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Biological Synthesis of Size-Controlled Cadmium Sulfide Nanoparticles Using Immobilized *Rhodobacter sphaeroides*

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Abstract Size-controlled cadmium sulfide nanoparticles were successfully synthesized by immobilized *Rhodobacter sphaeroides* in the study. The dynamic process that Cd^{2+} was transported from solution into cell by living *R. sphaeroides* was characterized by transmission electron microscopy (TEM). Culture time, as an important physiological parameter for *R. sphaeroides* growth, could significantly control the size of cadmium sulfide nanoparticles. TEM demonstrated that the average sizes of spherical cadmium sulfide nanoparticles were 2.3 ± 0.15, 6.8 ± 0.22, and 36.8 ± 0.25 nm at culture times of 36, 42, and 48 h, respectively. Also, the UV–vis and photoluminescence spectral analysis of cadmium sulfide nanoparticles were performed.

Keywords Biosynthesis · Cadmium sulfide · Nanoparticles · *Rhodobacter sphaeroides*

Introduction

Biosynthesis of nanomaterials as a novel nanoparticle synthesizing technology attracts increasing attention. It is well known that many organisms can provide inorganic materials either intra- or extracellularly [1, 2]. For example, unicellular organisms such as magnetotactic bacteria produce magnetite nanoparticles [3], and diatoms synthesize siliceous materials [4]. Even live plants such as *Alfalfa* are able to produce gold clusters surrounded by a shell of organic ligands [5]. Bansal et al. [6] have synthesized 4–5 nm barium titanate (BT) nanoparticles using a fungus-mediated approach. As far as the biosynthesis of cadmium sulfide (CdS) nanoparticles is concerned, a number of biosynthesis methods have been reported. For example, CdS nanoparticles can be synthesized intracellularly by the yeasts *Schizosaccharomyces pombe* [7]. However, intracellular synthesis of CdS nanoparticles makes the job of downstream processing difficult and beats the purpose of developing a simple and economical process. The extracellular enzyme secreted by the fungus *Fusarium oxysporum* can mediate extracellular synthesis of CdS nanoparticles [8]. But live organisms have the endogenous ability to exquisitely regulate synthesis of inorganic materials. For example, shape control of inorganic materials in biological systems was achieved either by formation of membrane vesicles [9] or through functional molecules such as aluminophosphates and polypeptides that bonded specifically to mineral surfaces [10]. On the other hand, the size, shape, and yield of biosynthesized nanoparticles significantly depend on physiological parameters, and remarkably are affected by growth conditions (including pH, temperature, culture time, and metal ions concentration) of live organisms. For example, gold nanowires with a network structure can be synthesized with the change of $HAuCl_4$ concentration by *Rhodopseudomonas capsulate* [11], and triangular gold nanoplates can be produced with adjusting the pH of initial solution by *Rhodopseudomonas capsulate* [12]. The exploitation of size- and shape-controlled biosynthesis of CdS nanoparticles using live photosynthetic bacteria is so far unexplored and underexploited. In this study, prokaryote photosynthetic bacteria *Rhodobacter sphaeroides*, recognized as one of the ecologically and environmentally

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important microorganisms, commonly existing in the natural environment, were investigated for producing CdS at room temperature with a single step process. Especially CdS nanoparticles were formed intracellularly and then were transported into extracellular solution. In addition, immobilized *R. sphaeroides* can be separated from cadmium sulfide nanoparticles easily.

Experimental

Organism and Cultivation

Rhodobacter sphaeroides were obtained from College of Life Science and Technology, Shanxi University, Taiyuan, China. *R. sphaeroides* were cultured in the medium containing (in 1 L) 2.0 g malic sodium, 0.15 g $MgSO_4 \cdot 7H_2O$, 1.2 g yeast extract, and 1.5 g $(NH_4)_2SO_4$ at pH 7 and 30 °C [13]. The bacteria were cultured for 72 h and separated from broth by centrifugation (5000 rpm) at 4 °C for 10 min. The collected bacteria were washed five times with distilled water to obtain about 1 g wet weight of bacteria.

