

Full Length Research Paper

Rapid biosynthesis of cadmium sulfide (CdS) nanoparticles using culture supernatants of *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633 and *Lactobacillus acidophilus* DSMZ 20079T

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Accepted 2 March, 2012

Cell-associated biosynthesis of cadmium sulfide (CdS) nanoparticles has been reported to be rather slow and costly. In this study, we report on a rapid and low cost biosynthesis of CdS using culture supernatants of *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633 and *Lactobacillus acidophilus* DSMZ 20079T. The synthesis was performed at room temperature in the laboratory ambience and CdS nanoparticles were formed within 24 h. Ultraviolet (UV)-visible spectroscopy study revealed the build-up of absorption bands at 419.5, 381.5 and 362.5 nm for *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T, respectively for assisted synthesis of CdS nanoparticles. X-ray diffraction (XRD), transmission electron microscopy (TEM) and fluorescence spectral analyses were performed to ascertain the formation of CdS nanoparticles. Individual nanoparticles as well as few aggregates having the size of 2.5 to 5.5 nm were found. The process of extracellular and fast biosynthesis may help in the development of an easy and eco-friendly route for the synthesis of CdS nanoparticles.

Key words: Nano-cadmium sulfide (CdS), nanomaterials, nanobiotechnology, biosynthesis, *Escherichia coli*, *Bacillus subtilis*, *Lactobacillus acidophilus*.

INTRODUCTION

Nanometer-sized binary chalcogenides have attracted considerable attention due to their unique properties compared to their bulk counterparts on account of the size quantization effects (Fukuoka et al., 200; Maleki et al., 2007). Among these, cadmium sulfide (CdS) has been extensively studied due to its potential technological applications in field effect transistors, solar cells, photovoltaics, light emitting diodes, photocatalysis, photoluminescence, infrared photodetectors, environmental and biological sensors (Ionov et al., 2006; David and Michael., 2006; Nag et al., 2008; Yang et al., 2009). The preparation of CdS nanoparticles was carried out using

various methods such as microwave heating (Wada et al., 2001), microemulsion synthesis (Talpin et al., 2002; Ohde et al., 2002), chemical synthesis (Monte et al., 2006; Yang et al., 2009), photoetching (Torimoto et al., 2003) and ultrasonic irradiation (Wang et al., 2001).

It is well established that many organisms can produce inorganic materials either on intra- or extra-cellular level (Williams et al., 1996; Ahamed et al., 2002; Kowshik et al., 2002). In order to meet the requirements and exponentially growing technological demand, there is a need to develop an eco-friendly approach for nanomaterial synthesis that is devoid of using toxic chemicals in the synthesis protocols. Recently, microorganisms have been explored as potential bio-factories for the synthesis of both semiconductor and metallic nanoparticles including sulphides, gold and silver nanoparticles (Prasad and Jha,

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2010). CdS nanoparticles. CdS nanoparticles belong to semiconductor nanoparticles when their diameter is less than a certain value known as the exciton Bohr radius, their spectral properties become size-dependent and CdS nanoparticles are then termed quantum dots (QDs). For CdS, the exciton Bohr radius is 2.8 nm (Titova et al., 2006).

There is a growing interest in QDs because of their unique luminescence properties. QDs are extensively applied as fluorescent labels of biological compounds because they have unique advantages over conventional organic fluorophores including photostability and multifluorescence maxima (Pinaud et al., 2006).

QDs also have the distinguished property that the position of their spectral fluorescence maxima depend on the particle size. This allows their application as multi-emitting species using the same excitation wavelength.

In the present work, *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633 and *Lactobacillus acidophilus* DSMZ 20079T were used in order to assess their potential as putative candidate bacteria for the synthesis of CdS nanoparticles. CdS nanoparticles obtained were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM) and Ultraviolet (UV)-visible and fluorescence spectra. An effort has also been made to understand the mechanism of nano transformation of accomplishing biosynthesis at the extra-cellular level.

MATERIALS AND METHODS

Bacteria used for the synthesis of CdS nanoparticles

The test strains *E. coli* ATCC 8739 and *B. subtilis* ATCC 6633 were obtained from our culture collection (Bacteriology Unit at Botany Department, Faculty of Science, Tanta University) and cultivated in Luria and Bertani (LB) broth containing tryptone 10.0; yeast extract 5.0; sodium chloride 10.0 g/l of distilled water according to Luria and Burrous (1957). *L. acidophilus* DSMZ 20079T was isolated from yoghurt and cultivated on ST broth containing g/l casein enzymic hydrolysate, 10.0; yeast extract 5.0; sucrose 10.0; dipotassium hydrogen phosphate 2.0 and 1000 ml distilled water according to Lee et al. (1974).

