Decolorization of crystal violet dye with zinc nanoparticles synthesized from endophytic fungus *Aspergillus niger*

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KEYWORDS

Endopytic fungi; Zinc nanoparticles; Biosynthesis; Dye decolorization

ABSTRACT:

Introduction: A common cationic triphenylmethane dye in many industrial applications is crystal violet (CV). Among these are the dyeing of cotton, silk, wool, nylon, and polyacrylonitrile textiles. CV is also utilized as an antibacterial and mutagenic agent, in veterinary medicine, and in biological staining. Despite its many applications, CV is a chemical that is resistant to environmental deterioration, leaving a long-term residue in soil and wastewater. Furthermore, it is impossible to overlook CV's harmful effects on both people and the environment, as even a small amount of it may produce a bright color. There is a need for an efficient degrading method because CV has been shown to be mutagenic, and carcinogenic in addition to being toxic to mammalian cells.

Objectives: Given the possible hazards that CV poses to both human health and the environment, it is critical to create an effective degradation process that can lessen both its toxicity and environmental impact.

Methods: After being first detected as a color shift, the creation of ZnNPs was later verified by Ultraviolet-visible spectroscopy, which revealed a distinctive silver absorption peak at 420 nm. Studies using transmission electron microscopy revealed that the ZnNPs ranged in size from 4 to 15 nm. The crystalline structure of the ZnNPs was discovered using X-ray diffraction research.

Results: CV dye was effectively decolored by ZnNPs up to 96% after 72 h of incubation.

Conclusions: As a result, ZnNPs may be employed as very cost-effective agents for the quick elimination of dye-based pollutants from the environment as well as for the management of other reducible pollutants.

1. Introduction

One of the most active research fields in contemporary materials science is nanotechnology. Based on certain attributes including size, distribution, and shape, nanoparticles display novel or enhanced features. Over the past ten years, there have been several noteworthy advancements in the field of nanotechnology, and a number of novel techniques have been created for the synthesis of nanoparticles with particular size and form properties based on their requirements. Additionally, the number of possible new uses for nanomaterials and nanoparticles keeps growing quickly.

Because of their wide range of applications across numerous research areas (Li et al. 2011), such as optics, electronics (Peto et al. 2002; Daniel and Astruc 2004), bioimaging, sensors, and diagnostics, as well as the development of novel therapeutic agents in biomedical research (Salata 2004), nanoparticles have a high surface area and a high fraction of surface atoms (Jeevan et al. 2012). They are also the fundamental building blocks of nanotechnology (Vahabi et al. 2011).

Since metallic silver and Zinc nanoparticles (ZnNPs) present a low risk of toxicity to humans, they have been used as antimicrobial agents in a variety of products, such as water purification systems (Choi et al. 2008), wound dressings (Fernandez et al. 2008), animal feed (Höjberg et al. 2005), cosmetics (Kokura et al. 2010), and anti-dimorphic materials (Yehia and Al-Sheikh 2014). Over the past ten years, the use of biological systems for the synthesis and assembly of nanoscale materials has gained traction as a feasible substitute for traditional physical and chemical techniques.

As they are less harmful to the environment, safe, economical, less time-consuming than conventional methods, green chemistry approaches for the production of nanoparticles using biological entities have a number of important advantages. Moreover, these kinds of reactions can be carried out in a single step (Patil et al. 2012), and molecules made by bacteria, fungi, yeasts, algae, and plants can be used in place of the reducing agents and stabilizers normally used in the synthesis of ZnNPs (Gade et al. 2010; Narayanan and Sakthivel 2010).

Although they release a lot of enzymes and their biomass is manageable, fungi can be used to synthesize metal nanoparticles, which makes their application in this field very fascinating (Bhainsa and D'Souza 2006). According to Hassan et al. (2011), Zhang et al. (2012), Junejo et al. (2013), and Kalwar et al. (2013), the majority of the nanoparticles produced in this manner can be employed in chemical reactions for the breakdown of organic molecules. For many companies, the degradation of dye-containing effluents is a serious issue (Hassan et al. 2011).

