1 Introduction

Semiconductor nanomaterials are invariably considered for application in solar energy conversion and for photovoltaic processes. These nanosized materials are often referred to as quantum dots (QDs), and they exhibit distinctive electronic and optical properties owing to size dependency when the size decreases to less than Bohr exciton radius. Cadmium sulfide (CdS) particularly holds immense potential for electromechanical and optoelectronic applications due to the direct band gap in the visible region; similarly, CdS QDs have been widely utilized for biomedical and biological studies. The optical properties, i.e. photoluminescence and photostability, of CdS nanoparticles can be tuned with the help of dispersion stability, shape, size, and structure. Various synthesis approaches (milling, intense heat treatment, electrochemical or photochemical reduction, etc.) have been attempted to synthesize CdS nanomaterials. These processes involve intense processing and environmentally perilous chemicals that restrict control over size, shape, and dispersity. The primary predicaments due to these conventional methods of nanosized material production remain in their detrimental effect on the environment. This leads to a perpetual quest for environmentally innocuous and acceptable ways of nanomaterial synthesis.

A biologically motivated green route of controlled CdS QD synthesis is a potentially effective and flexible approach utilizing biological molecules such as cysteine and organic thiols as capping agents. Plants, yeasts, and bacteria utilize these molecules to detoxify the harmful cadmium into CdS nanocrystals. Microbe-reinforced mineralization of metals into biocompatible materials is a potential drive towards the green synthesis of materials. However, metal biomodification into nanoparticles verily exhibits non-uniformity of the nanoparticles due to limited study of the cell behavior and bio-mechanism, which mitigate their applications in the optoelectronics field. In the present study, we utilized the self-assembly behavior of chemical species in a biological environment to produce mono-disperse and well-controlled cadmium sulfide quantum dots (CdS QDs). Cadmium quenching (up to 85%) by organic molecules, exported from bacterial (Pseudomonas aeruginosa) cells, has been established by studying the influence of biological growth parameters (cell age) and precursors (cysteine and cadmium) on the formation of CdS QDs. Herein, we elucidate the mechanism involved in the bacterial precipitation of cadmium into CdS QDs using AFM and TEM profiles, which confirm the hypothesis of QD formation involving the cell wall in the medium and its interaction with precursors. The green synthesis of CdS QDs with controllable surface functionalities (studied via FTIR spectroscopy) results in tunable photoluminescence.
2 Experimental

2.1 Microorganism and culture conditions

*Pseudomonas aeruginosa* was recovered from Microbial Biotechnology and Downstream Processing Laboratory, Indian Institute of Technology, Kharagpur, India, and was cultivated at 37 °C in a modified P2 medium containing 0.25 g L\(^{-1}\) yeast extract. For energy sources, 0.4 M glucose was used. Magnesium sulfate heptahydrate (0.5 g L\(^{-1}\)) and 0.01 g L\(^{-1}\) each of sodium chloride, ferrous sulfate heptahydrate and manganese sulfate monohydrate were used as a salt mixture into the media. Sodium β-glycerophosphate (1 g L\(^{-1}\)) was used as a phosphorus source replacing KH\(_2\)PO\(_4\) in the cadmium precipitation experiments to prevent cadmium phosphate formation. Sodium bicarbonate (2.5 g L\(^{-1}\)) was used to maintain the pH of the culture during the experiment. Buffers used in the study included phosphate buffer (a mixture of KH\(_2\)PO\(_4\) and K\(_2\)HPO\(_4\) and pH 7.2 maintained with NaCl) and a wash buffer containing Tris HCl (100 mM pH 7.2), 10 mM tetrasodium EDTA and 400 mM NaCl. Cysteine hydrochloride monohydrate (0.2% (w/v)) was included in the media components to act as a reducing agent and a sulphur source. Pure cadmium chloride monohydrate was obtained from Merck. Cadmium standard for atomic absorption spectroscopy (AAS) was purchased from Agilent technologies.

2.2 Preparation of CdS nano precipitates

CdS precipitates were synthesized via a green route using anaerobically grown *Pseudomonas aeruginosa* cells. Late log-phase to early stationary-phase cells were used as the inoculum for precipitation. During the experiment, 1 mM CdCl\(_2\) was added to the actively growing cell culture. The obtained precipitates were further characterized optically, physically and structurally to check for the efficiency of the method.