Preparation of Immobilized *Rhodobacter sphaeroides*

The concentrated pure-culture *R. sphaeroides* were then mixed with polyvinylalcohol (PVA) (10 g PVA/100 mL distilled water). The initial concentration of cells was 30 mg/L. The gel beads with wrapped microbial cells were formed in a solution of 10% H_3BO_3, and the average diameter was about 3 mm. The beads were "annealed" in the H_3BO_3 solution for 18 h. After activation in growth medium, the immobilized beads were washed twice with distilled water and were prepared for use [14].

Biological Synthesis of Cadmium Sulfide Nanoparticles

Synthesis was conducted in a 1000 mL sterile serum bottle containing 20 g immobilized *R. sphaeroides* and 500 culture medium of 1.0 mM $CdCl_2$. The resulting solution was incubated at 30 °C under the dark and aerobic (DO = 5 mg L^{-1}) conditions for 36 h. After the bio-transformation reaction was completed, the precipitate was washed several times with distilled water. The final precipitate was dried at 50 °C for 3 h in a vacuum kiln. The products were obtained in about 85% yield based on Cd.

The CdS nanoparticles synthesized by immobilized *R. sphaeroides* were used for powder X-ray diffraction (XRD) analysis. The spectra were recorded on a Rigaku Dmax-γA automatic instrument. The diffracted intensities were recorded from 10° to 70° 2θ angles. The sample was prepared by drop coating onto a carbon-coated copper grid for transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM), and selected area electron diffraction (SAED). TEM was performed on a Hitachi H-600 instrument operated at an accelerating voltage of 120 kV while HRTEM and SAED were performed on a Hitachi H-2010 instrument operated at a lattice image resolution of 0.14 nm. The cells were analyzed by transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDXS), using a 100CX scanning transmission electron microscope and a Kevex 8000 EDX system. The cell samples were prepared as previously described [15]. Ultraviolet and visible (UV–vis) absorption spectrum was collected at room temperature on Shimadzu UV-2101PC using $BaSO_4$ powder as a standard. The photoluminescence emission and excitation spectra were recorded with a Hitachi F-850 fluorescence spectrometer.

Different Forms of Cadmium Separated by Different Centrifugation Speed

Nanocrystal formation was initiated by adding $CdCl_2$ (1 mM) to a cell sample (about 1 g wet weight) suspended in growing medium. The solutions were incubated on an orbital shaker at 30 °C and agitated at 150 rpm. Samples were taken at predefined time intervals (0, 12, 24, 36, 42, and 48 h). The sample was centrifuged at 4000×*g* for 20 min. The biomass pellet (P_1) was collected and the medium without cells was centrifuged at 15000×*g* at 4 °C for 60 min. The supernatant (S_1) was collected, and the pellet (P_2) with the CdS-containing particles was washed with deionized water three times. Each experiment was repeated three times. The contents of cadmium in different forms of P_1, S_1, and P_2 were determined using Shimadzu AA-6300 atomic absorption spectrophotometer in an air-acetylene flame at 228.8 nm wavelength [16].

Cysteine Desulfhydrase Assay

Cysteine desulfhydrase activity of the cell was measured using a colorimetric assay adapted from Chu et al. [17]. Samples of *R. sphaeroides* were centrifuged at 4000×*g* for 20 min. The pellet was resuspended in phosphate buffer (10 mM, 1 ml, pH 7.5). The reaction was started by the addition of Tris (0.1 M buffered to pH 7.6) and cysteine hydrochloride (100 mM, pH 8.6), then the mixture was incubated at 37 °C for 1 h. Sulfide formation was determined by adding *N*,*N*-dimethyl-p-phenylenediamine sulfate (20 mM, in 7.2 M HCl) and $FeCl_3$ (30 mM, in 7.2 M HCl) to the reaction tubes. Absorbance was measured at 650 nm and the concentration of sulfide was determined according to a standard sodium sulfide

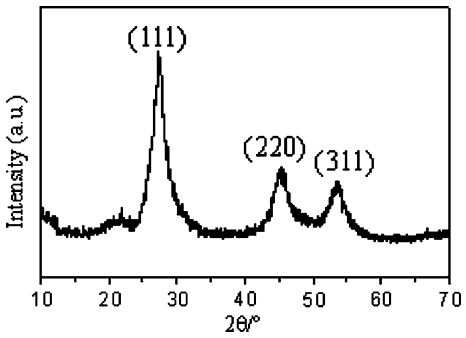

Fig. 1 X-ray diffraction pattern of CdS nanoparticles synthesized by immobilized *R. sphaeroides* at 42 h

calibration curve. Total protein was measured by the method of Chen et al. [18].

