et al., 2011), which gives *Shewanella* the potential to produce sulfide nanomaterials. Recently, *Shewanella* has been proven capable of producing metal sulfide nanomaterials, including ZnS (Xiao et al., 2015), Ag_2S (Suresh et al., 2011), MnS (Lee et al., 2011), FeS (Xiao et al., 2016), and CuS (Zhou et al., 2016). In these studies, metal sulfide nanomaterials are formed as granules or aggregates in the medium, at the cell surface or in the cytoplasm. But the architecture of biogenic metal sulfide nanomaterials reported in these studies was not complex. In addition, these studies mainly focused on the characteristics of biogenic nanomaterials, but they seldom explored the dynamics of biosynthesis. Although complex architectures like As-S nanotubes (Lee et al., 2007; Jiang et al., 2009), tellurium nanorods (Kim et al., 2013) and selenium nanowires (Ho et al., 2010) have been observed in the biofabrication by *Shewanella*, the assembly of advanced architectures of metal sulfide nanomaterial have not been reported up to now. The lack of relevant reports has limit the further understanding of the mechanisms involved in the bacterial biofabrication of nanomaterials.

In the present study, we investigated the self-assembly bioprocess and the architecture of a metal sulfide nanomaterials biofabricated. For these purposes, *Shewanella oneidensis* MR-1, a typical electrochemically active bacterium, was employed to biofabricate CuS nanomaterials under anaerobic conditions. The as-synthesized nanomaterials were characterized by transmission electron microscopy (TEM), high resolution TEM (HRTEM), field emission scanning electron microscope (FESEM) and powder X-Ray diffractometer (XRD). The self-assembly of metal sulfide complex micro/nanomaterials was monitored for the first time. The effect of surface modification by CuS nanomaterials on the biosorption ability of *S. oneidensis* MR-1 was also investigated. This work may facilitate a better understanding of the synthetic mechanisms involved in the biologically-mediated sulfide precipitation, which may ultimately serve for the large-scale production of biofabricated nanomaterials.

2. Materials and methods

2.1. Biofabrication of CuS nanomaterials

S. oneidensis MR-1 used in this study was kindly provided by Prof. K. H. Nealson from the University of Southern California (Bretschger et al., 2007). This strain was aerobically cultured in LB medium (tryptone 1%, yeast extract 0.5%, sodium chloride 1%) at 30 °C until the late stationary. The bacterial cells were collected after centrifugation at 5000 rpm for 15 min, and then washed 2 times with sterile deionized water.

A mineral medium without phosphate was used for the CuS biofabrication under anaerobic conditions as previously described (Bretschger et al., 2007). Lactate (18 mM) and thiosulfate (14 mM) were used as the electron donor and acceptor, respectively. The medium was buffered at pH 7.0 with 50 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and supplemented with 2 mM $CuSO_4$. Bacterial cells prepared as above were added and the initial concentration was adjusted to $4–6 \times 10^6$ CFU ml^{-1}. Aliquots of 80 ml of the suspensions were filled into 100-ml serum bottles, bubbled with N_2 for 10 min, and then sealed with butyl rubber stoppers.

2.2. Characterization of nanoparticles

All bottles were incubated on a rotary shaker (200 rpm) at 30 °C. Culture samples were collected periodically, under anaerobic condition. The samples were prepared on carbon-coated copper grids and observed by TEM/HRTEM (JEOL, Model JEM-2100) to monitor the biosynthetic process. The spatial distribution of biogenic nanomaterials was examined by TEM. After centrifugation at 5000 rpm for 10 min, the bacterial cells were collected and washed with 0.1 M PBS, and then subsequently fixed with 1% osmium tetraoxide in distilled water at 4 °C for 1.5 h. After washing again with 0.1 M PBS, the cells were dehydrated at room temperature with ethanol (70–100%) and then embedded using a mixture of resin (Epon 812), hardeners (dodecenyl succinic anhydride and methyl nadic anhydride), and accelerator (dimethylaminomethyl phenol). The mixture was polymerized at 60 °C for 2 d. Ultrathin sections of 70 nm were cut using an ultramicrotome (EMUC6, Leica Co., Germany), and mounted on a carbon-coated copper TEM grid. All sections were stained with 2% uranyl acetate in distilled water prior to TEM analysis (Tecnai 12, Philips Inc., Netherlands).

After incubation for 15 d, the precipitates were collected by centrifugation at 5000 rpm for 10 min, and washed with Milli-Q water for 3 times. A portion of the sample was dehydrated with 50% ethanol, 75% ethanol, 100% ethanol and acetone, sequentially. The dried sample was characterized by X-ray diffraction (XRD) using a D/MAX Ultima III diffractometer (Rigaku, Japan) with Cu Kα radiation (λ = 1.54056 Å). The other portion of the sample was fixed with 2.5% glutaraldehyde in distilled water for 1 h at 4 °C, and then gradually dehydrated at room temperature with ethanol (70–100%). After critical point drying with CO_2, these samples were sputter-coated with gold and then examined in a Hitachi S-450 scanning electron microscope (SEM) with energy dispersive X-ray analysis (EDX).

2.3. Removal of Cr(VI)

The absorption capacity of bacterial cells covered with CuS nanomaterials was evaluated from the removal of Cr(VI) in solution. 0.01 g of CuS-covered cells were added into 20 ml $K_2Cr_2O_7$ solution (30 mg L^{-1}). The suspension was shaken at room temperature. 1 ml of the suspension was sampled periodically and centrifuged at 6000 rpm for 2 min. The untreated *S. oneidensis* MR-1 cells were employed as control under the same condition. The Cr(VI) concentration was determined by UV spectrophotometer (Sarin and Pant, 2006). The Cr(VI) removal efficiency was determined using the following expression:

$$\text{Cr(VI) removal}(\%) = [(C_o - C_t)/C_o] \times 100\%$$

where C_o and C_t represent concentrations of Cr(VI) at the initial phase and different sampling point, respectively.

3. Results and discussion

3.1. Biosynthesis and characterization of CuS nanomaterials

The biofabrication of CuS nanomaterials by *S. oneidensis* MR-1 under anaerobic conditions was investigated. As shown in the supplementary materials (Fig. S1), the color of solution gradually changed from colorless to black over the incubation time. After 15 d of incubation, the suspended solids, including bacterial cells were collected and examined by SEM-EDX. The rod-like cells were observed with a length of 2.5 μm and a diameter of 0.75 μm. No