Avestia Publishing
International Journal of Theoretical and Applied Nanotechnology (IJTAN)
Volume 10, Year 2022
Journal ISSN: 1929-1248
DOI: 10.11159/ijtan.2022.001

Evaluation of the Culture Medium in the Synthesis of Cadmium Sulfide Nanoparticles by the Fungus *Fusarium Oxysporum*

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Abstract **-** *Fusarium oxysporum* f. sp. *lycopersici* is a species reported to synthesize silver, gold, and cadmium nanoparticles when using biomass; it can produce and secrete many secondary metabolites hydrolytic enzymes involved in nanoparticle synthesis processes. However, the production of these components depends on the substrate where the fungus grows. The present work aims to evaluate the effect of the growth medium of *Fusarium oxysporum* in the synthesis of cadmium sulfide nanoparticles (CdS-NPs). The fungal biomass was obtained from a growth medium with nitrogenous components of higher and lower complexity (MGYP and DS respectively) and used for the synthesis of CdS-NPs using cadmium nitrate (Cd $(NO_3)_2$*$4H_2O$) and elemental sulfur (S°). CdS-NPs exhibit two absorbance bands at 320 and 450 nm, with a fluorescence emission at 520 nm when excited with a wavelength at 365 nm. Transmission electron microscopy, energy-dispersive X-ray spectroscopy, and X-ray diffraction confirmed the presence of CdS-NPs with a mean diameter of 4.7 ± 0.7 and 4.9 ± 0.9 nm when MGYP and S media were used for biomass production, as well as atomic columns of CdS-NPs with A similar crystalline structure. However, it was also possible to appreciate fewer CdS-NPs when using the fungal biomass obtained from DS medium, which was confirmed by spectrophotometry through the absorption and emission bands at 450 and 520 nm.

Keywords: Nanoparticles, biosynthesis of nanoparticles, cadmium sulfide, *Fusarium oxysporum*

Date Received: 2022-09-08
Date Accepted: 2022-09-16
Date Published: 2022-09-25

1. Introduction

The biosynthesis of nanoparticles (NPs) is achieved by living organisms such as bacteria, plants and fungi through organic compounds [1], enzymatic and chemical reactions by nucleophilic processes, which reduces the use of materials and harmful compounds to health and the environment [2], [3]. The biosynthesis of cadmium sulfide nanoparticles (CdS-NPs) through fungal route by extracellular processes has been investigated in filamentous fungi *Pleurotus ostreatus* [4], *Phanerochaete chrysosporium* [5], *Coriolus versicolor* [6], *Trichoderma harzianum* [7], *Aspergillus versicolor* [8], *Aspergillus niger* and *Fusarium oxysporum* [9], [10], since they produce the biomolecules and enzymes necessary to perform the synthesis and coating of NPs by oxide-reduction processes [11], [12].

Filamentous fungi within the genus *Fusarium* are good candidates for the synthesis of metal NPs due to the wide variety of primary and secondary metabolites that they produce [13], [14], as a result of the extensive ecological, biological and genetic diversity of the genus [15]. *Fusarium oxysporum* f. sp. *lycopersici* is one of the most widely studied species reported for its ability to produce different NPs when its biomass is put in contact with silver [16], [17], gold, and cadmium ions [10], [18]. Therefore, the substrate or culture medium where the fungus grows is essential for the biosynthesis of CdS-NPs [3], [14], since it is responsible

not only for providing the basic requirements of the fungus growth and survival for the production of biomass, which contains the enzymes and metabolites that are involved in the processes of synthesis of NPs [19]. Thus, this study aims to evaluate the effect of growth medium of *Fusarium oxysporum* f. sp. *lycopersici* in the synthesis of CdS-NPs.

2. Materials and Methods

2.1. Production of fungal biomass and synthesis of CdS-NPs

Biomass production of Fusarium oxysporum f. sp. lycopersici was done in 1.0 L flasks with 250 mL of MGYP medium (g/L: 6; malt extract, 10; glucose, 3; yeast extract, 5; gelatin peptone) or Dextrose Sabouraud medium (DS, Cat. No. 7031) with a pre-inoculum of 1×10^6 spores/mL. After four days of incubation at 150 rpm and 30°C, the biomass was washed with 15 mL of sterile water and recovered by vacuum filtration [20]. The negative inorganic control (Inorganic) was performed in 250 mL flasks with 60 mL of $Cd(NO_3)_2*4H_2O$ 1.0 mM and S° 0.1 M, while the biological control was carried out using 6.0 g of biomass grown in MGYP (Biological MGYP) or DS (Biological DS) medium and 60 mL of sterile distilled water at 24 h, 150 rpm, and 30°C .