Results and Discussion

Biosynthesis of CdS Nanoparticles

Figure 1 displays the XRD pattern of the CdS synthesized by immobilized *R. sphaeroides* at 42 h. Three diffraction peaks at ca. 26.58, 44.16, and 52.39 can be indexed as cubic CdS (1 1 1), (2 2 0), and (3 1 1) faces by comparison with the data from JCPDS file no. 42-1411, which indicates that CdS nanoparticles have been successfully prepared by immobilized *R. sphaeroides*. The widened peaks imply a small particle size of the product. According to Debye-Scherrer equation, the mean grain size is calculated to be approximately 4.3 nm. Typical EDX pattern shows that the CdS nanoparticles are composed of the elements Cd and S, and the ratio of Cd:S is 0.97:1.00, being in with the expected value.

A representative HRTEM image at low amplificatory times of the CdS nanoparticles obtained at 42 h is given in Fig. 2a. The particles are essentially spherical, and the average particle size is 6.8 ± 0.20 nm selecting one hundred particles of TEM. However, HRTEM at high amplificatory times shows that the nanocrystals have a size of 4.3 nm at the place I. The size of nanocrystals observed by HRTEM at high amplificatory times is smaller than that at low amplificatory times due to a few gathered nanocrystals. HRTEM at high amplificatory times and lattice images reveal that the nanocrystals are cubic with a *d* spacing of 0.36 nm, corresponding to the (111) plane of cubic CdS (Fig. 2b, c). The SAED pattern of these particles indicates that they are the face-centered cubic (fcc) crystalline structure (Fig. 2d).

