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Article

Copper Nanoparticles Synthesis from Electroplating Industry Effluent

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Abstract

In present investigation, samples from wastewaters of electroplating industry were collected and analyzed for the concentration of Cu^{2+} heavy metal. For the synthesis of copper nanoparticles, *Pseudomonas stutzeri* bacterial strain was used. The bacterial strain was isolated from soil and found that it produced 50-150 nm sized cubical copper nanoparticles from electroplating waste water. The nanoparticles have been characterized by UV-visible Spectrophotometer, X-ray diffraction, Scanning Electron Microscopy and Energy Dispersive X-ray Analysis.

Keywords: Copper, Heavy Metals, Soil Bacteria, Wastewaters, Nanoparticles.

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1. Introduction

The presence of heavy metals in the environment is of major concern because of their toxicity, bio-accumulating tendency, and threat to human life and environment. The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The anthropogenic sources of heavy metals include wastes from the electroplating and metal finishing industries, metallurgical industries, tannery operations, chemical manufacturing, mine drainage, battery manufacturing, leather tanning industries, fertilizer industries, pigment manufacturing industries, leachates from landfills and contaminated ground water from hazardous waste sites [1-4]. Wastewaters from these industries include metal ions having permanent toxic effect. In recent years, many low cost sorbents such as algae, fungi, bacteria and lingo-cellulosic agricultural by-products have been investigated for their biosorption capacity towards heavy metals. Algae, fungi, yeast and bacteria remove heavy metals from wastewaters through functional groups such as ketones, aldehydes, carboxyls; on their cell walls [5, 6]. Some strains exhibited a novel property of metal bioaccumulation with simultaneous synthesis of nanoparticles [7]. As in the current context, importance is being given to the fabrication of a wide range of nanomaterials for developing environmentally benign technologies in material synthesis [8]. Despite of their minute structure, they trigger the chemical activity due to their distinctive crystallographic nature that increases surface area, hence the scope of reactivity [9, 10]. In recent times, synthesis of inorganic nanoparticles has been demonstrated by many physical and chemical means. But the importance of biological synthesis is being emphasized globally at present because chemical methods are capital intensive, toxic, non eco-friendly and have low productivity [11].

The most important challenge in nanotechnology today is to cost effectively tailor the optical, electric and electronic property of nanoparticles by controlling the configuration as well as monodispersity. This goal could be achieved using bacterial organisms in an organized manner [12]. Pseudomonas stutzeri AG259 has been reported to fabricate Ag particles [13], which are accumulated within the periplasmic space of bacterial cell of 200 nm.

Copper nanoparticles were widely used as alternative catalysts [14]. such as selective hydrogenation and methanol synthesis reactions, which make them suitable for application in the field of catalysis. There are several reports available of physical and chemical synthesis of

copper and copper based nanomaterials. Chuncheng Hao et al. have reported the preparation of copper nanoparticles encapsulated in graphitic carbon shells using modified arc plasma method [15]. Surfactant-assisted electrochemical procedures have been shown to be a powerful tool for preparing stable nanoparticles composed of a wide range of metals and compounds [16-21]. We have recently reported a novel biological method using non-pathogenic bacterial strain Pseudomonas stutzeri isolated from soil for synthesizing copper nanoparticles [22]. It is, therefore, important to develop synthetic strategies which are simple, cost-effective, environment friendly, easily scalable and at the same time with parameters to control size and shape of the materials. Now an attempt has been made in the present investigation to synthesize copper nanoparticles from electroplating waste waters by a bacterial method using non-pathogenic bacterial strain *Pseudomonas stutzeri* isolated from soil and characterize them for their properties.

