

Nanobiotechnology for the removal of heavy metals from industrial wastewater

SHAFIQ S. *, MUNIR A.

The Department of Biotechnology, The University of Faisalabad, Amin campus, Faisalabad, Pakistan

*corresponding author:

e-mail: s.shafiq@hotmail.com; hod.biotechnol@tuf.edu.pk

Abstract Nanobiotechnology offers promising solution to treat industrial effluent. Industrial effluent contains significant levels of toxic heavy metals including chromium, lead, zinc and cadmium. Current physical and chemical treatment methods are expensive and non-environmental friendly. It is crucial to explore potential green techniques that are cost-effective and reduce carbon footprints. Therefore, in the present study, bio-synthesized ZnO nanoparticles (NPs) were analysed for their potential to remove or degrade chromium (VI) ions to chromium (III) ions to decrease its' toxicity in water. The NPs were synthesized using *Bacillus subtilis* and were characterized by FTIR and zetasizer to identify the shape, size and average particle size. The spectroscopic analyses confirmed the removal of chromium ions through bio-synthesized ZnO NPs. The study showed that biologically synthesized nanoparticles have significant potential to remove and/or degrade chromium ions. It is concluded that Nanobiotechnology is an efficient technique to clean waste water and biologically synthesized nanoparticeles presents environmetal friendly and cost effective method that must be explored in future for other toxic heavy metals also.

Keywords: Industrial effluent, wastewater, nanobiotechnology, heavy metals, green synthesis

1. Introduction

Industrialization and urbanization have led to significant water contamination from industrial and household wastewater, introducing pollutants like heavy metals and pathogens (Babuji et al., 2023). Traditional water treatment methods face limitations, such as high costs and toxic sludge, while nanotechnology, particularly zinc oxide (ZnO) nanoparticles, offers an efficient, cost-effective solution for water purification (Sagir et al., 2021). Green synthesis of ZnO nanoparticles (NPs) using microorganisms like *Bacillus subtilis* provides an eco-friendly alternative (Phogat et al., 2018). The potential of these NPs needs to explore for the sustainable water treatment for removing toxic pollutants like chromium (Puay et al., 2015).

2. Materials and Method

2.1. Preparation of Zinc Oxide Nanoparticles by Green Synthesis

Bacillus strains were isolated using nutrient agar plates (Fig 1) and incubated at 33°C for 24 hours. A colony was then transferred into a 1000 mL Erlenmeyer flask containing 300 mL of nutrient broth, which was incubated at 33°C for 24 hrs. The culture was then centrifuged for 20 min, and the supernatant was used for the biosynthesis of ZnO NPs. Then 6 mM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ with pH 7.5 was incubated at 33°C for 72 hrs in a shaker at 120 rpm. A white precipitate formed during the reaction, indicating the formation of ZnO NPs. This precipitate was centrifuged, and the supernatant was discarded. The pellet was washed with deionized water and ethanol. The product was dried overnight in a hot air oven, and then ground with a pestle and mortar (Fig 2).

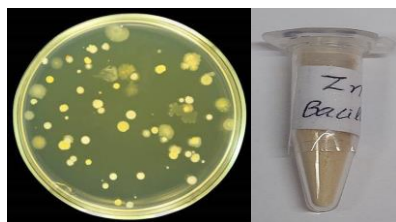


Fig (1) *Bacillus Subtilis* growth on agar plate (2) Zinc Oxide Nanoparticles

2.2 Purification and Characterization of Zinc Oxide Nanoparticles

The sample was washed with deionized water and ethanol at 7000 rpm for 15 min. The sample was then autoclaved, dried overnight at 120°C, and sonicated for 30 min to reduce nanoparticle size. The synthesized nanoparticles were characterized and analyzed using AFM, TEM, X-ray diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, ZetaSizer and Zeta potential.

2.3. Wastewater Collection and Analysis

Effluent samples were collected from various industrial sites and analyzed for the concentration of chromium, pH,

and conductivity, Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), and Total Suspended Solids (TSS).

2.4 Determination of Chromium (VI) in Standard Sample

A 1000 ppm solution of $K_2Cr_2O_7$ and various dilutions were prepared through serial dilution. Diphenyl Carbazide (DPC) solution was prepared by dissolving 0.5g of DPC in 200 mL of propanone, along with 3M sulfuric acid for the analysis. Chromium (VI) and (III) concentrations were determined using DPC and ascorbic acid in spectrophotometric analysis.