The synthesis of CdS-NPs was made in 250 mL flasks with 6.0 g of biomass grown in MGYP (CdS-NPs MGYP) or DS (CdS-NPs DS) medium, 60 mL of $Cd(NO_3)2*4H_2O$ (1.0 mM) and S° 0.1 M at 24 h, 150 rpm, and 30°C [21]. At the end, reactions were filtered with 0.45 µm nylon membranes for physicochemical characterization.

2.2. Physicochemical characterization of CdS-NPs

Optical characterization of CdS-NPs was performed on a Genesys 10S, Thermo Scientific UV-Visible and F96Pro, Luzeren fluorometer, with spectrum readings every 1 nm from 400 to 700 nm, photomultiplexing (PMT) of 5 with an excitation at 365 nm [21]. The size, structure and composition of the CdS-NPs were analyzed by high-resolution transmission electron microscopy (HRTEM) and EDX spectroscopy on a JEOL microscope, JEM-ARM200F. The polydispersity index, hydrodynamic radius, and Z-potential of the CdS-NPs were analyzed by dynamic light scattering (DLS) ina Litesizer 500 analyzer, Anton Paar [22].

3. Results and Discussion

3.1. Optical characterization of the CdS-NPs

UV-Vis absorption analysis showed that inorganic precursors used in the CdS-NPs synthesis, not react by themselves, since the negative inorganic control (Inorganic) containing $Cd(NO_3)2*4H_2O$ and S°, showed an absorption band at 225 nm (Fig 1A), corresponding to $(NO_3)_2{}^{2-}$ a group of cadmium nitrate, indicating that there is no abiotic reaction between both components for CdS-NPs formation [23]. Similarly, the biological controls containing the fungal biomass in distilled water without the presence of the inorganic precursors (Biological MGYP and Biological DS) did not exhibit CdS-NPs formation, as they only showed absorption bands at 230 and 280 nm (Figure 1A), which suggest the presence of biomolecules such as carbohydrates, proteins and polar compounds secreted by the biomass [24], [25]. On the other hand, samples containing fungal biomass and inorganic precursors (CdS-NPs MGYP and CdS-NPs DS), showed an absorption band at 320 and 450 nm corresponding to the formation of CdS-NPs (Figure 1A). However, absorption was higher in the synthesis reactions when biomass was obtained from MGYP [4], [10], [21], [26].

The fluorescence analysis showed an emission band at 450 nm in Biological MGYP, Biological DS, CdS-NPs MGYP, and CdS-NPs DS (Figure 1B), which corresponds to some aromatic amino acids present in the proteins [27], as well as to the enzymatic cofactors NADH and NADPH released by the fungal biomass [28]. Nevertheless, only in CdS-NPs MGYP and CdS-NPs DS, did the fluorescence emitted at 450 nm decrease, and an emission band at 520 nm appeared (Figure 1B), which corresponds to CdS-NPs formation [29], [30]. According to previous data and based on Hietzschold results [31], we can suggest that the oxidation of NADH and NADPH cofactors occurs due to their participation in the reduction processes of metal ions to synthesize the CdS-NPs.