Preparation of supernatants

LB broth and ST broths were prepared, sterilized and inoculated with freshly grown inoculums of the test strains *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T. The culture flasks were incubated for 24 h at 35°C. At the end of the incubation period, the cultures were centrifuged at 6000 rpm and their supernatants were used for further experiments.

Synthesis of CdS nanoparticles

Synthesis of CdS nanoparticles using *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T was undertaken by allowing these bacteria to grow in sterile distilled water containing

nutrients for 36 h and this was diluted as source culture.

This diluted culture solution was again allowed to grow for another 24 h and the supernatant was obtained by separating cells by centrifugation at 6000 rpm. Twenty milliliter of 0.25 M cadmium chloride (CdCl_2) solution was taken and 5 ml of 0.5 M aqueous solution of Na_2S giving an orange-yellow color of cadmium sulphide suspension.

The suspension was added to the culture supernatant and heated on steam bath up to 60°C for 10 to 20 min until fluffy orange-yellow deposition starts to appear at the bottom of the flask, indicating the initiation of transformation into nanoparticles. Then, the culture solution was cooled and allowed to incubate at room temperature in the laboratory ambience overnight. Next day, the mixture solution was observed to have distinctly remarkable coalescent orange-yellow clusters deposited at the bottom of the flask leaving the colloidal supernatant at the top. It was filtered for further studies.

UV-visible and fluorescence spectroscopy

The formation of CdS nanoparticles was characterized by UV-visible spectroscopy using a Shimadzu UV-160A Spectrophotometer. Steady-state emission spectra were measured using a Shimadzu RF 510 spectrofluorophotometer. The fluorescence spectra were not corrected for machine response.

Transmission electron microscopy (TEM)

The CdS nanoparticles formed by the culture supernatants of the test bacteria were imaged using TEM; Joel, 100SX, Japan with AMT digital camera. Each specimen was dispersed ultrasonically to separate individual particles, and one or two drops of the suspension was deposited onto holey-carbon coated copper grids and dried under infrared lamp. The nanoparticles film was observed and photographed.

X-ray diffraction

The formation of CdS nanoparticles was checked by XRD technique using an X-ray diffractometer (Phillips PW 1729/40 generator, diffractometer, one line detector) with Cu K_α radiation ($\lambda = 1.5405 \text{ \AA}$ over a wide range of Bragg angles (20 to 80°). Glass slides coated with CdS nanoparticles were tested. For more efficient XRD studies, slides were primarily coated with silica gel then loaded with CdS nanoparticles solution prior to drying.

RESULTS

Figure 1 shows the XRD profiles of CdS nanoparticles synthesized using *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T. The XRD patterns showed the diffraction peaks at 2θ values of 25, 30, 35, 37, 44.5, 48, 52.9, 55, 58 and 69° which match earlier reported values for CdS nanoparticles (Prasad and Jha, 2010).

The TEM micrographs of the CdS nanoparticles formed by *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T is shown in Figure 2. The micrographs clearly illustrate individual nanoparticles as well as some aggregates. The nanoparticles are almost spherical in shape. The difference in size may possibly

