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Characterization and optimization of biosynthesized silver nanoparticles by resting cells of *Aspergillus niger* using Taguchi method

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Introduction

Microbial nanoparticle synthesis is a green chemistry process that integrates nanotechnology with microbial biotechnology. Compared to other methods, microbial synthesis offers superior control over the size distribution of the resulting nanoparticles. Silver nanoparticles (AgNPs), known for their high specific surface area and dense surface atom distribution, are utilized across various fields, including medicine, agriculture, animal husbandry, household products, cosmetics, packaging, and military applications. This study investigates the extracellular synthesis of AgNPs using resting cells of the fungal strain *A. niger* ZRS14. Additionally, the Taguchi design approach was employed to evaluate and optimize several factors influencing AgNP synthesis.

Methods and Materials:

The *A. niger* strain ZRS14 (accession number KF414527) was isolated from soil samples collected from the Angoran zinc and lead mines in Zanjan Province and is part of the microbial collection at Kurdistan University. The study investigated the effects of various factors on the synthesis rate of AgNPs, including silver nitrate precursor concentration (1, 2, 3, and 4 mM), wet biomass concentration (4, 8, 12, 16, and 20 g/L), temperature (24, 28, 32, and 36 °C), pH (5, 6, 7, 8, and 9), and incubation period (12, 24, 36, 48, 60, 72, and 84 hours), using a one-factor-at-a-time approach. In the Taguchi experimental design, four factors, including silver concentration, were selected based on the results from the single-factor optimization studies. To purify the AgNPs synthesized during the transformation reaction with resting cells of A. niger, the supernatant was first filtered through 0.2 µm filters to remove fungal biomass, followed by centrifugation at 13,000 rpm for 40 minutes. The resulting sediment was washed three times with sterile deionized water and ethanol. The samples were then dried using a freeze dryer for further analysis. Surface examination of the produced nanoparticles, along with their elemental composition, was performed using field emission scanning electron microscopy (FESEM) equipped with an energy-dispersive X-ray (EDX) detector to assess size and shape. X-ray diffraction (XRD) analysis determined the chemical content and crystal structure of the AgNPs produced by fungal biomass. Fourier-transform infrared spectroscopy (FTIR) was used to investigate the binding of organic compounds to the surface of the nanoparticles, as well as their potential role in reducing the silver precursor and serving as a stabilizing agent for the biosynthesized nanoparticles.

Results & Discussion

The synthesized AgNPs were initially confirmed through visual observation and analysis of the UV-visible absorption spectra. The reaction solution changed to a brown color due to the biological reduction of AgNO3 to AgNPs, indicating the formation of a colloidal suspension. After 24 hours of incubation using the resting cell strategy, ultraviolet-visible spectroscopy revealed a distinct absorption peak at 428 nm, confirming the presence of elemental AgNPs in the reaction mixture. Optimization of various factors—including temperature, pH, fungal biomass weight, silver nitrate concentration, and incubation time—was performed using the "one factor at a time" method. The optimal conditions identified were 2 mM silver nitrate, 16 g/L biomass, pH 6, and a temperature of 32 °C, resulting in spherical AgNPs with good dispersion. The Taguchi method was employed for experimental design to minimize the number of experiments and costs while optimizing the effective factors in AgNP synthesis. The highest synthesis efficiency was observed at 3 mM silver nitrate, 16 g/L fungal biomass, pH 6, and an incubation time of 72 hours. Using Qualitek-4 software, the contribution of each factor to the synthesis rate of AgNPs was determined. Results indicated that pH was the most influential factor, followed by fungal biomass concentration and incubation time, while silver nitrate concentration had the least impact on the biosynthesis process. Further studies were conducted to investigate the shape and size of the nanoparticles under optimal conditions. Spherical AgNPs with an average diameter of 24.8 nm were observed after 48 hours of incubation. Extending the incubation time to 72 hours increased the average size to 31.5 nm

, and after 96 hours, the size reached 38.1 nm. Thus, the size of the AgNPs exhibited a direct correlation with increased incubation time.

Conclusion

This study explored the ability of *A. niger* ZRS14 to synthesize AgNPs extracellularly. The findings suggest that this fungus is a cost-effective and eco-friendly biocatalyst, offering a viable alternative to traditional physicochemical methods for AgNP production. Using Taguchi software, the optimal synthesis conditions were validated, showing alignment with predicted values. Spectroscopic analysis confirmed that the synthesized AgNPs are spherical silver crystals under 38 nm in size, stabilized by proteins produced and secreted by the fungus.

Keywords: Fungal biosynthesis, Spectroscopy, Electron microscope, Silver nanoparticle

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Declaration of conflict of interest:

The authors declare that they have no conflicts of interest