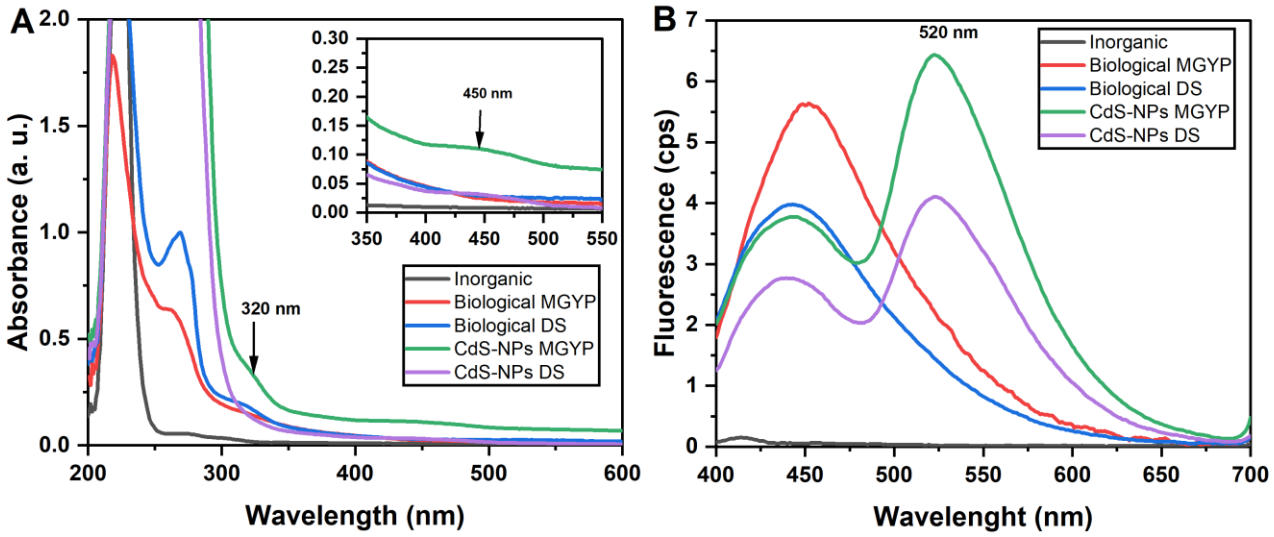

Figure 1. UV-Vis absorbance spectroscopy (A) and fluorescence (B) analysis of CdS-NPs synthesized from biomass of *Fusarium oxysporum* f. sp. *lycopersici*. Inorganic; $Cd(NO_3)_2$*$4H_2O$ 1 mM and S° 0.1 M, Biological MGYP and DS; biological control with the biomass obtained from MGYP or DS media; CdS-NPs MGYP and DS, cadmium sulfide nanoparticles synthesized by biomass obtained from MGYP or DS media.

3.2. Morphological characterization of the CdS-NPs

The DLS analysis showed that CdS-NPs have a wide range of hydrodynamic radius with a polydispersity index of 28%, despite the growth medium used to produce Fusarium oxysporum f. sp. lycopersici biomass (Figure 2). However, growth media (MGYP and DS) showed particles below 100 nm with a relative frequency of 2.63 ± 0.74 and 12.00 ± 3.78%, respectively. NPs below 10 nm were only observed at 0.2% of relative frequency when using the biomass produced in MGYP medium, evidencing the formation CdS-NPs. In addition, particles greater than 200 nm were also observed with a frequency higher than 20% in both culture media. It can be due to the presence of NPs clusters [22] and traces of organic material from the biomass, which is supported by the analysis of CdS-NPs at pH 5.5 by Z potential, suggesting the presence of colloidal instability of CdS-NPs MGYP and CdS-NPs DS samples which values were of 13.2 ± 1.0 mV and 9.0 ± 1.0 mV, respectively [32], [33].

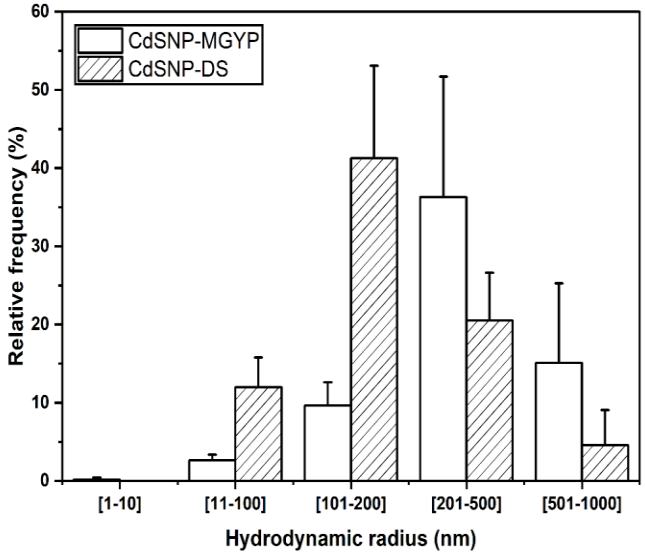

Figure 2. Determination of the hydrodynamic radius of CdS-NPs. CdS-NPs-MGYP and CdS-NPS-DS; cadmium sulfide nanoparticles synthesized by *Fusarium oxysporum* f. sp. *lycopersici* biomass obtained from MGYP and DS media.