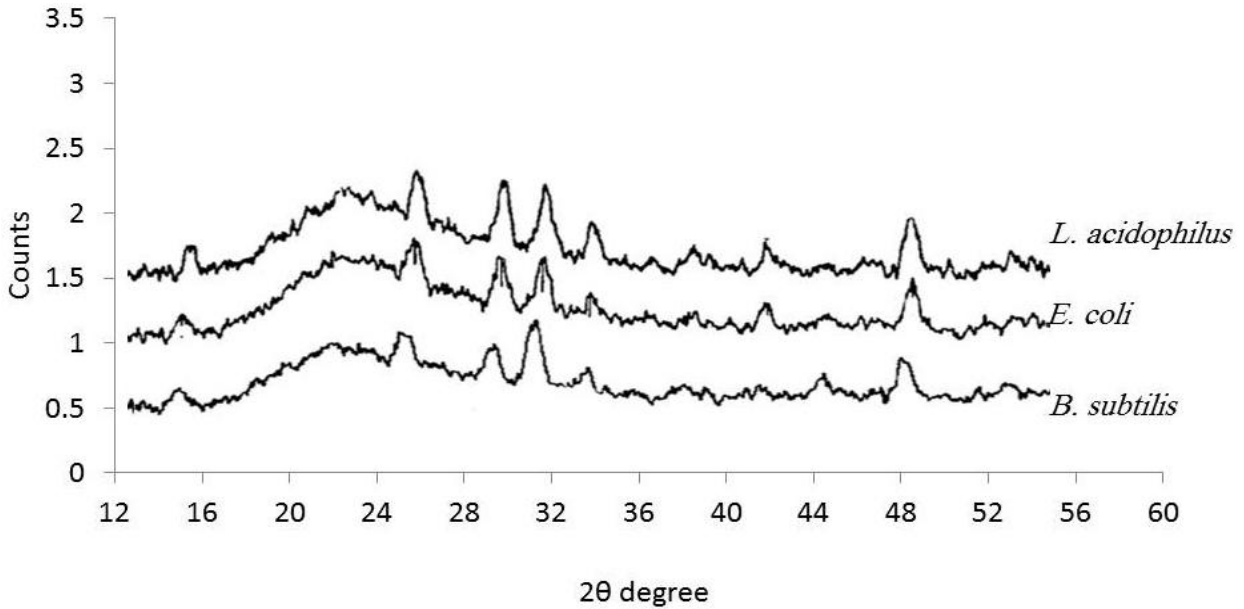


Figure 1. X-ray diffraction patterns of CdS nanoparticles prepared by *E. coli*, *L. acidophilus* and *B. Subtilis* at room temperature.

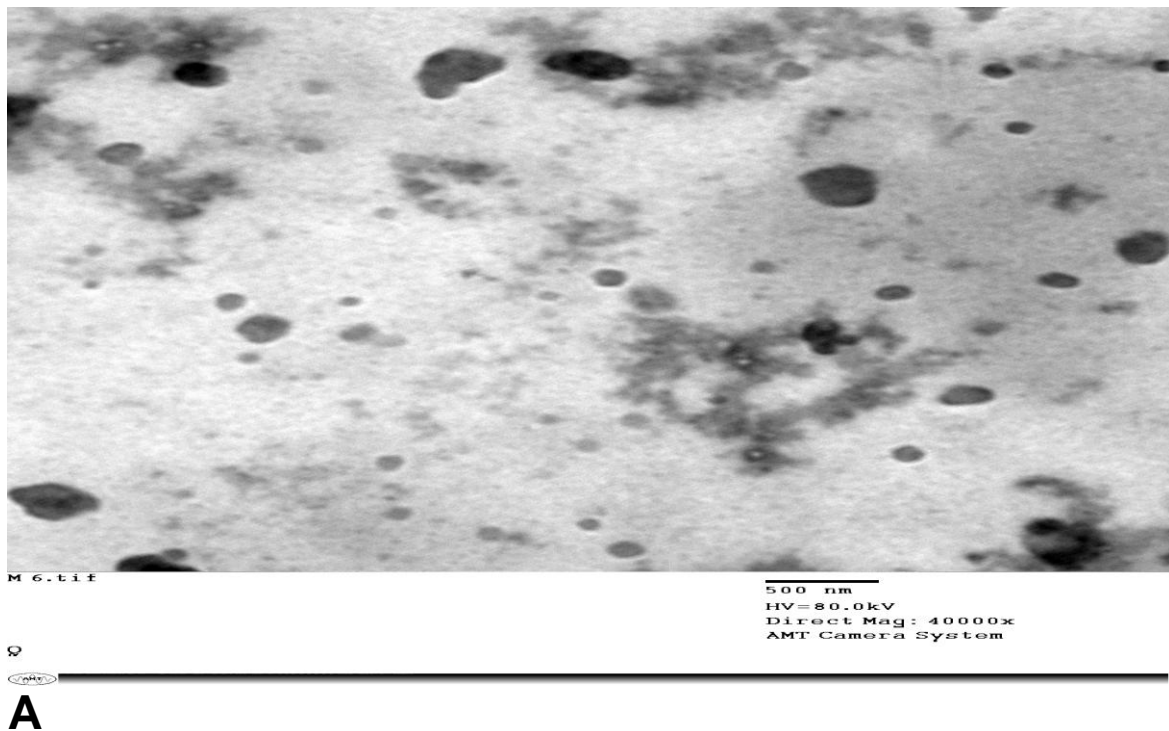
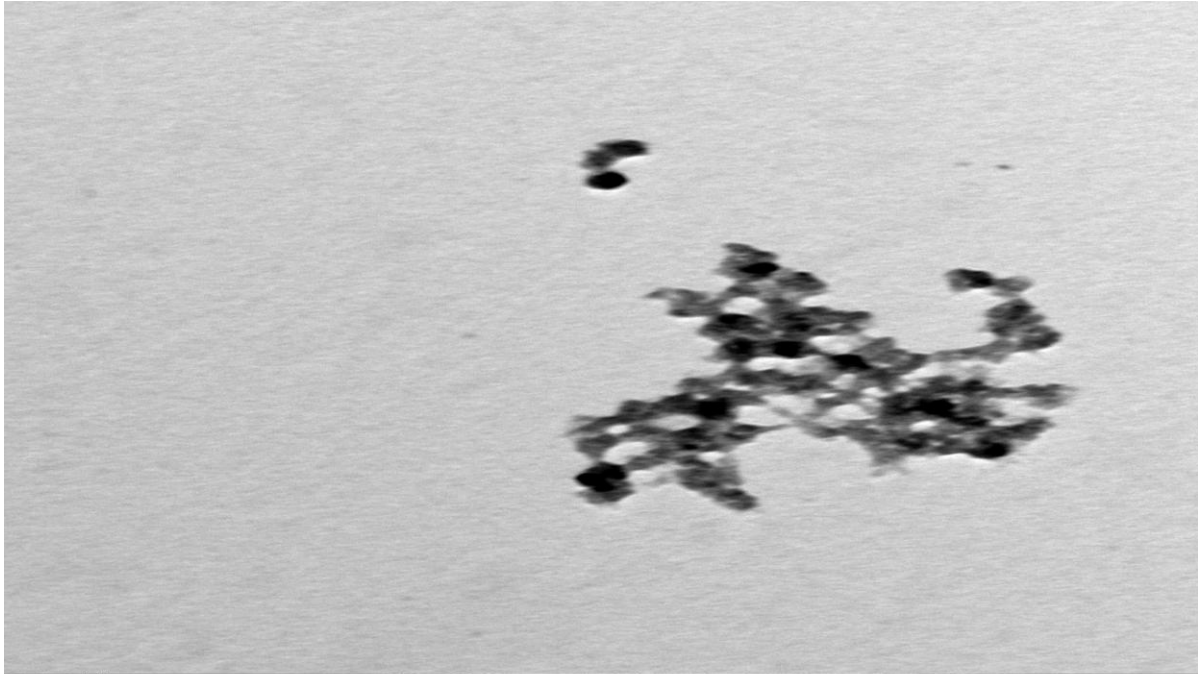