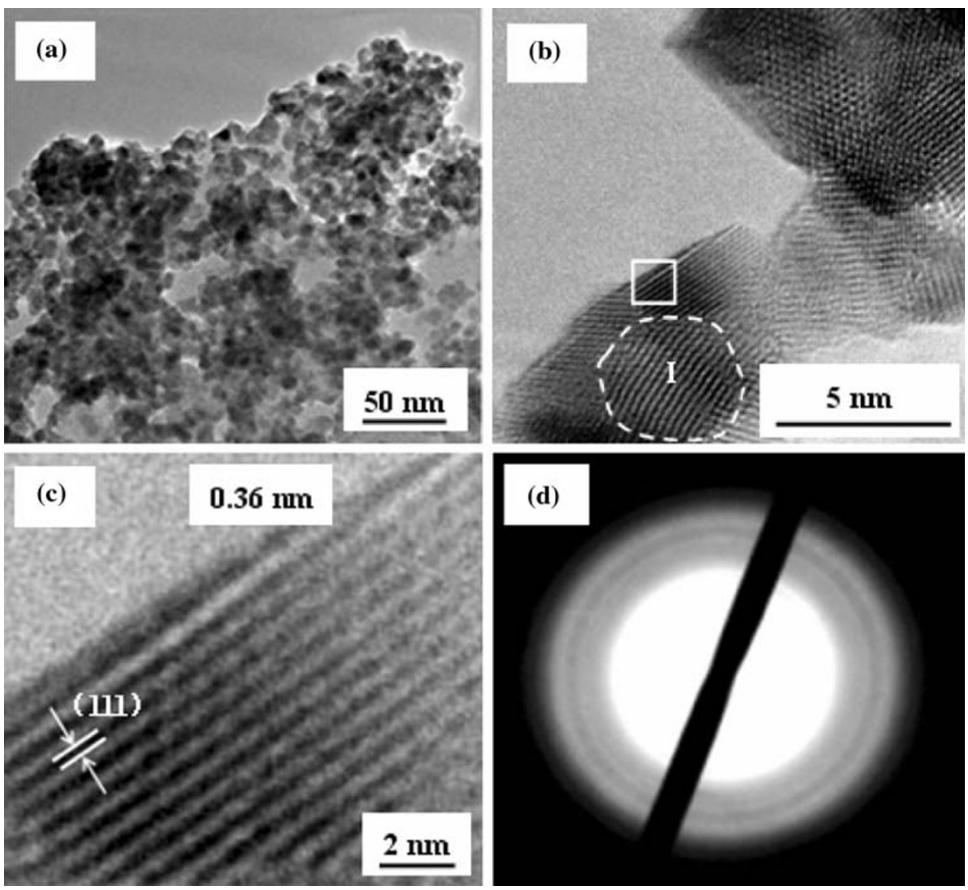

Fig. 2 The product of CdS nanoparticles synthesized by immobilized *R. sphaeroides* at 42 h **a** HRTEM image at low amplificatory times, **b** HRTEM image at high amplificatory times, **c** (111) lattice fringes of denoted area ($d_{111} = 0.36$ nm), **d** the corresponding SAED pattern

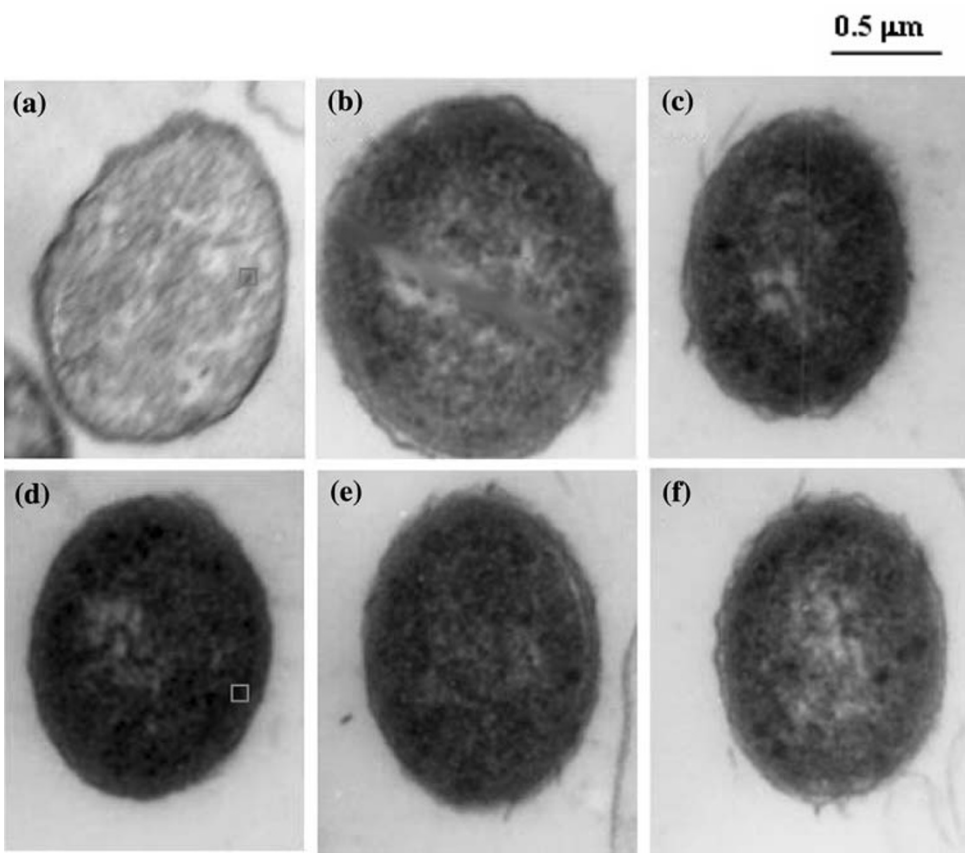

Fig. 3 TEM images recorded from thin sections of *R. sphaeroides* cells after reaction with $CdCl_2$ at different times. **a** 0 h, **b** 12 h, **c** 24 h, **d** 36 h, **e** 42 h, **f** 48 h