2. Experimental Procedures

Bacterial strains were enumerated from soil around the sewage outfall of the small scale electroplating industry from Dhakran, Agra by Serial dilution-agar plating method [23]. 1×10^{-3} dm^3 inoculum was transferred into 50×10^{-3} dm^3 medium containing (g L^{-1}) Peptone, 5; Beef extract, 3; Sodium chloride, 5; Agar, 15; pH 7.2 in Erlenmeyer flasks. Cells were grown at 37 °C for 24 h and then harvested by centrifugation (8000 r min^{-1}, 10 min at room temperature). The cell pellet was re-suspended and centrifuged three times in deionized water. Samples of effluent wastewater were collected from the electroplating Industry from a small-scale industry at Dhakran, Agra. Effluents used in this study were filtered with Whatman filter paper 44 (Himedia) to eliminate the suspended matter and then filtered with the 0.45 µm nylon membrane filter (Millipore) after a partial lime treatment to pH 6 and then samples were kept at 4 °C. The concentration of heavy metal in the effluent water was determined by atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 400). In a typical synthesis for nanoparticles using *Pseudomonas stutzeri*, the carefully weighted 0.1 g biomass was added to 100 ml of effluent solution, in conical flasks of 250 ml content. The flasks were thereafter incubated in incubator-shaker at 150 rpm at room temperature. To confirm the synthesis of nanoparticles, characterization was done by UV-Vis spectroscopy, X-ray diffraction, Scanning Electron Microscopy and EDX analysis. The bioreduction of copper ions in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring Ultra violet visible (UV-vis) spectra of the resulting diluents. UV–vis spectroscopy analyses of copper nanoparticles produced were carried out on ELICO UV spectrophotometer at a resolution of 1 nm. X-Ray diffraction (XRD) measurements of the bioreduced solution, drop-coated onto glass substrate, were done by an X'Pert Pro X-ray diffractometer instrument operating at a voltage of 45 kV and a current of 40 mA with Cu Ka radiation. The biomass after reaction spontaneously precipitated at the bottom of the conical flasks in 1 h. After the precipitation, the suspension above the precipitate was centrifuged at 8000 rpm for 10 min and sampled for SEM-EDX observations. SEM samples of the aqueous suspension of nanoparticles were fabricated by dropping the suspension onto clean electric glass and allowing water to completely evaporate. Samples were coated by carbon and SEM analyses were performed on a Zeiss EVO 40 Electron Microscope with Bruker EDX.

3. Results & Discussion

The serial dilution technique was adopted for the isolation of bacterial strains from the electroplating effluent enriched soil and sediment. The colonies of bacteria that appeared on agar plate were isolated and further purified on a nutrient agar plate by the process of spot inoculation. Three isolates were isolated and tested for their ability for bioaccumulation of copper from the medium containing effluent solution. *Pseudomonas stutzeri* has been used for the biosynthesis of copper nanoparticles in effluent waste water. In Industrial effluent heavy metal analysis, electroplating industry found to contain 28.6 µg mL^{-1} Cu^{2+}. The results revealed that the biomass showed absorptive capacity of 73 µg g^{-1} after the completion of reaction and also synthesized cubical and spherical shaped copper nanoparticles.

The synthesis of copper nanoparticles can be clearly seen with the change of solution color. The solution incubated with deionized water (control) retained its original color (i.e. yellow) while the biomass treated effluent solution turned red (as shown in Fig. 1 inset) after 10 h due to the formation of copper nanoparticles extracellularly. As indicated in the experimental section, the bioreduction was carried out at room temperature and clearly, the formation of copper nanoparticles was due to the bacterial biomass.

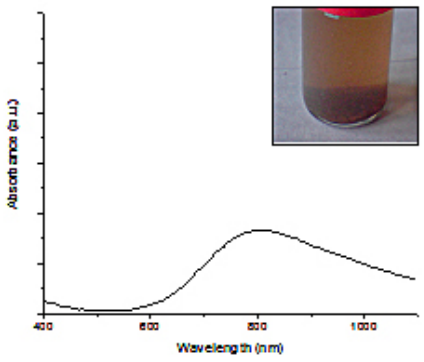

Fig. 1 UV-Vis spectra recorded as a function of time of reaction of copper nanoparticles. Inset shows copper nanoparticles.