TEM micrographs show the presence of CdS-NPs (Fig 3A and Fig 4A). Also, Fig 3B and 4B show the histograms of size distribution, showing a diameter of 4.7 ± 0.7 and 4.9 ± 0.9 nm respectively, for MGYP and DS media. Besides, Fig 3C and 4C show the HRTEM images of CdS-NPs showing the crystalline planes with interplanar distances of 3.47 Å. For DS medium, fewer

particles below 10 nm were observed [4], [34]. It was impossible to detect CdS-NPs by DLS, which could be attributed to the growth medium components since MGYP media contains more structurally complex components and diverse nitrogen sources than the DS medium, such as gelatin peptone, yeast extract and malt extract [35]. The composition of media, in this case MGYP, could induced a higher production and secretion of hydrolytic enzymes necessary for the formation of simple molecules from complex compounds that the fungus can capture to achieve its growth and reproduction, resulting in biomass rich in biomolecules and enzymes that help in the nucleophilic processes involved in the synthesis of CdS-NPs [36], [37].

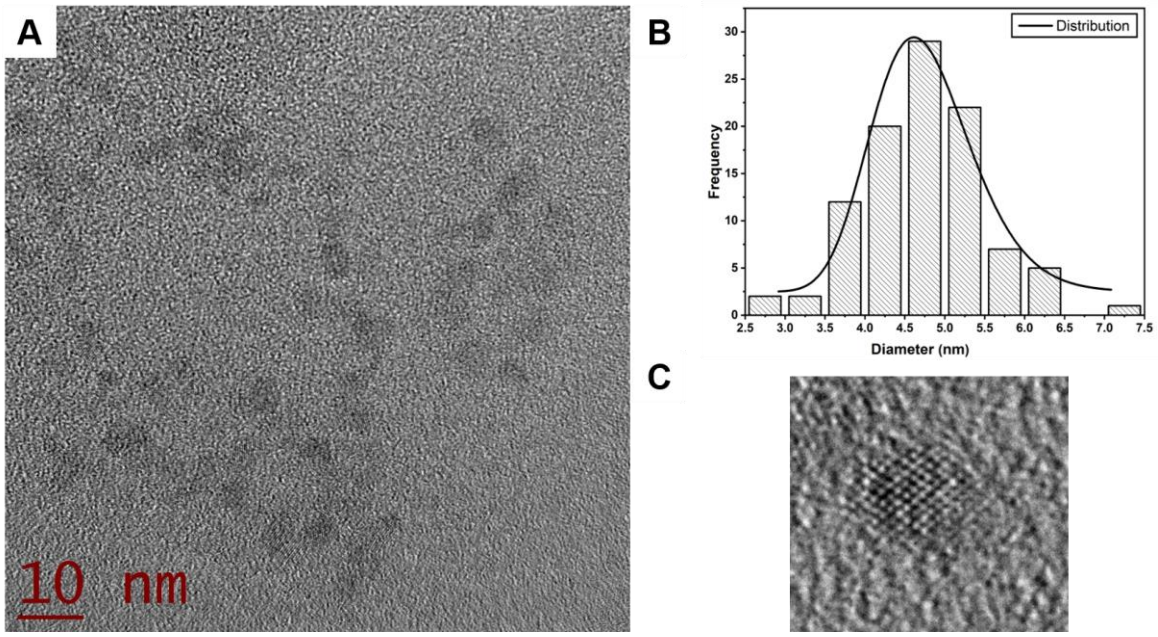

Figure 3. HRTEM of CdS-NPs synthesized by *Fusarium oxysporum* f. sp. *lycopersici* biomass obtained from MGYP media. A; CDS-NPs, B; size distribution of CDs-NPs, C; HRTEM images shows a single crystal structure of CDS-NPs.