Biosynthesis Kinetics of CdS

To understand the synthesis process of CdS in a greater detail, the kinetics of the formation of CdS by living *R. sphaeroides* exposed to 1 mM $CdCl_2$ culture medium at 30 °C was followed by TEM. Figure 3a, b shows the thin sections of CdS nano-*R. sphaeroides* cell as a function of reaction time. At the beginning of reaction, the Cd cannot be seen (Fig. 3a). At very early stage of reaction, the Cd can be seen as dense population from the TEM images (Fig. 3b). The result indicates that only a little of Cd^{2+} is carried into the *R. sphaeroides* cells. After 24 h of reaction, the relative quantity of Cd^{2+} are transported into the cell and result in the increasing of Cd^{2+} (Fig. 3c), but little CdS deposits are obtained from extracellular resolution, and most of Cd^{2+} are in solution (Fig. 4). At 36 h, a lot of Cd^{2+} is carried into the cell (Fig. 3d), much CdS deposits are gained from extracellular resolution, and Cd^{2+} in solution are reduced to half of initial concentration (Fig. 4). At 42 h, the intracellular Cd decreases (Fig. 3e), and a large population of CdS are visible in extracellular solution (Fig. 4). At 48 h, the intracellular CdS is little (Fig. 3f), and the CdS in extracellular resolution are observed in large population (Fig. 4). The dynamic process of intracellular Cd (including Cd^{2+} and CdS) transported by living *R. sphaeroides*, characterized by TEM, is allowed for the observation of key intermediates and characteristics of the carrying process of Cd^{2+} from solution into cell.

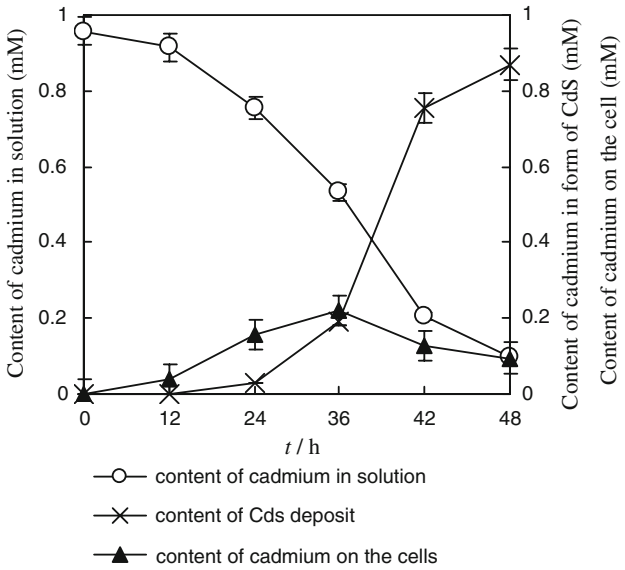

Fig. 4 Relations among content of cadmium in solution, CdS deposit, and on the cell

At the same time, the chemical analysis of cell ultra thin section of *R. sphaeroides* was performed by EDS. Figure 5 shows X-ray EDS analysis of *R. sphaeroides* cultivated in

culture medium in the absence or in the presence of 1 mM Cd^{2+} (circled in red, Fig. 3a, d). The strong signals in Fig. 5b indicate the presence of Cd and S, and the ratio of Cd:S is 0.97:1.00. The result shows that the deposit of CdS has been synthesized in cells. However, there are not the signals of Cd and S in Fig. 5 a. The presence of C and O in Fig. 5 suggests the biomolecules in the *R. sphaeroides* cells.

Size-Controlled Biosynthesis of CdS Nanoparticles

The growing phase of cells was found to be an important factor in modulating the morphology of CdS nanoparticles because they evidently affected the physiological parameters of living *E. coli* [19]. Figure 6 shows TEM images of the CdS nanoparticles formed by living immobilized *R. sphaeroides* exposed to 1 mM culture medium of $CdCl_2$ at different culture times. The spherical CdS nanoparticles with the average size of 2.3 ± 0.15, 6.8 ± 0.22, and 36.8 ± 0.25 nm were formed at 36, 42, and 48 h, respectively, which indicates that the size of CdS nanoparticles increases with the increasing culture time.