Nanosized copper together with other noble metals as gold and silver are the most studied metallic nanoparticles

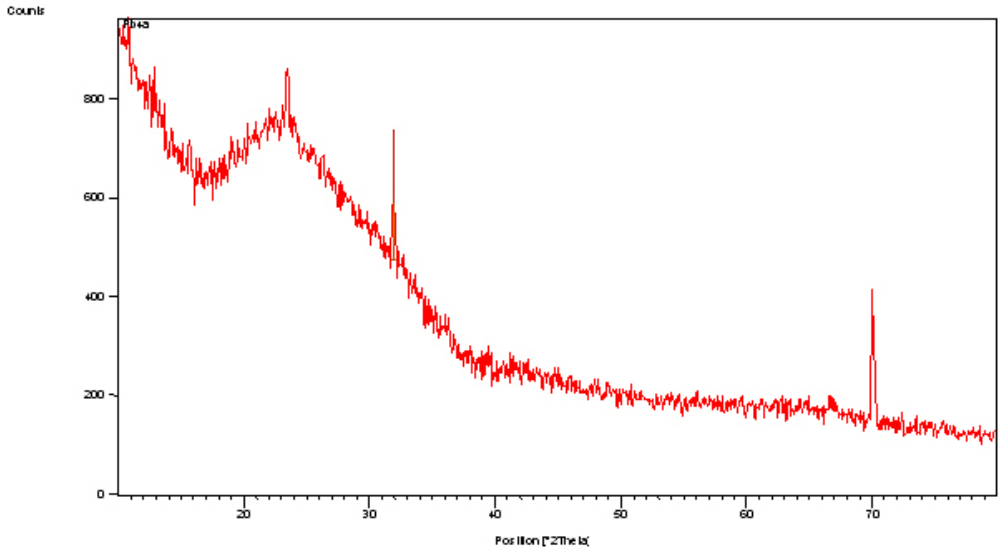

Fig. 2 XRD patterns recorded from drop-coated films of copper nanoparticles on glass substrates

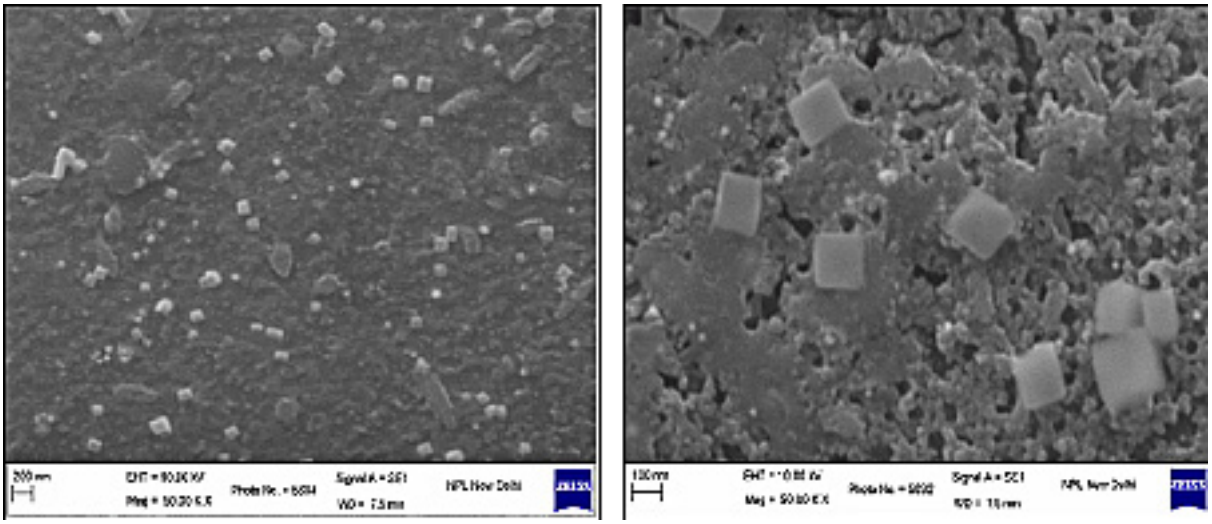

Fig. 3 SEM images of copper nanoparticles formed extracellularly from electroplating industry effluent by bacteria Pseudomonas stutzeri. [Scale Bar: 200 nm, 100 nm.]

