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A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus

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ABSTRACT

A novel "green" route to prepare CdS nanoparticles under ambient conditions is reported. The long-term studies were carried out with immobilized fungus *Coriolus versicolor* in continuous column mode. The immobilized fungus served a dual purpose of both bioremediating cadmium as well as synthesizing stable CdS nanoparticles in aqueous conditions. The fungus immobilized in the column could remove 98% cadmium within 2 h. The continuous and extracelluar production of autocapped CdS nanoparticles is an added advantage of this system. Interestingly, no external source of sulfur is required for the transformation of toxic Cd to non-toxic CdS. The thiol group of the fungal protein was found mainly responsible for the production of such highly stable and autocapped CdS nanoparticles. The physico-chemical properties of the particles were studied by FTIR, XRD, SEM, TEM, AFM, TGA and PL.

1. Introduction

In recent years, semiconductor nanomaterials have attracted much attention in both fundamental research and technical applications because of their interesting and novel electronic and optical properties [1]. As a typical semiconductor material of the II–VI group, cadmium sulfide (CdS) nanocrystal has been the subject of intense interest because of its unique properties and potential applications in solar cells, laser light-emitting diodes and photoelectric devices [2].

In the past decade, a number of synthetic methods have been developed for the synthesis of CdS nanocrystals. Various approaches, such as polymer template-guided synthesis [3], hydrothermal and solvothermal methods [4] have been applied to achieve CdS nanocrystals. Most of these methods are still in the development stage and problems are often experienced with stability of the nanoparticles preparations, control of the crystal growth and aggregation of the particles. Hybrid composites of CdS nanoparticles embedded in epoxy resin matrixes have been also prepared by coprecipitate method [5]. Also, for biological and environmental applications, it is essential to prepare CdS NCs in water directly. The use of biological organisms in this area is rapidly gaining importance due to its growing success and ease of formation of nanoparticles. Presently, both prokaryotic (bacteria) and eukaryotic organisms such as yeast have been found to produce semiconductor nanoparticles within the cell wall of the microorganisms. The use of white rot fungi in the synthesis of nanoparticles is a relatively recent addition to the list of microorganisms. The use of fungi is potentially exciting since they are simpler to deal with in the laboratory and secrete large amounts of enzymes which are capable of metal–ion reduction, thereby significantly increasing the productivity of this biosynthetic approach. To prevent the damage caused by excess heavy metals, a fungus can immobilize the metal ions by simple adsorption or precipitation in cell wall or extracellular matrix, such as formation of calcium oxalate crystals or cadmium sulfide [6]. In response to cellular metal stress, fungi utilize intracellular sequestration of metal by cysteine-rich molecules, namely glutamyl peptides, metallothioneins and glutathione [7]. There have been recent reports on the intracellular and extracellular synthesis of metal nanoparticles such as Au and Ag by *Verticillium* [8], synthesis of zirconia nanoparticles and the extracellular synthesis of gold nanoparticles by *Fusarium oxysporum* [9].

In our present work, attempt has been made for synthesizing CdS nanoparticles without any additional stabilizer and to explore the possibilities of having more stable nanoparticles. We report here our discovery that the fungus *Coriolus versicolor* could successively reduce long-term cadmium to CdS nanoparticles in continuous column mode by a purely enzymatic process. Attempt has been made in this work to investigate the nature of binding of an amino acid to the surface of CdS particles.

To the best of our knowledge, there have been no papers describing the synthesis of autocapped CdS nanoparticles in continuous column mode. The potential of white rot fungus for large-scale

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production of semiconductor nanoparticles, an entire "green" process has been explored in these studies. This is a safe, low cost and more convenient approach as it does not involve special instrumentation, poisonous intermediates and the growing rate can also be easily controlled. The CdS nanoparticles obtained were characterized by XRD, FTIR, TGA, SEM, AFM, UV–vis and photoluminescence (PL) spectra.

2. Materials and methods

2.1. Growth of the fungus

C. versicolor was obtained from Institute of Microbial Technology, Chandigarh, India. The strain was maintained at 4 °C on malt agar slants. The growth medium used contained (g/l) 10 glucose and 5 malt extract. The medium was autoclaved (WidWo-Cat. AVD 500 horizontal autoclave) at 15 psi for 30 min and cooled to room temperature before use and the pH after autoclaving was 5.6.

