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Biosynthesis and structural characteristics of selenium nanoparticles by *Pseudomonas alcaliphila*

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ABSTRACT

In this paper, we report that selenium (Se) nanoparticles were first biosynthesized by *Pseudomonas alcaliphila* with a simple and eco-friendly biological method. The structural characteristics of Se nanoparticles were examined. The results showed that spherical particles appeared with diameters ranging from 50 to 500 nm during incubation and Se nanorods were present after incubating in an aqueous reaction solution for 24 h. However, the formation of Se nanorods was interrupted when 5% (w/v) poly(vinyl pyrrolidone) (PVP) was added in the aqueous reaction solution, obtaining stable spherical Se nanoparticles with a diameter of about 200 nm.

1. Introduction

Nanoparticles ranging from 0.1 nm to 1000 nm in size have demonstrated new physical and chemical properties [1,2]. Semiconductor nanoparticles have excellent nonlinear properties, saturable absorption, and optical bistability. Selenium (Se), as a functional material, is an important elemental semiconductor attracting more attention owing to its special physical properties such as the anisotropy of thermo-conductivity, high photoconductivity (ca. 8×10^4 S cm^{-1}), thermoelectric response, and nonlinear optical response [3]. Therefore, Se is used in many applications ranging from photocells, photographic exposure meters, and solar cells to semiconductor rectifiers [4]. Furthermore, Se, as one of the essential trace elements, is confirmed to improve the activity of the seleno-enzyme and glutathione peroxidase prevention of free radical damage to cells and tissues in vivo [5,6]. Since surface area-to-volume ratio increases with decreasing particle size, selenium nanoparticles have high biological activity [7], including anti-hydroxyl radical property [8] and a protective effect against the oxidation of DNA [9].

In the past few years, many methods for the synthesis of Se nanomaterials have been developed. Song has reported a method to produce stable amorphous selenium nanospheres capped with poly(vinyl pyrrolidone) (PVP) with a size of 100 nm [10]. These amorphous Se nanospheres could be transformed into Se nanorods at the liquid–liquid interface. Xie has reported a new approach to synthesize Se nanobelts with a diameter of ca. 80 nm and a length of 5 μm on a large scale under room temperatures [11]. Quintana has reported a method to synthesize selenium nanoparticles of 120 nm in size by pulsed laser ablation [12]. A soft, solution-phase approach to the large-scale synthesis of uniform nanowires of trigonal selenium (t-Se) has been described by Gates et al. [13]. Wang has reported a general procedure for directing the assembly of Se nanowires into macroscopic fibers by electro-kinetic techniques [14]. Wang and his team have reported the formation of Se microwire networks by hydrothermal treatment in a tungstosilicic acid solution [15]. Filippo et al. has reported a simple and rapid vapor deposition route to synthesize trigonal selenium microtubes in a horizontal tubular furnace under argon gas flow [16].

However, most methods used to synthesize Se nanomaterials are characterized by elevated temperatures, high pressures and are hazardous to the environment. Therefore, the development of clean, nontoxic and eco-friendly methods to synthesize nanoparticles deserves merit. As a result, the biological synthesis of nanomaterials has attracted more attention. For example, Li has described the formation of amorphous selenium (alpha-Se)/protein composites using *Capsicum annuum* L. extract to reduce selenium ions at room temperature [17]. Gurunathan et al. have reported an approach to synthesize silver nanoparticles using *Escherichia coli* [18]. Wang et al. have described a method to synthesize selenium

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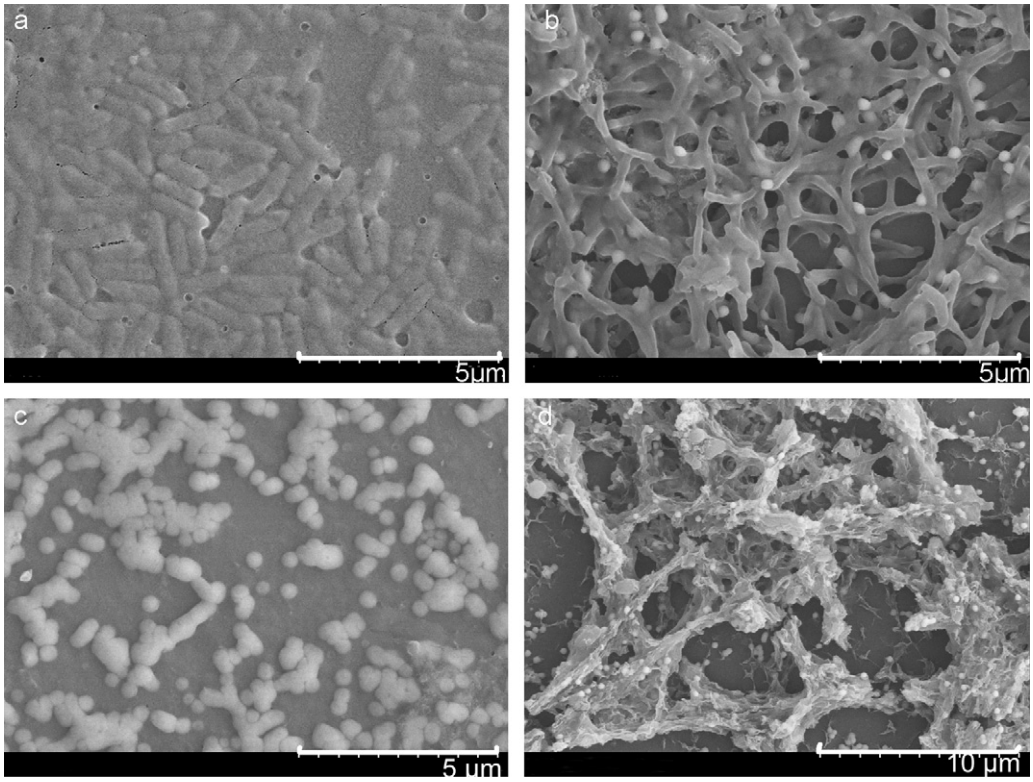