Prolonged exposure to dyes can irritate the gastrointestinal and respiratory systems and negatively impact anaerobic biomass. In light of this, it is crucial to develop physical and chemical treatment methods for eliminating dyes from wastewater systems (Parimala and Santhanalakshmi 2013). The majority of the materials used in the pigmentation of cosmetics, textiles, paper, ceramics, leather, inks, and food processing items are azo dyes. Dyes are utilized in many different industries. Because these colors are hazardous, 15% of the dyes used in these sectors are released into natural water sources such rivers and streams, posing a serious risk to both people and the environment (Mahmoud et al. 2009). Many methods, such as adsorption (Kamboh et al. 2011), nanofiltration (Lau and Ismail 2009), and biological treatment procedures (Nisola et al. 2010), have been developed for the removal of colors from water. Methyl green is the most hazardous of the several dye varieties (Shadi et al. 2009). The triphenylmethylene functional group of methyl green, a heterocyclic aromatic molecule that is also cationic in nature (Jayaraj et al. 2001), poses a serious risk of pollution to natural water sources (Parimala and Santhanalakshmi 2013). Research on the application of metals and metal nanoparticles as heterogeneous catalysts for waste water treatment has produced a number of positive outcomes (Ghosh et al. 2002; Ibhadon et al. 2008).

In the current study, endophytic fungi were used to produce ZnNPs, and the nanoparticles' capacity to degrade dyes was assessed.

2. Methods

Isolation of endophytic fungi

The technique outlined by Petrini et al. (1986) was used to isolate endophytic fungi. The leaves of the *Ocimum balilicum* L. plant were sampled. To get rid of any dust, the leaves were gently washed under running water before being surface sterilized. Following washing, the leaves—both with and without the midrib—were chopped into tiny (0.5 × 0.5 mm) pieces using a flame-sterilized cork borer in an aseptic setting. Using the imprint approach outlined by Schulz et al. (1993), the effectiveness of the surface sterilization process was assessed for each tissue section. The dishes were covered with parafilm, cultured for three weeks at 28±2°C, and five segments were put on potato dextrose agar supplemented with 50 mg L^{-1} ampicillin. Based on their microscopic and macro structures, the fungi that were emerging from the plant segments were identified and purified(Raper and Fennel 1965; Kenneth et al. 1965). By analyzing the nucleotide sequences of the internal transcribed spacer (ITS) regions as outlined by Sambrook and Russell (2001), endophytic fungi that produced the most ZnNPs were found. ITS1 and ITS4 primers were used for PCR amplification, and the resultant sequence was utilized as a query sequence to use the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) to look for similar sequences from GenBank.

Biosynthesis and characterization of ZnNPs

According to Sanghi and Verma (2009), the fungal biomasses utilized in the biosynthetic studies were cultivated aerobically in a liquid growth medium that contained 10.0 g L^{-1} glucose and 5.0 g L^{-1} malt extract. After being inoculated with culture, the flasks were incubated for 72 h at 25°C in an orbital shaker set at 100 rpm. Plastic sieves were used to harvest the ensuing fungal biomass, and any remaining medium was then thoroughly cleaned with distilled water. Following weighing, the freshly cleaned biomasses were incubated for 72 h at 25°C in 200 mL of MilliQ-purified water on a shaker set to 100 rpm. Following filtration of the resultant biomass using Whatman filter paper No. 1, the cell-free filtrate was gathered for use in further studies.

After adding 8.4 mg of silver nitrate ($ZnNO_3$) to a 250 mL conical flask containing 50 mL of the cell-free filtrate to create a 1 mM solution, the mixture was shaken at 150 rpm at 25°C without light. Simultaneously, a control experiment was carried out with the same setup but without ZnNO3 (i.e., only the cell-free filtrate). The identical settings as previously mentioned were used in a negative control experiment that used solely $ZnNO_3$ in water.

Using a UV-Vis spectrophotometer (Optizen 2120 UV; Mecasys, Korea), ultraviolet-visible (UV-Vis) analysis verified the nanoparticle production in these studies. Every spectrum was captured at wavelengths between 300 and 700nm.