2.2. Column reactors and growth media

The columns were made of borosilicate glass with 1.8 cm ID, 30 cm height, 15 cm bed length and were packed with tubular ceramic beads. The filamentous live fungus is able to grow inside and around the hollow ceramic beads and thus firmly adhere to it so that it does not come out along with the effluent. Thus the ceramic beads not only provide large surface area for the fungal growth and its immobilization but it also does not interfere with any adsorption and/or reduction studies. The flow rate of the column was maintained at 35 ± 5 ml/h and air was continuously sparged into the column bed with an aquarium pump through a capillary Teflon tube fitted at the bottom of the column.

Composition of the feed was decided such as to fulfill the macro and micro nutrient requirement of the fungi as reported in the literature. Feed Composition (g/l): Dextrose anhydrous 2, Malt Extract 1, Magnesium Sulfate (hydrated) $MgSO_4 \cdot 7H_2O$ 4.1, potassium dihydrogen orthophosphate KH_2PO_4 4, peptone 1, calcium chloride $CaCl_2$ (fused) 0.26, manganese chloride $MnCl_2$ 0.36. Initially, the columns were conditioned by recycling fungus-containing media for a week followed by feeding of growth media until the COD reduction reached a steady state. Although a fresh feed was prepared every third day to inhibit the growth of microorganisms in the influent, the column was continuously being fed by the influent feed.

To eliminate the possibility of any external source of sulfur, $MgSO_4$ was removed from the media composition before spiking with cadmium. The column was thereafter run without $MgSO_4$. Thus, the CdS particles are prepared with immobilized fungus *C. versicolor* in continuous column mode with no external source of 'sulfur'. The immobilized fungus served a dual purpose of both bioremediating cadmium as well as synthesizing stable CdS nanopartilces. Thereafter, the influent was spiked with known concentration of cadmium every third day and the absorbance of influent, effluent, was monitored every day. A control column as well as two replicate CdS columns were also set up whose COD was regularly monitored. In the control column Cd was never spiked and the replicate columns in which Cd was spiked were used to assess the reproducibility of the process.

2.3. Synthesis of CdS nanoparticles (CDSy)

For comparison purpose CdS nanoparticles was also prepared by conventional synthetic method. The typical synthesis procedure for CdS nanoparticles is described as follows. The aqueous solution of sodium sulfide Na_2S (2.5×10^{-3} M) was mixed with the aqueous solution of cadmium nitrate $Cd(NO_3)_2$ (1.8×10^{-3} M) followed by the addition of acetonitrile as solvent in different proportion. Rapid decrease of absorbance at 240 nm was monitored for 3 h. Complete precipitation of CdS occurred within 3 h of preparation. Subsequently, the resulting yellow precipitate (CdSy) was centrifuged, washed with deionized water and acetone remove excessive byproducts. Finally the products were dried in room temperature for 24 h [10].

2.4. Characterization

For the measurement of the UV–vis absorbance, the dried particles were dispersed in deionized water were recorded with a UV/vis, Spectrophotometer Lambda 40 (PerkinElmer, USA) in the wavelength range of 200–800 nm. Photoluminescence (PL) spectra were measured by using Fluorolog-3 spectrofluometer (Jobin Yvon, USA) at room temperature. The samples were excited at wavelength 300 nm and spectra were recorded in the range 240–850 nm with the help of a gated photomultiplier tube as a detector. Infrared (IR) spectra were recorded on a BRUCKER, VERTEX-70, Infrared spectrophotometer making KBr pellets in reflectance mode. The XRD spectra were recorded in ARL X TRA X-ray Diffractometer and the X-ray diffracted intensities were recorded from 10° to 80° 2θ angles. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) microanalysis were carried out on gold-coated samples, using a FEI (QUANTA 200) instrument operating at a voltage of 10–17.5 kV with a tilt angle of 45°.

The morphology and particles size of CdS nanoparticle was determined by transmission electron micro Philips® EM208 200 kV. Atomic force microscopy (AFM) images were obtained using a Picoscan™ Molecular imaging (USA). The samples for AFM analysis were prepared by dropping solution of nanoparticles on mica film. Thermo gravimetric analysis (TGA) was performed with a TGA/DTA 6300, SII Nano Technology, Inc. (Japan) instrument under a stream of air. The sample was heated at 10 °C/min from 35 to 1000 °C.