2.3 Material characterization of CdS precipitates

Structural characterization of the microbially produced CdS QDs was carried out by using powder XRD with monochromatic nickel-filtered Cu K\(_{x}\) radiation in the 2\(θ\) range of 20 to 80°. The FTIR spectrum of the nano precipitates was recorded using a commercially available spectrophotometer. The sample was prepared by placing one drop of the dilute solution of the precipitates in the media onto a copper grid supported on a thin film of amorphous carbon followed by drying the sample in a vacuum desiccator.

2.4 Optical characterization

Optical spectra of CdS quantum dots were recorded using a UV-1800 spectrophotometer, Shimadzu, Japan. The band gap was estimated using Tauc plots generated from the absorption spectra with an assumption of direct bandgap for CdS and 1 cm path length as the cuvette width. Photoluminescence
excitation (PLE) spectrum of the synthesized samples was recorded at different emission wavelengths using a spectrofluorimeter LS 55 (PerkinElmer).

2.5 Bio-mechanism of CdS QDs
Green synthesis of CdS QDs involves an enzymatic pathway to carry out cadmium sulfide precipitation. However, a study on the impact of biological parameters was required to understand the biological mechanism of cadmium precipitation. Cadmium was provided to bacterial cells at different phases of their growth, and subsequently, the cadmium sulfide precipitation was recorded at each phase to establish the impact of cell age on QD formation. Cadmium precipitation in the form of CdS was detected by AAS. The effect of cysteine concentration on CdS synthesis was measured by examining CdS precipitation by cells grown in media with a gradient concentration of cysteine, i.e. 0.01–0.5%. CdCl₂ was added to the cell culture at a concentration of 0.5–5 mM to check for the threshold concentration. Samples were withdrawn after 24 h and analyzed for viable cells and lag-phase duration. Controls were also prepared without addition of cadmium. The co-ordinated effect of cysteine and cadmium on the precipitation process was found out when log-phase 
P. aeruginosa
 cells were inoculated in the media with different concentrations of Cys-HCl and cadmium chloride solution. The synchronized effect of cysteine and cadmium was estimated in the form of CdS precipitation. Cysteine desulfhydrase specific activity was also measured to signify the direct impact of CdS on cell metabolic activity, i.e. enzyme production.

To evaluate the time-regulated formation of CdS QDs, absorption spectra were recorded at different time intervals post cadmium addition to the media. Cell surface morphology was studied by atomic force microscopy (AFM, Agilent Technologies, USA, Model 5100), where cells were dried on a glass substrate and scanned to obtain the high-resolution micrographs of the cells with nano-precipitates. All the measurements were carried out in air at room temperature (25 °C) using a silicon nitride tip (PPP-NCL) in the intermittent contact mode.

3 Results and discussion
3.1 Biological synthesis of CdS QDs
To study the synthesis of CdS QDs using bacterial cells, a series of experiments were designed keeping the cell age constant. An actively growing culture of Pseudomonas aeruginosa (turbid white solution in Fig. 2(A)) was mixed with CdCl₂ and incubated for 6 h, resulting in bright yellow precipitates, as shown in Fig. 2(A). The precipitate was collected and further characterization was carried out. The mass balance experiments were simultaneously performed to endorse the cadmium removal from the medium in the form of CdS precipitates (Table 1). From the table, it can be concluded that 85% cadmium was removed from the inoculated medium in the form of CdS.

For controlled experiments, approximately 5% cadmium removal was observed, which may be due to the presence of cadmium phosphate or cadmium carbonate (quantified by dissolving in EDTA-containing wash buffer).

3.2 Material characterization of microbially synthesized CdS
The yellow-colored colloids were analyzed by spectroscopy (UV-vis absorption, photoluminescence, and FTIR) and microscopy (TEM and EDX) methods.

![Physical characterization of bio-synthesised CdS QDs](image-url)
The XRD pattern shown in Fig. 2(B) reveals the peaks at 2θ values of 26.60, 43.90 and 51.62° corresponding to the (111), (220), and (311) planes of the diamond cubic phase of CdS (with lattice constant \( a = 5.32 \, \text{Å} \) (JCPDS File No. 10-154)). The broad XRD peaks implied the small crystallite size of \( 6 \pm 0.3 \, \text{nm} \), which was calculated using the Scherrer equation,\(^{18}\) and further confirmed by the TEM micrographs and the absorption spectra in subsequent sections. The surface functional groups of CdS QDs and their interaction with \textit{Pseudomonas aeruginosa} were studied by FTIR spectroscopy (shown in Fig. 2(C)). A peak at 3443 cm\(^{-1}\) could be assigned to the O–H stretching vibration present as absorbed water on the CdS surface. The surface ligands on the CdS QDs were attributed to the bands at 2924, 2846, 1635, 1393, and 1021 cm\(^{-1}\), coming from the organic functional groups (alkyl, alken, carbonyl, and amine) present in the media.\(^ {19,19}\) Bands obtained at 490 cm\(^{-1}\) and 650 cm\(^{-1}\) signified the S–S and C–S stretching bonds, whereas peaks at 1237 and 2353 cm\(^{-1}\) were assigned to the organic sulfate group and CO\(_2\), respectively.\(^ {20}\)