Figure 2. TEM photograph of CdS nanoparticles synthesized using (A), *E. coli*; (B), *B. Subtilis*; (C), *L. bacillus*.

be due to the fact that the nanoparticles are being formed at different times. UV-vis absorption spectra of CdS nanoparticles formed by *E. coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T is shown in Figure 3. Absorbance maxima were observed at

419.5, 381.5 and 362.5 nm for the CdS nanoparticles formed by *E. Coli* ATCC 8739, *B. subtilis* ATCC 6633 and *L. acidophilus* DSMZ 20079T, respectively. The absorption tail is broadened to longer wavelengths which may be due to the size distribution of the particles



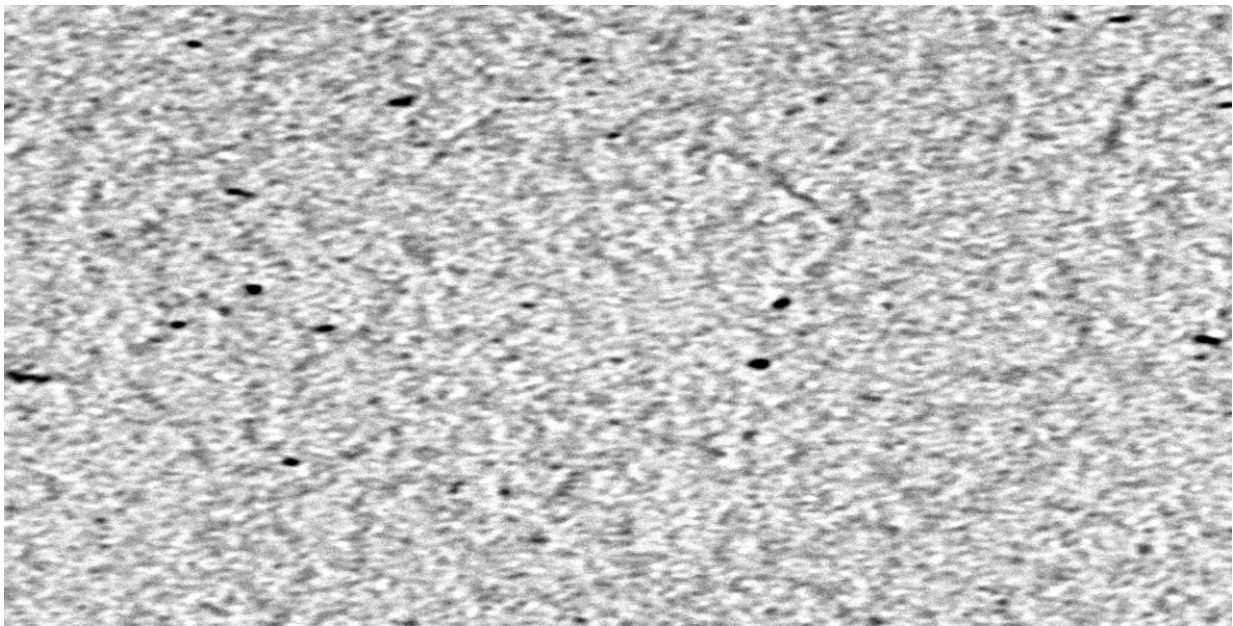
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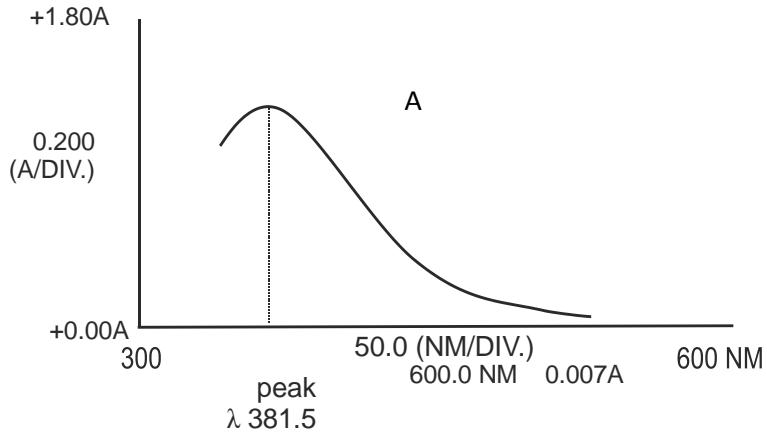
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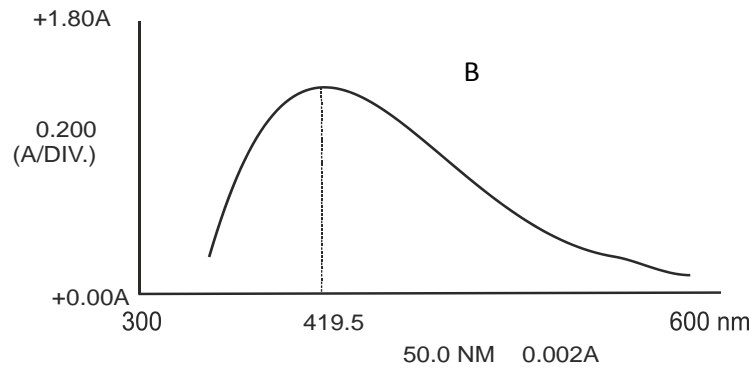


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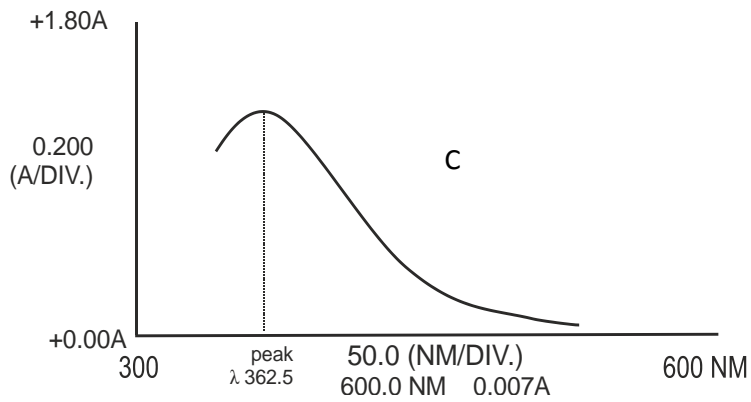
Figure 2. Contd.



E. coli



B. subtilis



L. acidophilus

Figure 3. UV-Vis spectra of CdS nanoparticles produced by (A) *E. coli*, (B) *B. Subtilis* and (C) *L. acidophilus*.

(Mulvaney, 1996). It is considered that the nanosized CdS particles should have a wider band gap than the

bulk material owing to the quantum confinement of the electron-hole pair that forms due to the absorption of a