3. Results and discussions

Initially a low concentration (10 ppm) of Cd was spiked every third day to see whether it gets toxic to the fungus. The fungus could completely remove the Cd as monitored in the effluent. The fungus could well sustain the toxic effect of repeated respike of the metal as evident by the 98% removal within 2 h of spiking the metal. After a month, the concentration of cadmium was slowly increased by a factor of 10 ppm fortnightly, from 10 to 60 ppm in a continuous mode to check the metal tolerance limit of the fungus. Even with the increase in the initial spiked concentration of cadmium, almost complete removal of Cd from the effluent was observed. A thin yellow precipitate seemed to be accumulating with time in the columns, with no clogging effect on the column. Due to the continuous production of CdS in a flowing column the yield of CdS cannot be quantified, unlike the conventionally synthesized CdSy. This colloidal precipitate (CdS) in the column was frequently collected and analyzed by various instrumental techniques. Fig. 1 shows fungus immobilized on ceramic beads in continuous up flow column reactors (a) before (b) after spiking of Cadmium ions. Our studies indicate that protein-bound SH-containing compounds might be involved in the detoxification of Cd as well in its successive reduction to CdS nanoparticles [11].

The CdS nanoparticles visualized by SEM (Fig. 2a) exhibited a uniform morphology showing micro- to nanospheres in the 100–200 nm range. Strong S signals were also observed in the EDAX spectrum recorded from the as prepared cadmium sulfide nanoparticles, indicating the presence of thiol proteins. Due to lower resolution and magnification compared to TEM, the size estimation by SEM cannot be totally relied upon as large variations in

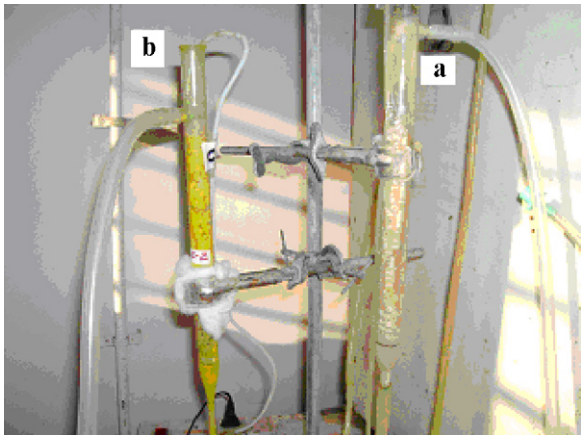

Fig. 1. Fungus immobilized on ceramic beads in continuous up flow column reactors (a) before (b) after spiking of cadmium ions.

particle size were observed from a few to approximately 100 nm in diameter. TEM and AFM images could give accurate and better understanding of the particle morphology. Fig. 2b represents TEM image of the CdS nanoparticles, embedded in the matrix indicating that the sample is composed of a large number of well dispersed spherical nanoparticles with uniform size and shape. The average size of the particles estimated from the TEM image is about 5–9 nm. The TEM image clearly showed the dots seen in SEM to be actually composed of several particles of different sizes grouped in clusters. Each cluster seemed to be surrounded by a thin outer not so dense layer which is perhaps the protein capping.

AFM studies were in agreement with the TEM studies indicating that most of the particles were in the size range from 8 to 15 nm, the average particle size of approximately 8 nm (Fig. 3). The particles were globular shaped and lie very close to each other in clusters. On comparison by AFM, both CdS and CdSy nanoparticles appeared to be similar sized with similar uniform morphology.

The XRD patterns of the CdS nanoparticles are considerably broadened due to very small size of the crystallites. Four diffraction peaks at 26.8°, 44.3°, 51.85°, and 72.5° found in CdS nanoparticles as well as in conventionally prepared CdSy and can be indexed as (1 1 1), (2 2 0), and (3 1 1) which are identified for cubic (c) CdS