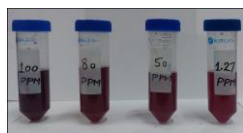


Fig 3 Chromium solutions with different ppm

3. Results

The reduction of Chromium (VI) with pH, Temperature and Zinc NPs concentrations, 2D image of NPs are shown in Figs 5A - C below;

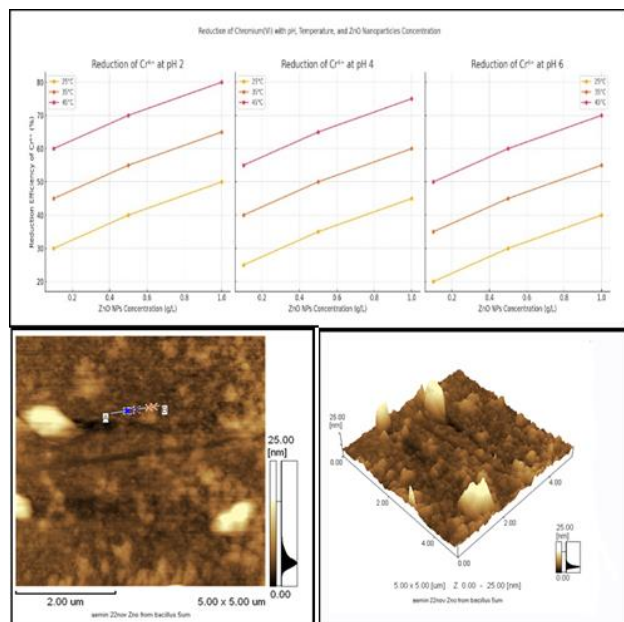


Fig 5 (A). Reduction of Chromium (VI) with various pH, temperature and Zinc NPs concentrations (B). 2D Topographical and Height Image of Zinc Oxide Nanoparticles by Green Synthesis. (C) 3D Image of Zinc Oxide Nanoparticles by Green Synthesis

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Wastewater Treatment with Synthesized Particles

ZnO NPs synthesized from *Bacillus subtilis* were used to treat industrial effluent containing Cr (VI) ions. The NPs with sizes ranging from 120 to 500 nm (Fig 6) were added to Cr (VI) solutions, and the pH was adjusted while exposing the samples to UV light. The highest Cr (VI) removal efficiency of 67.6% was achieved at pH 2. The treatment process showed improved efficiency as the pH decreased, and absorbance analysis confirmed the results.

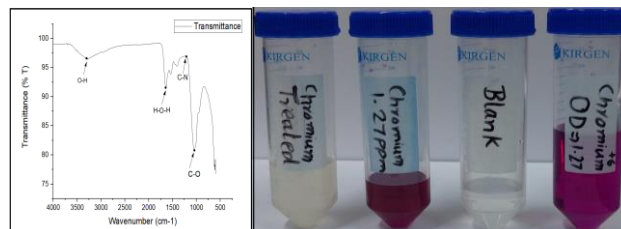


Fig (6) FTIR spectra of Zinc oxide Nanoparticles (7) Chromium after treatment under spectrophotometric analysis.

Atomic Absorption Spectroscopy (AAS) results showed that at the 5ppm Cr solution concentration, the adsorption efficiency of ZnO NPs exceeded 86%. The results indicated that the adsorption efficiency is directly associated with the amount of ZnO concentration (Fig 7).

4. Discussion

The "Reduce, Reuse, and Recycle" (3R) principles are crucial for sustainable waste management. Urbanization and industrialization contribute to water scarcity, with industrial wastewater being a major polluter. This pollution, especially from industries like textiles, petrochemicals, and refineries, harms water quality and ecosystems. Nanotechnology, especially nanoparticles, offers a promising solution for wastewater treatment. Zinc oxide nanoparticles (ZnO NPs), with their large surface area and photocatalytic properties, are effective in removing various contaminants, including metals and organic pollutants. Therefore, it is concluded that these nanoparticles can be synthesized through eco-friendly biological processes, such as using *Bacillus subtilis*, making them cost-effective and environmentally sustainable (Puay et al., 2015). ZnO NPs are particularly effective in treating toxic contaminants like Chromium and outperform traditional chemical methods in terms of sustainability and toxicity.

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