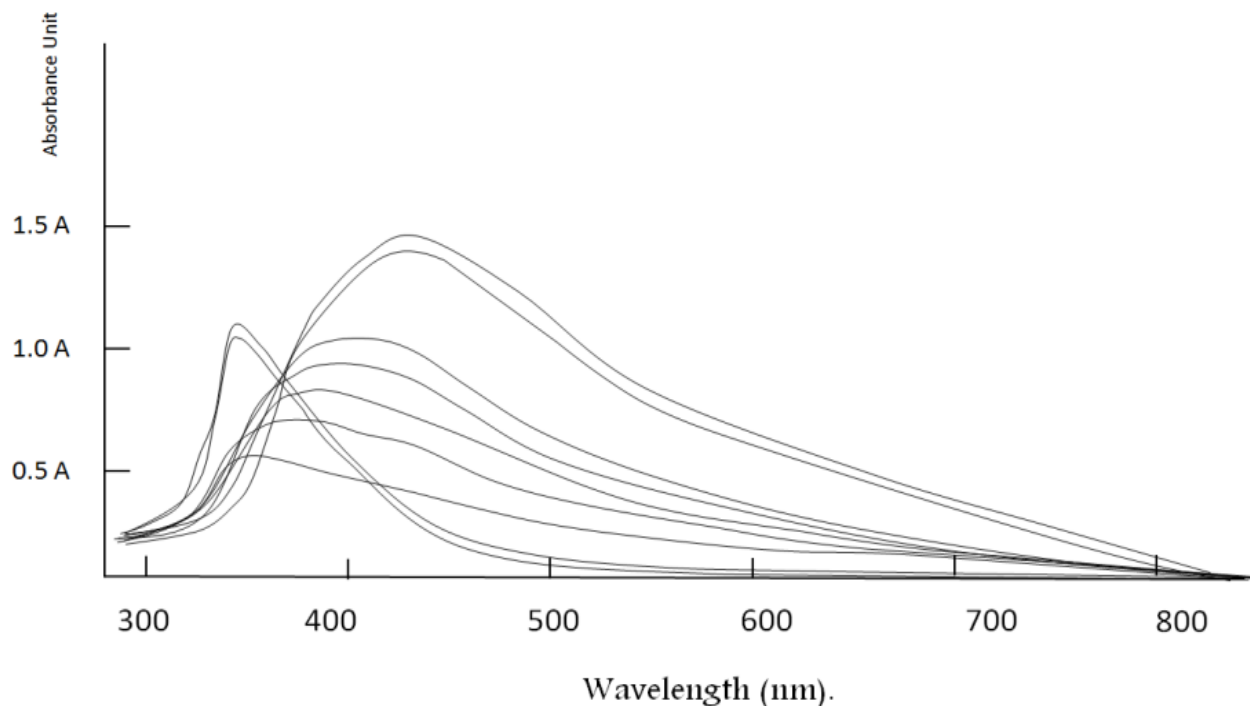


Figure 4. The build-up of absorbance for CdS nanoparticles formed (at increasing absorbances above 400 nm) at 10 min time intervals using *B. subtilis* supernatant.

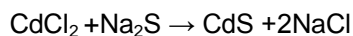
sufficiently energetic photon. The larger energy difference causes a shift in the UV-visible absorption spectrum. Optical excitation of electrons across the band gap is strongly allowed, producing an abrupt increase in absorbance at the wavelength corresponding to the gap energy. This feature in the optical spectrum is known as the optical absorption edge which can be determined by diffuse reflectance spectroscopy (Kumar et al., 1999). The build-up of absorbance is shown in Figure 4 in which a change was observed in absorption spectra of CdS nanoparticles formed at a 10 min time interval using *B. subtilis* bacteria supernatant.

The fluorescence emission of CdS nanocrystals is shown in Figure 5. An intense fluorescence peak is obtained at 440 nm ($\lambda_{ex.} = 365$) for CdS synthesised by *E. coli* and *L. acidophilus*. CdS nanoparticles prepared by *B. subtilis* gave rise to fluorescence maximum at 450 nm ($\lambda_{ex.} = 365$ nm). The shift of 10 nm in fluorescence maximum is attributed to larger size CdS particles produced by *B. subtilis*. Intense fluorescence peaks were obtained for CdS nanoparticles synthesised by *B. subtilis* and *L. acidophilus*. CdS nanoparticles were prepared by *E. coli*. Figure 6 shows the build-up of fluorescence intensity ($\lambda_{ex.} = 340$ nm) for CdS nanoparticles prepared culture supernatant of *B. subtilis*. The fluorescence maxima at 470, 462 and 452 nm were recorded, respectively (at increasing intensities) after 1, 1.5 and 2 h of mixing with culture supernatant of *B. subtilis* bacteria. A shift to shorter wavelengths occurs as the synthesis time

increases reflecting more quantum confinement due to nanoparticle decrease in size.

DISCUSSION

The underlying action of bacteria is to transform micro-size particles into nanosize ones. CdS possesses a low solubility product value of 1×10^{-27} (mol/l)². The bacterial culture clearly shuttles this particle size transformation in a dynamic process including precipitation of nanoparticles and replenishment of depleted Cd²⁺ and S²⁻ ions at the expense of micro-size CdS particles. The chemical reactions which proceed in the culture medium is as follows:



It is known that *E. coli* usually accumulates Cd²⁺ via a zinc transport system which is less efficient than the manganese transport system (Dar, 1999) and is used to explain Cd²⁺ uptake in Gram-negative bacteria (Dar, 1999; Park, 1998; Yoon, 1998). The high uptake of Cd²⁺ showed that Cd²⁺ could be more efficiently transported by the expression of the manganese transport gene in *E. coli*.