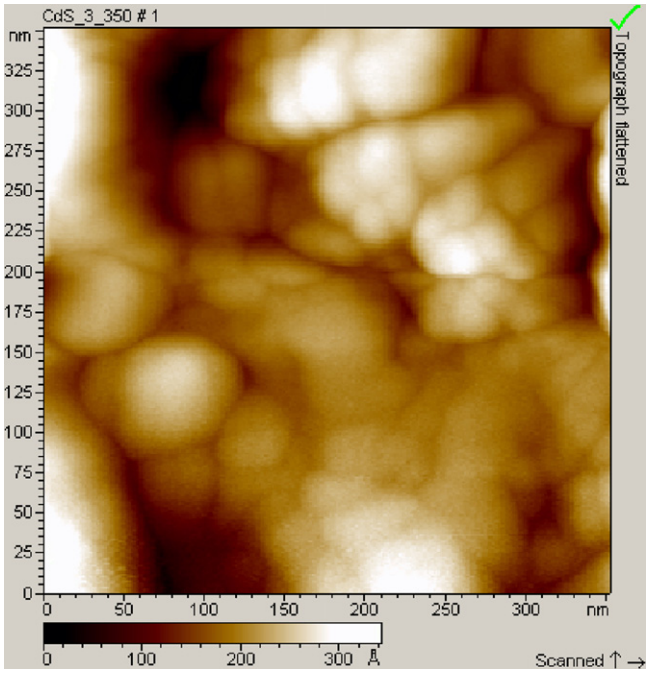

Fig. 3. AFM image of CdS nanoparticles.

phase (JCPDS Powder Diffraction File no. 10–454) for uncapped CdS nanoparticles. In case of protein-capped nanoparticle, small fractions of hexagonal phase also appear with the greenockite structure (JSPDC no.06-0314) at 36.95°, 43.60°, 52.80°, 67.90°, 69.60°, and 75.80°. These hexagonal peaks are absent in conventionally prepared CdSy nanoparticles. It reveals that the capping agents play a special role in affecting the crystal field distortion, symmetry breaking and an important role on the modification of crystal phase of CdS nanoparticles during synthesis [12].

The thermo gravimetric analysis (TGA) was carried out to understand the thermal stability of the CdS nanoparticles as well as conventionally prepared CdSy as shown in Fig. 4. The small weight loss of 9% between 140 and 160 °C is that of the physically adsorbed water and hydroxyls contained in the CdS nanoparticles. A marked weight loss of 50% occurring at 380–450 °C is attributed to the

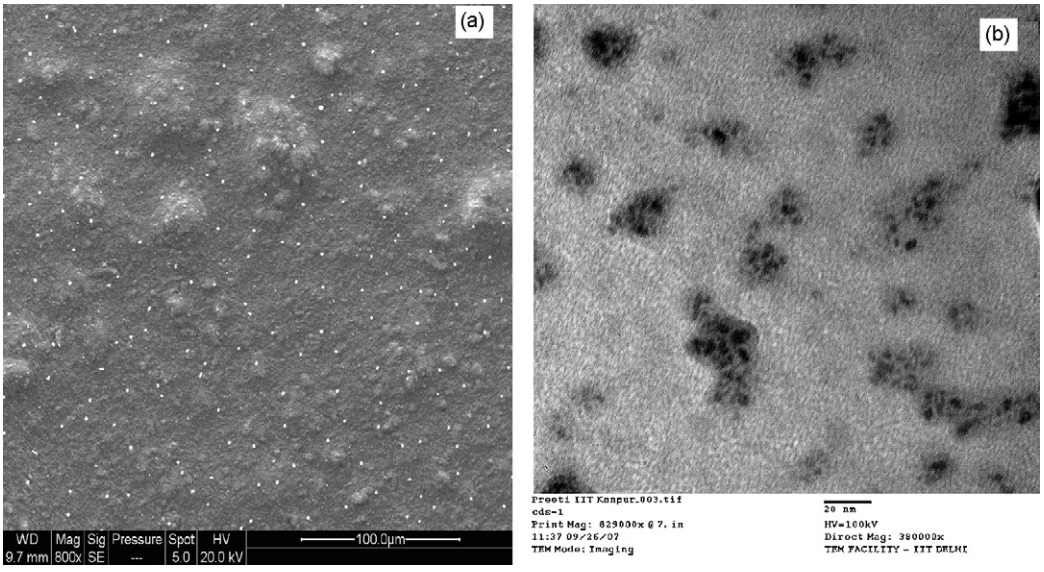

Fig. 2. CdS nanoparticles. (a) Scanning electron micrographs with scale bar 100 μm at 800 magnification. (b) Transmission electron micrographs of CdS nanoparticles with scale bar 20 nm at 3×10^5 magnification.