Using Cu Kα radiation ($\lambda = 0.15408$ nm) at 30 mA and 45 kV, X-ray diffraction (XRD) examinations of the freeze-dried ZnNP powders were performed at 2θ values in the 20° to 80° range (Philips PW 1830). The following is the preparation of samples for transmission electron microscopy (TEM) investigation. For five minutes, the samples were sonicated using a Vibronics VS 80. After loading ZnNPs onto carbon-coated copper grids, the solvent was let to evaporate for half an hour under infrared light.

A Phillips model CM 20 apparatus operating at a 200 kV accelerating voltage was used to record TEM pictures, and the sizes and forms of the ZnNPs were thoroughly described.

Dye decolorization properties of ZnNPs derived from endophytic fungi

In a standard experiment, 1000 mL of double-distilled water were mixed with 10 milligrams of CV dye, and the resultant mixture served as a stock solution. After adding 25, 50, and 100 µg of the biosynthesized ZnNPs to 100 mL of the methylene blue dye stock solution, the solutions were incubated for 72 h at 30°C. With the exception of not adding ZnNPs to the stock solution, a blank experiment was also carried out under the same circumstances. The absorbance value of the solution at 650 nm was used to determine the dye concentration during the degradation. The following formula was used to determine the percentage of dye degradation in each experiment:

Decolorization (%) = $(C_0 - C)/C_0 \times 100$,

where C_0 represents the dye solution's starting concentration and C represents its concentration following degradation.

Results and Discussion

Biosynthesis and characterization of ZnNPs

For the creation of metal nanoparticles, fungi make excellent candidates. The reduction of aqueous Ag^+ ions in cell-free extracts of four endophytic fungal isolates at 25°C was used in this investigation to create ZnNPs. In contrast to the negative control flasks that contained only water and $ZnNO_3$, the mixture turned dark brown when $ZnNO_3$ was incubated in the cell-free endophytic fungal isolate. For the structural characterization of ZnNPs, UV-Vis spectroscopy has been a popular analytical method (Junejo et al. 2013).

The pale yellow-brown silver colloids that were produced after $ZnNO_3$ was incubated for 72 h in the cell-free endophytic fungal isolates showed surface plasmon resonance (SPR) in their absorption spectra (Sun et al. 2003; Wiley et al. 2006; Lu et al. 2011). As particle size grows, SPR has been shown to shift to longer wavelengths (Zhao et al. 2006). Colloidal zinc is characterized by a high SPR peak at around 420 nm in the UV-Vis spectrum of the particles produced in the presence of the cell-free extract of *Aspergillus* sp. After 72 h of incubation, these findings showed that *Aspergillus* sp. produced the most ZnNPs, while *Alternaria* sp. produced the worst outcomes out of the four isolates examined (Figure 1). These findings were in line with those of Huang et al. (2007), Verma et al. (2010), and Yehia and Al-Sheikh (2014), who observed SPR peaks in the UV-Vis spectra of ZnNPs made from *Pleurotus ostreatus* at approximately 450 nm. There has been prior discussion of the function of nitrate reductases originating from microbes in the manufacture of ZnNPs elsewhere (Kumar et al. 2007; Bai et al. 2011; Yehia and Al-Sheikh 2014). *Aspergillus* sp. was found to be a strong producer of zinc nanoparticles in this investigation, and as a result, the ZnNPs generated by this fungus were chosen for additional testing.

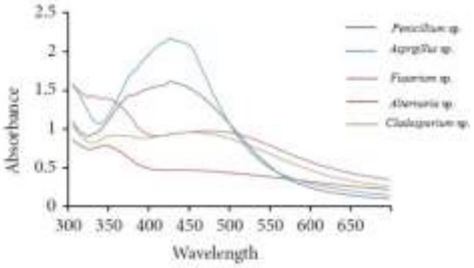
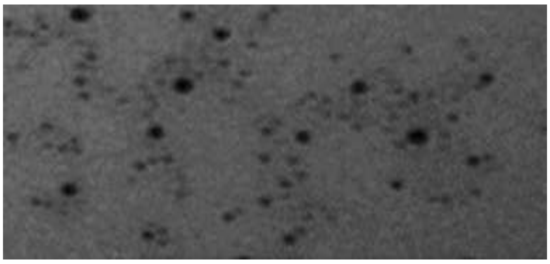


Fig 1. UV-Vis spectrum of the ZnNPs produced by different endophytic fungi after 72 h.