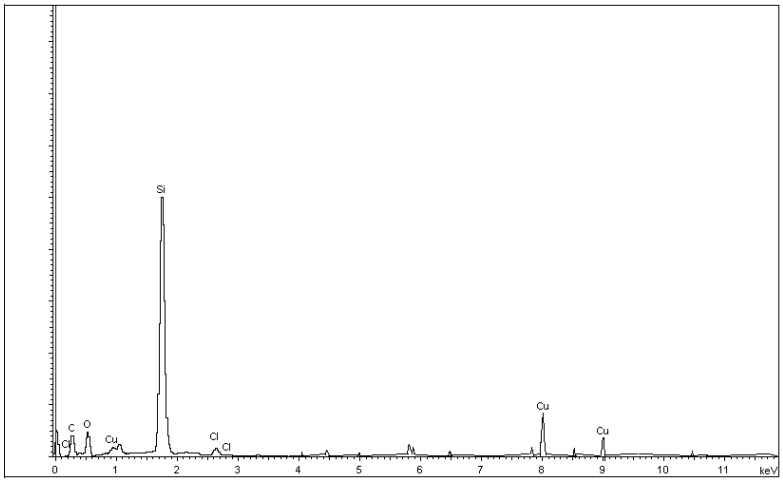

Fig. 4 EDX spectrum recorded from drop-coated films of copper nanoparticles formed extracellularly from electroplating industry effluent by bacteria Pseudomonas stutzeri.

as the surface plasmon resonances are clearly featured in the optical spectra, and are located in visible region.[24] The first indication of synthesis of copper nanoparticles was the appearance of red color after 10 h. This is due to Plasmon resonance, with a significant contribution from inter-band transition which produces red color hydrosol having λ_{max} at 780 nm (Fig. 1). Copper particles have a broad absorption at 780 nm, this is due to the aggregation of smaller particles that lead to the formation of cubic nanoparticles. Red color of colloidal solution remains stable after six months of synthesis.

X-ray diffraction (XRD) spectral data in Fig. 2, showed face centred cubic (FCC) Cu corresponding to the 24.20 (111), 32.4 (200), and 70 (220) reflections. XRD spectrum of the copper nanoparticles exhibited 2θ values corresponding to the copper nanocrystal. No peaks of impurities are observed in XRD data. The crystallite size can be found by applying Sherrer's equation and the average crystallite size is found to be 87 nm. The observation of diffraction peaks for the copper nanoparticles indicates that these are crystalline in nature.

In order to identify the existence of Cu nanoparticles structure SEM analysis was performed for the sample. The SEM images recorded from drop-coated films of the copper nanoparticles synthesized by treating electroplating effluent solution with *Pseudomonas stutzeri* for 10 h were shown in Fig. 3. A number of cubical copper nanoparticles can be seen and the nanoparticles are quite ranged in size from 50-150 nm. Interestingly, particles are quite separated; this may be due to some bioorganic component secreted by the bacteria acting as a stabilizing agent for the nanoparticles. The micrograph also demonstrates that as-synthesized copper nanoparticles are well-dispersed with no conspicuous agglomeration and stable even up to six months; since the absorption band does not change over this period. This indicates that the bacterial surface acts both as reducing as well as capping agent.

The biosynthesis of copper nanoparticles in the solution was quite evident in SEM micrographs, which were further confirmed by their EDAX analysis (Fig. 4). EDAX signals confirmed that the accumulated particles were indeed the copper particles. While signals from C, O and Cl atoms are also recorded which are likely to be due to X-ray emission from proteins/enzymes present in the cell wall of biomass. Signal from Si is due to glass on which sample was prepared.

The mechanism of nanoparticles formation by microorganism is yet to be fully understood. It is known that microbes detoxify the metal by (i) effluxing it out (ii) accumulating in cytoplasm and (iii) converting into less toxic form. The synthesis of nanosized particles around the metal centre could be mediated through reductases, followed by aggregation with other cellular proteins [25, 26].

4. Conclusion

An analysis of the results leads to an interesting conclusion; that cubical copper nanoparticles in size range of 50-150 nm were prepared by a novel biological synthesis technique which is simple and environmentally benign and synthesized copper nanoparticles with very good stability. It is an easy, fast and cost effective technique and doesn't involve any harmful and environmentally toxic chemicals used previously in conventional chemical reduction methods. It has been also discussed that bacterial biomass acts as capping agent for the metal nanoparticles by preventing them from aggregation.

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