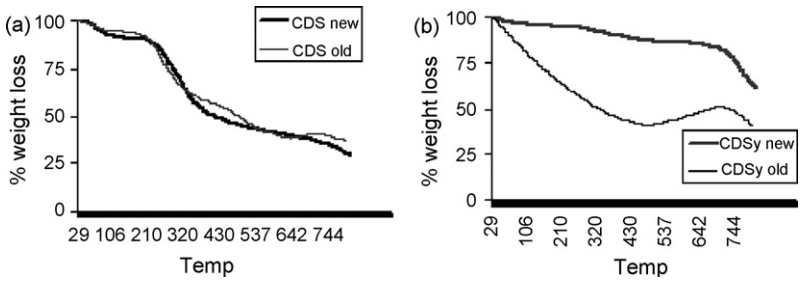

Fig. 4. Thermogravimetric analysis of (a) CdS nanoparticles new (freshly prepared) as well as CdS nanoparticles old (prepared one year before). (b) Conventionally prepared CdSy nanoparticles new (freshly prepared) as well as conventionally prepared CdSy nanoparticles old (prepared one year before).

decomposition of the protein moiety in the CdS nanoparticles. The very high stability of CdS nanoparticles is evident from Fig. 4 where no weight loss or degradation was observed even after one year of storing while conventionally prepared CdSy nanoparticles showed a weight loss of 50–60% within a month of storing. This result indicates that the CdS nanoparticles are very stable, and could be stored for a long time with no deterioration under ambient conditions [13]. The stability factor is attributed to the protein capping.

FTIR measurements (Fig. 5) were carried out both for the native fungus as well as for the capped CdS nanoparticles, to identify the possible interactions between Cd^{2+} with fungal proteins. The strong narrow peak at 3300–3500 cm^{-1} in the fungus is characteristic of the N–H and the O–H stretching vibrations. The broad band at 2500–3500 cm^{-1}, was due to the strong hydrogen bonding. The band at 1456 cm^{-1} is assigned to methylene scissoring vibrations from the proteins in the solution. Two small features occurring at about 2919 and 2848 cm^{-1} are assigned to the methylene antisymmetric and symmetric vibrations of the hydrocarbons present in the fungal protein. This is much intensified in CdS nanoparticles indicating the involvement of –CH groups.

On comparison of the spectra of native fungus and CdS nanoparticles, the most significant point to be noted is that the band at 2552 and 921 cm^{-1} corresponding to –SH stretching and bending mode are absent in the spectrum of CdS nanoparticles while these spectra are present in native fungal mycelium. It may be attributed to cleavage of S–H bond and formation of a new bond, i.e., –S–Cd bond of Cd-thiolate ($Cd–S–CH_2COOH$) complex on the nanoparticle surface.

It is reported earlier that proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins and via the electrostatic attraction of negatively charged carboxylate groups in enzymes present in the cell wall of mycelia and therefore, stabilization of the cadmium sulfide nanoparticles by protein is a possibility. It can also be seen that the band at 1367 corresponding to the free symmetric stretch of COO^- group [14], in the native fungus has been utilized in ester formation as evident by peak at 1735. An aromatic C–C stretch at 1157 cm^{-1} and a C–H bend at 825 cm^{-1} could be well assigned to the aromatic residue tyrosine and the C–H bending mode of the aromatic residue tryptophan detected at 742 cm^{-1}. Apart from this the spectrum also shows peaks at 663 and 715 cm^{-1} due to C–S stretching; these C–S stretching modes confirm the presence of sulfur-bearing proteins residues like cysteine and methionine. This observation confirms the protein capping of nanoparticles surface via thiol group.

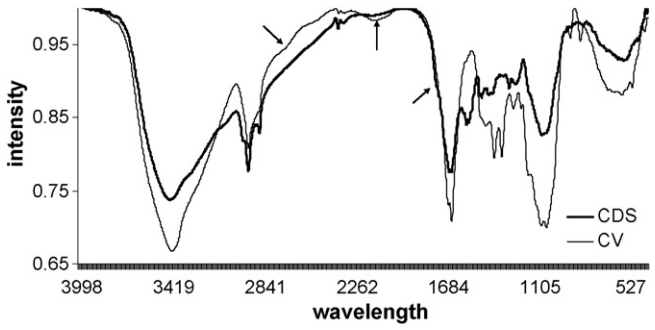

Fig. 5. FTIR spectra of CdS nanoparticles as well as spectra of plain fungus (CV).