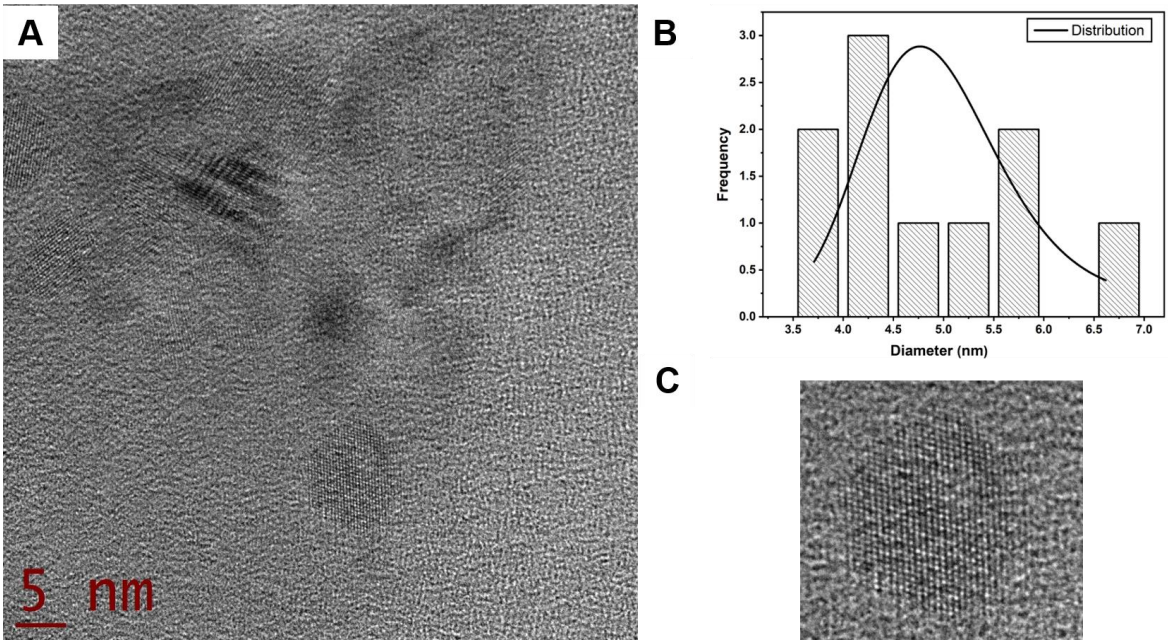

Figure 4. HRTEM of CdSNPs synthesized by *Fusarium oxysporum* f. sp. *lycopersici* biomass obtained from DS media. A; Cds-NPS, B; size distribution of CdS-NPs, C; HRTEM images shows a single crystal structure of CDS-NPs.

The EDX of CdS-NPs showed the presence of cadmium and sulfur, which form the CdS-NPs (Fig 5), and other elements present in the sample, like Cu, Si, and Fe, due to the TEM grid. The rest of the elements, such as C, O, and P, belong to biomolecules secreted by the fungal biomass.

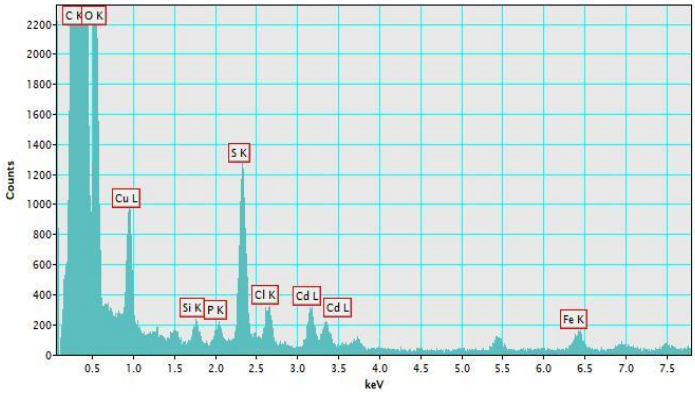

Figure 5. EDX spectroscopy of CdS-NPs samples synthesized by biomass of *Fusarium oxysporum* f. sp. *lycopersici.*

4. Conclusion

The synthesis of CdS-NPs from *Fusarium oxysporum* f. sp. *lycopersici* biomass obtained in MGYP and DS medium was demonstrated by UV-Vis and fluorescence spectrophotometry, HRTEM, and DLS. It was found that the size, structure, and crystallinity of the CdS-NPs obtained are not influenced by the growth medium since no significant changes in these parameters were found. However, by using HRTEM and DLS, it was possible to appreciate that the efficiency of NPs synthesis is reduced when the fungal biomass obtained from DS medium is used since the relative frequency of CdS-NPs below 200 nm is considerably decreased. We attributed it to the organic components present in the fungal biomass, which change according to the fungus's medium.

Acknowledgements

The project was funded by the National Polytechnic Institute and Sectoral Fund for Educational Research, National Council of Science and Technology by Project A1-S-31777. Author thanks to Dr. Raúl Borja-Urby for his support with HRTEM micrographs.

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