Previous studies indicated that cysteine desulfhydrase was an important factor in the biosynthesis of metal sulfide nanoparticles [15]. Also, we had confirmed that *R. sphaeroides* could secrete cysteine desulfhydrase (C–S-lyase) being responsible for producing S^{2-} [20]. The result shows that the activity of cysteine desulfhydrase in *R. sphaeroides* depends on culture time, and the activities at 36, 42, and 48 h are 32.6, 45.1, and 50.8 U g^{-1}, respectively. Namely, the activity of C–S-lyase at 36 h is lower than the ones at 42 h and 48 h. Hence, the reaction rate between cadmium ions and S^{2-} is very slow at 36 h, resulting in the formation of CdS nanoparticles with small diameter. With the increasing culture time, the enzyme activities and reaction rate correspondingly increase, contributing to the formation of thermodynamic-favored spherical particles. Thus, the size-controlled biosynthesis of CdS nanoparticles using immobilized *R. sphaeroides* could be obtained by simply changing the culture time.

Optical Properties of CdS Nanoparticles

Moreover, the samples obtained at different culture times exhibit excellent optical properties (see Fig. 7). The absorption peaks of the products obtained at 36 and 42 h are about 282 and 332 nm. The absorption peaks of CdS are blue-shifted from the absorption peak of bulk CdS

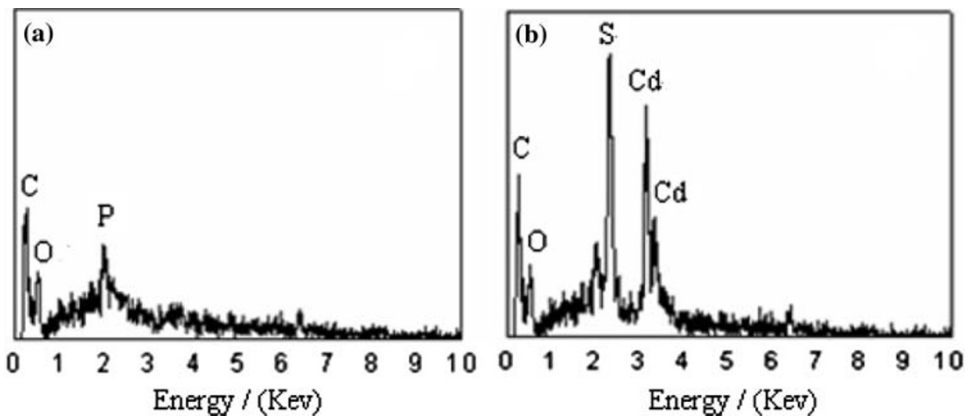

Fig. 5 The X-ray EDS analysis of cell ultra thin section of *R. sphaeroides* cultivated in culture medium in the absence of Cd^{2+} or containing 1 mM Cd^{2+}. **a** Circled in red, Fig. 3a, **b** circled in red, Fig. 3d

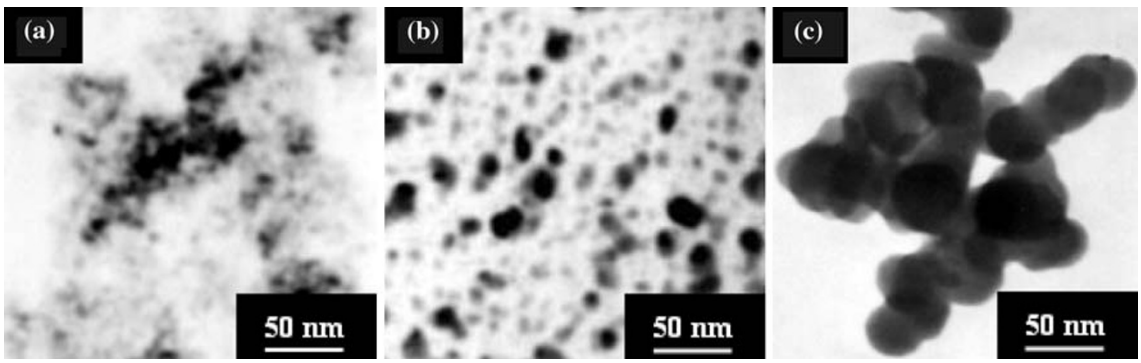

Fig. 6 TEM images of the obtained CdS samples at different culture times. **a** 36 h, **b** 42 h, and **c** 48 h