The amide linkages between amino acid residues in polypeptides and proteins give rise to well known signatures in the infrared region of the electromagnetic spectrum. The strong peak at 1656 cm^{-1} is due to the presence of amide I band which is primarily a C=O stretching mode. The amide II band due to the N–H stretching modes of vibration in the amide linkage was not visible in the native fungus but showed up at 1544 cm^{-1} in the cadmium sulfide samples. A shift of μ cm^{-1} 27 is also seen in the more complex Amide III band located near 1243–1270 cm^{-1} in CdS nanoparticles. The position of these bands is close to that reported for native proteins in earlier papers [15]. The FTIR results indicate that the secondary structure of the proteins is affected as a consequence of binding with the CdS nanopartilces.

The strong absorption band at 2105, 2160 and 2190 cm^{-1} associated with the isonitrile in native fungal mycelium was absent in CdS nanoparticles indicating the strong role of –N=C group in CdS nanoparticles synthesis [16]. Isonitriles are effective in causing condensation of amino acids having masked amino groups with compounds having active hydroxyl groups to the corresponding amino acid esters. The compounds having active hydroxyl groups that may be combined with N-protected amino acids in the presence of an isonitrile to form esters are a broad class [16]. The masked amide II band of the fungus was clearly observed in CdS at 1544. The new band seen as a shoulder at 1735 cm^{-1} corresponding to carbonyl stretch vibrations of the amino acid ester formed, indicating that the reduction of the cadmium ions is coupled to the oxidation of the hydroxyl groups in fungal mycelium molecular and/or its hydrolyzates. With the overall observations, it can be concluded that the proteins or peptides through either free amine groups or cysteine residues might have formed a coating over the cadmium sulfide nanoparticles, which in turn supports their stabilization.

In order to understand the utilization of thiol proteins in the process, the total thiol (TSH) concentration of samples from inside the column as well as in the effluent sample was determined with 5,5′-dithio-bis (2-nitrobenzoic acid) [17] by measuring the absorbance at 412 nm. In the control column, i.e., when cadmium was not spiked, the T–SH level of the sample from inside the column as well as that of the effluent sample of the column was very high, indicating the continuous release of thiol proteins inside the column. The production of thiol compounds is known to increase after exposure to cadmium metal [18] but in the current studies after cadmium was spiked in the column, almost negligible TSH was found both from inside the column as well as that in the effluent sample. This is because most of the SH-containing protein which was being released by the fungus in the control columns, gets utilized in the synthesis of CdS nanoparticles. The SH group binds to the Cd metal and is responsible for the formation of CdS nanoparticles. This was also confirmed by C, H, N, S analysis. In native

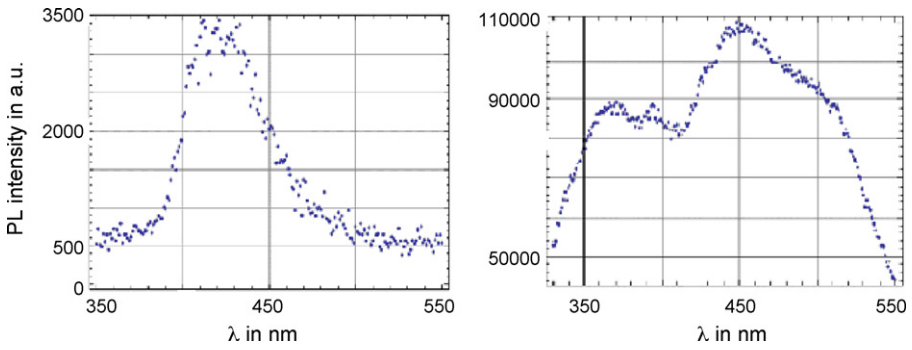

Fig. 6. Spectra of PL (a) CdS nanoparticles (b) conventionally prepared CdSy nanoparticles.

immobilized fungus the percentage of components is, C 52.45%, S 1.50%, N 10.71% whereas when cadmium was spiked the S content in the CdS nanoparticles increased four folds.