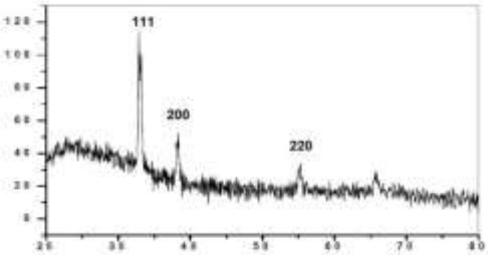


Fig 2. Transmission electron micrograph showing ZnNPs.

To understand more about the dimensions and form of the ZnNPs produced by *Aspergillus* sp., TEM investigation was carried out. Figure 2 displays a TEM picture of the ZnNPs positioned on a copper TEM grid coated with carbon. ZnNPs, which are made up of spherically shaped, polydispersed nanospheres, have the potential to be very effective catalysts in a variety of applications. The majority of the ZnNPs produced by *Aspergillus* sp. that were visible in the micrograph had a diameter of 4–15 nm.

To ascertain the crystalline size of the ZnNPs generated by *Aspergillus* sp., XRD examination was performed (Figure 3). The analysis's findings supported earlier findings from the literature by demonstrating that the ZnNP crystals have a face-centered cubic (FCC) structure (Fu and Viraraghavan 2001; Peng et al. 2013). The observed Bragg reflections were attributed to the (1 1 1), (2 0 0), and (2 2 0) sets of the lattice planes of the FCC structures of silver. These reflections had 2θ values of 32.6, 38.8, and 55.06°. The crystalline character of the ZnNPs produced by *Aspergillus* sp. was therefore verified by these XRD patterns (Evanoff et al. 2004).

With the aid of an identification key, the *Aspergillus* sp. that produced ZnNPs efficiently was located based on its morphological traits and assigned to the *Aspergillus niger* species. Additionally, the fungus' identity was verified by the use of 18S rRNA gene sequencing. DNA sequencing procedures were used to get the nucleotide sequence data, which were then uploaded to GenBank [GenBank: LC009511.1]. *Aspergillus niger* [GenBank: EF661058.1], and *Aspergillus niger* [GenBank: EF661059.1] were all 99% similar to certain amplicons following BLAST analysis.

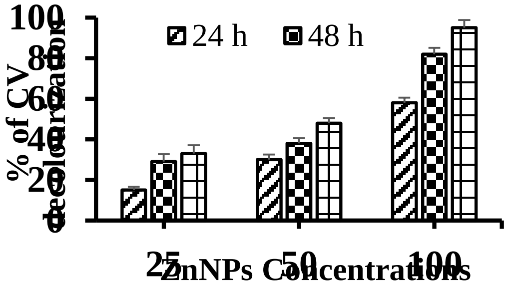


Fig 3. XRD of ZnNPs produced by *A. niger*

Decolorization studies

Using CV dye, the ability of the ZnNPs produced by *Aspergillus niger* to degrade dye was assessed. CV degradation was conducted over varying time periods and in the presence of varying ZnNP concentrations. At 72 h, the ZnNPs' percentage decolorization efficiency was 95% (Table 2). Interestingly, when the ZnNP concentration rose, so did the decolorization efficiency. These findings imply that the ZnNPs produced in this investigation may be effective catalysts for the CV dye's breakdown. Additionally, this study offers a cost-effective way to remove dyes from contaminated water systems, which may also be used to the removal of a number of other contaminants.

Fig 3. Decolorization rate of CV after 72 h.

Declaration of Conflicting Interests

The author hereby declares that there is no conflict of interest with respect to the research authorship and/or publication of the results of this work.

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