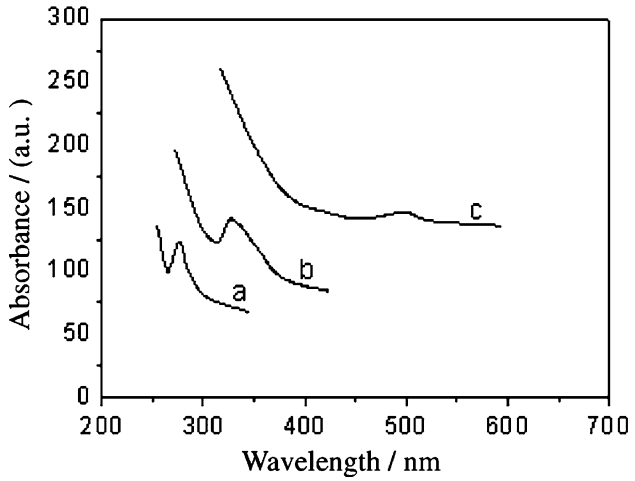

Fig. 7 Room-temperature UV–vis absorption spectra of the CdS samples prepared at different culture times. **a** 36 h, **b** 42 h, and **c** 48 h

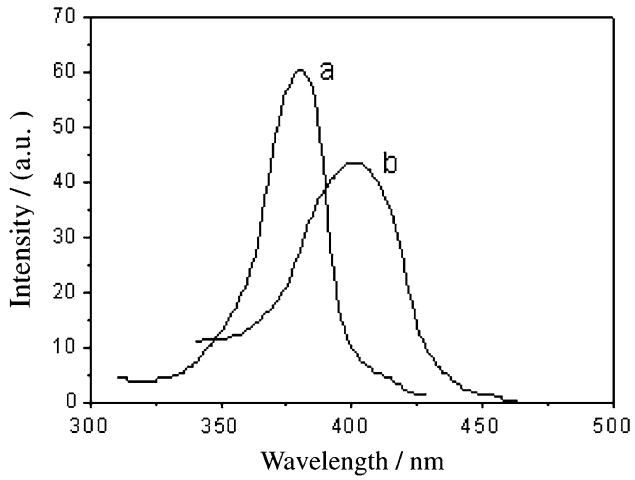

Fig. 8 Photoluminescence spectra excited by 345 nm of the CdS samples prepared at different culture times. **a** 36 h and **b** 42 h

(512 nm, $E_g = 2.43$ eV). According to the spectrum, we estimate the bandgap of CdS varied from 2.32 to 3.56 eV when the grain size reduces from 6.8 ± 0.22 to 2.3 ± 0.15 nm. This clearly indicates the presence of quantum size effects in the prepared CdS by the novel and simple route. However, the product obtained at 48 h with the grain size 36.8 ± 0.25 nm has a weak absorption at 506 nm, which is near the absorption peak of bulk CdS [7].

The photoluminescence spectra measurements of CdS nanoparticles synthesized at 36 and 42 h were carried using the same excitation wavelength of 345 nm at room temperature (see Fig. 8). The emission peaks at 382 and 406 nm correspond to the samples obtained at 36 and 42 h, respectively. The emission peaks at 382 and 406 nm are usually observed from the excitonic emission luminescence of semiconductor nanoparticles [21]. With increasing culture time, the fluorescence intensity remarkably decreases and the emission peak is red shifted. The result shows the change of bandgap of CdS nanoparticles and the presence of size-dependent quantum confinement effects.

Conclusions

The present study demonstrated that size-controlled CdS nanoparticles had been synthesized by living immobilized *R. sphaeroides*. Also, the result showed that *R. sphaeroides* could transport Cd^{2+} into cell from solution and then produced CdS. Finally, the CdS was carried to the extracellular solution and formed nanoparticles. The size of CdS nanoparticles biosynthesized by living immobilized *R. sphaeroides* could vary with the culture time. The way of the size-controlled biosynthesis of CdS nanoparticles by simply changing culture time provides a fully green approach for the biosynthesis modulation of nanomaterials. Moreover, the UV–vis absorption spectra and photoluminescence spectra showed that CdS nanoparticles exhibited unique optical properties.

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