Along with these peaks, a peak appeared at 1540 cm\(^{-1}\) ascribed to the aliphatic nitro groups on the surface of CdS QDs. The presence of all organic groups can be credited to the media components and cell metabolites produced and released during bacterial growth. This organically modified surface of QDs results in producing monodispersive nanoparticles.\(^ {21-25}\) From Fig. 2(G), the method "B" reported first extracellular CdS QD preparation using fungus, which involved 5–20 nm QD production in 4–6 days. However, current work manifests the synthesis of monodispersive (4–7 nm) CdS QDs in an incubation period of 6 h.

### 3.3 Optical characterization

UV-visible absorption spectroscopy was utilized to monitor the optical absorption properties of the QDs. The absorption spectra of \textit{Pseudomonas aeruginosa} with and without green synthesized CdS QDs are shown in Fig. 3(B). The absorption peak was observed at \( \sim 260 \, \text{nm} \) corresponding to the \( \pi \rightarrow \pi^* \) transition in the surface ligands attached to the surface of CdS QDs. The blue shift in the peak was detected as compared to the bulk CdS (at 514 nm). The band gap of the CdS (given by the Tauc relation) was obtained from \( \alpha h^2 \) versus \( h \), as shown in Fig. 3(A). The direct band gap value of green synthesized CdS was estimated to be \( \sim 3 \, \text{eV} \); this value was shifted compared with the bulk value and this could be a sign of a size quantization effect in the synthesized sample. The particle size of the CdS QDs was estimated using the formula given by Brus,\(^ {19,26}\) From the calculations, the radius of the precipitated nanoparticles is 3.18 nm which was found to be in parity with the particle size observed in the TEM micrographs and calculated from the X-ray diffractions. The absorption tail in the range of 300–600 nm is due to CdS.\(^ {27}\) These absorption bands contribute to the observed PL emission in CdS at 475 nm as supported by the PLE spectra in Fig. 3(C). However, unlike the traditional inorganic semiconductor QDs, the green synthesized CdS QDs emit excitation wavelength-dependent tunable PL, as shown in Fig. 3(D). Such tunable PL emission is due to the presence of organic ligands in the as-prepared colloidal solutions of CdS QDs.\(^ {28}\) There is a clear shift in case of absorption and PL emission peaks. The origin of such shift of the PL emission center in comparison to that of the absorption maxima can be explained with the help of basic theory of spectroscopy; the optical absorption is not necessary with the presence of PL, but PL emission requires absorption: (i) when the energy from the light is absorbed, the material can relax the energy through non-radiative paths (i.e. without light emission), (ii) PL emission can come from the electronic energetic level well, which is lower than the absorption gap, as shown in

### Table 1: Mass balance of the cadmium precipitate

| Cysteine concentration (mM) | Removal from media \( \Delta \) [cadmium] \( \pm \) S.E. (mM) | EDTA-soluble fraction | Pellet | % CdS precipitation |
|-----------------------------|----------------------------------------------------------|----------------------|-------|---------------------|
| 11.78 Inoculated             | 2.70 \( \pm \) 0.05                                       | 0.42 \( \pm \) 0.05   | 2.28 \( \pm \) 0.05 | 84.6               |
| Un-inoculated               | 1.61 \( \pm \) 0.05                                       | 1.6 \( \pm \) 0.05    | 0.01 \( \pm \) 0.03 | 0.3                |

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ligands in the biosynthesized CdS QDs. To confirm this, the measurement of emission wavelength-dependent PLE spectra of the material was performed (Fig. 3(E)). The as-observed shift in the PLE band generally occurs when PL emission occurs from multiple surface defect states (contributed by surface ligands (as observed in FTIR)) along with the intrinsic band gap of the material.29

### 3.4 Bio-mechanism of CdS QDs

The CdS QD formation involves quenching of cadmium ions in the presence of *Pseudomonas aeruginosa*, where Cys-HCl was catalyzed by l-cysteine desulphhydrase resulting in the release of sulfides that got precipitated in the form of CdS.