The presence of an absorption edge at 280 nm in the reaction medium is indicative of the presence of proteins [19]. The colloidal solution of CdS nanoparticles was extremely stable with no evidence for aggregation even after 10 months of continuously running the column.

The long-term stability of the CdS nanoparticle solution is due to the presence of the proteins in the nanoparticle solution that bind to the surface of the nanoparticles and prevent aggregation.

The presence of the broad absorption band at around 350–550 nm is characteristic of CdS nanoparticles in the quantum size regime [20]. The conventionally prepared CdSy nanoparticles exhibit the exciton absorption peak at 400 nm. This is due to 1s–1s′ absorption and the peak position varies between 350 and 460 nm. UV–vis spectra of CdS nanoparticles exhibit the appearance of a broad absorption band. The absence of strong excitonic structures may be attributed to two main factors; the weak exciton binding energy due to strong coulomb screening and/or broad size distributions. However, due to surface modification of CdS nanoparticles, it shows featureless absorption edges. It indicates that the surface modification dramatically influences the optical properties of CdS nanoparticles.

Fig. 6 shows the photoluminescence spectra of CdS nanoparticles as well as conventionally prepared CdSy nanoparticles. The emission spectra of conventionally prepared CdSy nanoparticles showed the dominant peak at 420 nm whereas the CdS nanoparticles exhibited a dominant blue emission peak at 450 nm (2.75 eV). A closer examination however indicated the appearance of an additional green emission band as shoulder at 500 nm (2.48 eV) at the excitation wavelength of 300 nm. It is suggested that the dominant emission peak at 450 nm is attributed to transition from conduction band to valance band. The peak at 500 nm was attributed to the intrinsic character of CdS nanoparticles, which had been reported by Butty and Peyghambarian [21].

The strength of the band edge emission peak at 365 and 390 nm adequately shows the presence of tryptophan and tyrosine residues of protein which strongly influence the optical property of CdS nanoparticles by acting as the capping agent. Since the intensity of fluorescence as well as the location of the emission peak is changed when the immediate environment around the fluorescing residues is altered, protein fluorescence has been used as a sensitive monitor of conformational changes in the protein [22].

3.1. Mechanism

As already discussed in the FTIR section, the formation of CdS NPs is through disulfide (cystine) bridges and may be attributed to cleavage of S–H bond and formation of a new bond, i.e., –S–Cd bond of Cd-thiolate (Cd–S–CH_2COOH) complex on the nanoparticle surface. The –COOH groups from the cadmium-thiolate complexes do not react with the –NH_2 groups of protein but interact with hydrogen bond. Therefore, the capped CdS nanoparticles are bonded to –NH_2 groups by hydrogen bond [23]. One of the oxygen atoms of the carboxylic group (–COOH) formed the co-ordinate bond between the oxygen atom and Cd^{2+} ions [24]. Thus competing with the thiol group to assemble onto the surfaces of the CdS nanoparticles. Also, the band at 1382 and 1544 cm^1, corresponding to COO– symmetric stretch and –NH^{+3} asymmetric bending mode is free to conjugate with suitable biomolecules. These molecules in turn are responsible for capping the CdS nanoparticles via hydrogen bonding and electrostatic interaction.

4. Conclusions

The detoxification potential of the live fungus could be harnessed into reliable waste treatment processes as well as used effectively for the synthesis of CdS nanoparticles. The biocatalytic and stability characteristics of the enzymes secreted by the fungus on exposure to cadmium ions clearly highlighted the potential of this approach both in bioremediation as well as large-scale mineral growth of CdS NPs. The utility of this process is underlined by the fact that even after continuous exposure to the toxic metal ions, the fungus readily grows and transforms the toxic conditions to non-toxic by reducing Cd to CdS without the use of any external source of sulfur. The thiol (SH) group of Cys in *C. versicolor* plays a critical role for binding to and sequestration of Cd^{2+} as well as reduction to CdS.

Another important potential benefit of the process described herein is the fact that the semiconductor CdS nanoparticles, which are quite stable in solution, are synthesized extracellularly in large quantities. This is thus a very important advantage over other biosynthetic methods where the nanoparticles are entrapped within the cell matrix in limited quantity whereby an additional processing is required to release them from the matrix.

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