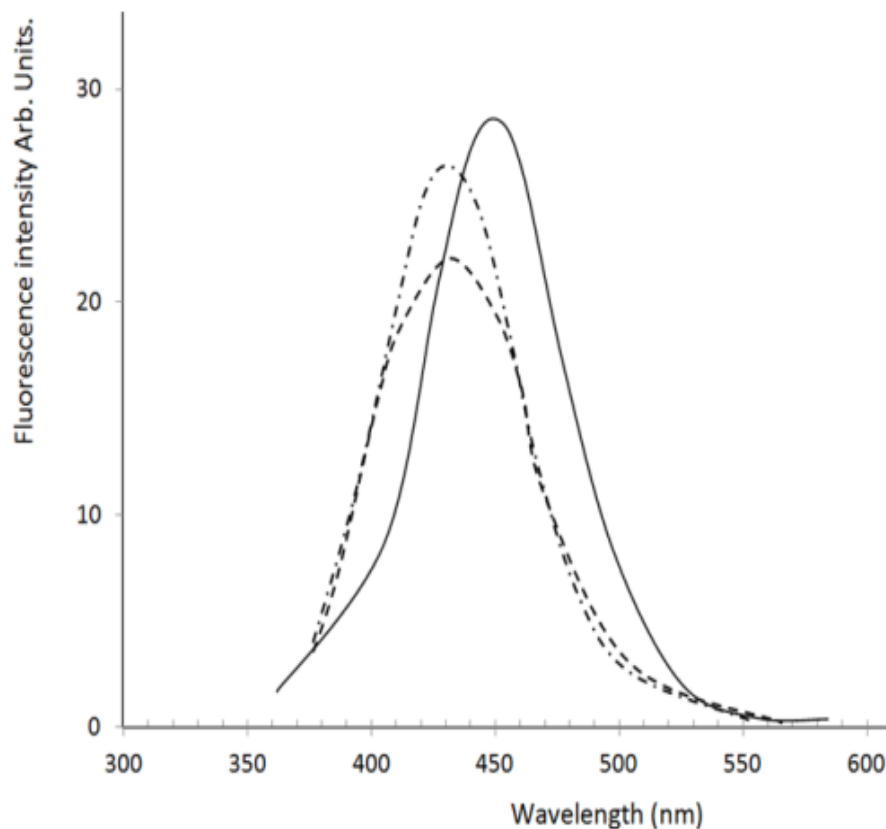


Figure 5. Fluorescence spectra ($\lambda_{\text{ex}} = 340 \text{ nm}$) of CdS nanoparticles prepared in culture supernatant of (—) *B. subtilis*, (-·-·-·-) *E. coli* and (----) *L. acidophilus*.

In case of *B. subtilis*, Cd^{2+} accumulation is performed after inducing the cells growth. The decrease in cell growth upon induction might be due to the metabolic burden in the induced cells. This indicates that the transport system has a high selectivity to Cd^{2+} and can be applied to the selective recovery of Cd^{2+} from a mixture of other metals.

The Cd^{2+} accumulation culture showed a similar accumulation under neutral and basic conditions (Volesky and Holan, 1993). *B. subtilis* was resistant to ambient conditions, such as pH, ionic strength and the presence of metal chelators or complexing agents. Once Cd^{2+} was transported into the cytoplasm, it formed a complex with the metallothionein (MT) protein. In the induction at the early and late logarithmic phases, Cd^{2+} was accumulated. It was speculated that this was due to the incorporation based on the polysaccharide composition of each particular organism, and is highly variable among distinct genera and even strains from the same species. Gram-positive cells has teichoic acids and acids associated to the cell wall, whose phosphate groups are key components for the uptake of metals (Beveridge, 1989).

Gram-positive cells accumulate a much higher amount of heavy metals than Gram-negative cells. Carboxyl groups are the main agents in the uptake of heavy metals. The sources of these carboxyl groups are the teichoic acids, associated to the peptidoglycan layers of the cell wall (Da Costa and De França, 1996; Da Costa, 1999).