Fig. 1. FESEM images of Se nanoparticles at 6 h (a), 12 h (b), 24 h (c), and 48 h (d) after adding selenite pentahydrate into the culture solution.

nanoparticles by *Bacillus subtilis* [19]. To date, about 16 diverse species of bacteria and Arechaea have been discovered to reduce selenium oxyanions to the red, amorphous or monoclinic allotropes of Se^0 [20,21].

In this paper, we report a facile, economical and green way to synthesize Se nanoparticles (SeNPs) by *Pseudomonas alcaliphila*, which exhibits a high resistance to Se^{2-} in the experiment, holding a promising alternative for the large-scale commercial synthesis of SeNPs. Furthermore, the process of synthesis is controlled to obtain distinct and highly regular spherical SeNPs by adding PVP.

2. Materials and methods

2.1. Pure strain and cultivation

P. alcaliphila was obtained from the Chengdu Institute of Biology, Chinese Academy of Science (Chengdu, China). Sodium selenite pentahydrate was obtained from the Chengdu Kelong Reagent Co. The growth medium contained the following components (per liter of distilled water): 1.0 g of KNO_3, 1.0 g of $MgSO_4$, 2.0 g of KH_2PO_4, 5.2 g of trisodium citrate per liter, and 0.005 g $FeCl_3$. The complete salts solution was composed of 17.5 g of NaCl per liter, 0.74 g of KCl per liter, 12.3 g of $MgSO_4{\cdot}7H_2O$ per liter, and 0.15 g of Tris buffer per liter and the pH was adjusted to 7.5. PVP was obtained from Sigma (Germany). The water used in the experiment was double distilled water.

Analytical grade chemicals and reagents were used in all experiments without further purification.

For activation of *P. alcaliphila*, the bacteria were aerobically cultivated in the growth medium mentioned above at 28 °C on a rotary shaker (150 rpm) for 24 h and harvested by centrifugation (8000 rpm at 4 °C for 20 min) before use.

2.2. Synthesis of Se nanomaterials using P. alcaliphila

Prior to the synthesis of Se nanomaterials, 1 ml activated *P. alcaliphila* with or without 5.0 g PVP (50 g/l) was aerobically cultivated under the same method as in the activation test. After 24 h of bacterial growth, 2.63 g sodium selenite pentahydrate (0.1 M) was added into the medium, and then the reaction started. After 48 h of the reaction, the culture solution was centrifuged (10,000 rpm for 10 min) to collect the precipitates, which were subsequently washed with double distilled water before further analysis. All harvested samples were stored at room temperature.

Some collected samples for investigating the composition of SeNPs were purified by sequential centrifugation at 10,000 rpm for 10 min in the complete salts solution, 0.25 M NaOH, 0.1 M NaOH, 10 mM Na_2HPO_4 (adjusted to pH 7.3) and carbon-free, distilled, deionized water.

Some samples were lyophilized for infrared spectroscopy analysis. We also prepared and incubated samples without Se as a control in the infrared spectroscopy analysis.

2.3. Characterization of selenium nanoparticles (SeNPs)

The size and morphology of the synthesized selenium nanomaterials were analyzed using a field emission scanning electron microscope (FESEM) (Hitachi Co., Japan S4800) at an accelerating voltage of 5.0 kV on a glass substrate. The spectrum of the energy dispersive X-ray spectroscopy (EDS) of the sample was carried out using an Oxford IE150 instrument. The X-ray diffraction (XRD) patterns of the samples were recorded using an X-ray diffractometer (Philips Co., Holland). Absorption spectra were collected on a UV–vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China TU-1901) at a wavelength range of 200–800 nm at a resolution of 2 nm. Further characterization involved Fourier Transform Infrared Spectroscopy (FTIR) analysis of the dried sample.