The impact of growth phase on the cadmium removal is demonstrated in Fig. 4(A). It is evident from the curve that CdS formation occurred until the late stationary phase. There was a cessation of CdS precipitation post stationary phase, which could be linked to the declined expression of the metabolic enzymes particularly cysteine desulphhydrase.30 The impact of cysteine concentration on the precipitation of CdS was quantified by preparing culture media with different concentrations (0.05–1% (w/v)) of cysteine (shown in Table 2). More than the optimal amount of cysteine had adverse effects on *P. aeruginosa* due to cysteine toxicity, which has resulted in less cadmium removal.31 The cadmium chloride solution was added to *Pseudomonas aeruginosa* cells to find out the tolerance threshold (Table 3). Cadmium toxicity in the case of bacterial cells has been speculated to be associated with DNA damage via reactive oxygen species generation, which is likely to cause interference in the synthesis of proteins and other metabolites.32 According to Ron *et al.*, 1992, cadmium mediates the stress response in the cells, which results in the production of a large number of proteins in response to stress.33

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The synchronized impact of cysteine and cadmium was estimated in the form of CdS precipitation, and growth parameters including cysteine desulphhydrase formation are presented in Fig. 4(B and C). Upon increasing the cadmium concentration above the optimized value, there was negligible CdS precipitation even when coupled with a tremendous increment in l-cysteine desulphhydrase specific activity. This may be due to the overproduction of l-cysteine desulphhydrase, which causes toxicity to the cell growth owing to the shift in cellular machinery. There has been an apparent diversion in the utilization of cysteine from protein production to sulfide generation. It is worth noting here that during escalation in the cadmium concentration, *P. aeruginosa* has encountered the metabolic burden of overproducing cysteine desulphhydrase and

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**Fig. 3** Optical characterization of CdS QDs: (A) Tauc plot obtained for band gap estimation; (B) absorption spectra for the cells with and without CdS; (C) photoluminescence emission and absorption spectra; (D) excitation wavelength-dependent tunable PL emission; (E) emission wavelength-dependent PLE spectra of CdS nanoparticles and (F) energy band diagram representing the plausible electronics transition responsible for PL in CdS QDs.

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52 4
21 0
Cadmium conc. (mM) Lag phase duration (h) Colony formation
1 0.5
0.5 2
1 4
2 10
4 24
5 24

Inoculated 0.05
/C6
Inoculated 0.080
/C6
Inoculated 0.623
/C6
Inoculated 0.860
/C6
Inoculated 0.05 0.06
/C6
Inoculated 0.05 0.070
/C6
Inoculated 0.05 0.499
/C6
Inoculated 0.05 0.507
/C6
Inoculated 0.05 0.06
/C6
Inoculated 0.05 0.087
/C6
Inoculated 0.05 0.553
/C6
Inoculated 0.05 0.773
/C6
Inoculated 0.05 0.829
/C6
Inoculated 0.05 0.01
/C6
Inoculated 0.05 0.773
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image of the cells immediately after (Fig. 5(A)) and 6 h (Fig. 5(B)) post metal addition shows the active formation of precipitates. The AFM and TEM images establish the distinctive behaviour of cells during QD formation. The QD deposition (given by height profile (Fig. 4(H))) on the cells is the manifestation of the cell’s resistance towards metal influx in the form of extracellular CdS formation.

The plausible mechanism of the cadmium sulfide precipitation is shown as a schematic in Fig. 5(C), as validated and suggested by the micrographs, absorption spectra, and other optimization results.

4 Conclusion

In this study, cadmium, a toxic metal, was precipitated (up to 85%) by Pseudomonas aeruginosa in the form of CdS, providing an eco-friendly circumvention for toxicity removal. The as-prepared nano precipitates were found to be monodisperse (6 ± 2 nm) as confirmed by XRD, TEM and UV-vis, and tunable photoluminescence was observed owing to surface-functionalized ligands (due to bacterial cells validated by FTIR spectroscopy). The functionalized surface state of QDs owing to media and growth components vouches it to be potential for biomedical applications including biosensing. The cell growth parameters were shown to positively influence QD precipitation when supplied with optimum CdCl₂ and Cys-HCl (sulfide source). The step-wise formation of QDs involved the cell wall interaction with the precursor, i.e. cadmium, and its conversion into insoluble CdS precipitates within 6 h, which was demonstrated by AFM micrographs and plotted height profiles. This is the first ever report of examining the control of cellular environment on metal detoxification and consequently QD synthesis and properties. It is considered an advanced step for the development of bio-based nano-material production with an insight into the cellular level mechanism involved in the process thus adds value to the greener ways of material fabrication.

Conflicts of interest

There are no conflicts to declare.

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