The capacity of Lactobacilli to grow even in the presence of oxygen makes it metabolically more capable. Addition of reducing agents like glucose tends to lower the value of oxidation-reduction potential. The oxidation-reduction potential expresses the quantitative character of degree of aerobiosis having a designated unit expressed as rH2 (the negative logarithm of the partial pressure of gaseous hydrogen). In this connection, monosaccharide and plant extracts were used as reducing agents for rapid chemical synthesis of nanoparticles (Shankar et al., 2004). Energy yielding material – glucose (which controls the value of rH2), the ionic status of the medium pH and overall oxidation-reduction potential (rH2) which is partially controlled by the bicarbonate, all these factors cumulatively negotiate the synthesis of CdS nanoparticles in the presence of *E. coli*,

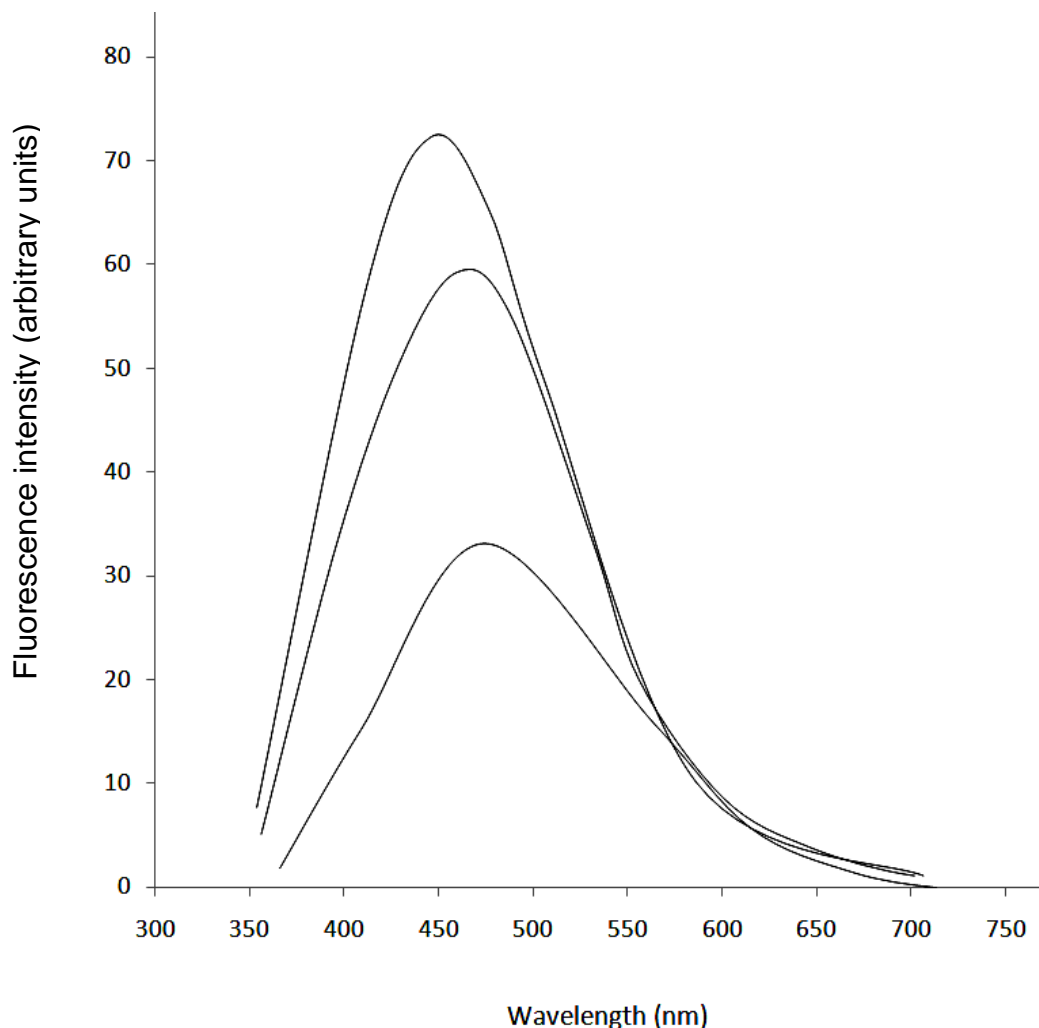


Figure 6. The build-up of fluorescence intensity ($\lambda_{ex.} = 340$ nm) for CdS nanoparticles prepared by culture supernatant of *B. subtilis*. The fluorescence maxima at 470, 462 and 452 nm are recorded, respectively (at increasing intensities) after 1, 1.5 and 2 h of mixing with culture supernatant.

B. subtilis and *L. acidophilus* strain. *Lactobacillus*, being a prokaryote has comparatively less developed detoxification machinery in the cytosol and circumvents any chemical challenge at the membrane level itself, a mildly acidic pH and lowered rH_2 activates the membrane bound oxidoreductase and makes the requisite ambience for an oxide nanoparticle synthesis.

ACKNOWLEDGMENTS

The authors We would like to express our gratitude to Dr. ElZeiny, M. Ebied Prof. physical chemistry, Chemistry Department, Faculty of Science, Tanta University, Egypt for help with facilities he offered in photochemistry laboratory. Thanks are also extended to Prof. M. El-Saadany, Alexandria University for help and support in this study

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