International Scientific Journal of Engineering and Management (ISJEM) ISSN: 2583-6129
Volume: 04 Issue: 06 | June – 2025 DOI: 10.55041/ISJEM04679
An International Scholarly || Multidisciplinary || Open Access || Indexing in all major Database & Metadata

Bio-Fabrication of Zinc Oxide Nanoparticles and Its Application in Dye Degradation

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Abstract - Every year large amount of untreated dye discharged as effluent by many industries create threat to the environment. To solve this problem, many phsico-chemical techniques are available. Because of their some limitations, biological methods are proved ecofriendly and sustainable. In this paper, photocatalyst based dye removal by nanoparticles (NP) is studied. Use of biologically synthesized NP have made this technique sustainable. Microorganisms from dye contaminated soil were isolated and screened out for heavy metal tolerance. Isolates were then used to synthesize zinc NP. They were then mixed with dye solution and allowed to degrade the dye under sun light. Nanoparticles acted as photocatalyst which improves dye degradation. Dye degraded significantly in NP treated flasks compared to control. A significant dye degradation of nearly 84% in chemical oxygen demand was also achieved. Collectively, bio-synthesized NP can be effectively used in dye degradation.

Key Words: Biodegradation, dye, nanoparticles, photocatalysts, *Bacillus*

1.INTRODUCTION

Globally, dye and color industries are one of the largest segments of chemical industry. They are used to impart colors to the fabrics, food, cosmetics, plastics, paper, leather, etc. Among these, textile industry is the largest one and discharge around 2-20% of unused dye into waterbodies. Water resources resulting into severe environmental concerns due to their high toxic impacts (Imran, 2019). It absorbs the sun light and reduces the photosynthesis, enters in the food chain of aquatic animals, and cause long term health hazards to its consumers. Some of the textile dyes and their products are mutagenic and carcinogenic, causing serious impacts on living components of ecosystem (Siddique, 2021; Sohaib, 2023).

Currently, there are many techniques available for dye removal or separation. It includes flocculation, adsorption, coagulation, precipitation, reverse osmosis, membrane filtration, nanofiltration, ultrafiltration, etc. Many of the techniques require sophisticated instruments and so become costly. Few of them uses chemical, results in the introduction of new chemicals in environment (Agustina, 2022). Moreover, some of the techniques can remove only partial pollutants and remaining will persist in the environment (Rathore, 2023). Thus, effective, and sustainable technique will be the solution to this problem.

Biodegradation can come to help here. It is a reliable and sustainable approach but it tends to slow. It can be improved by using photocatalysts. They are the molecules that accelerates a chemical reaction in the presence of light. It could efficiently degrade or mineralize dyes into nontoxic or less toxic form (Agustina, 2022). Photocatalysts are very effective in their nanoforms, as they have large surface area. Nanoparticles of metals like zinc, titanium, silver, copper, magnesium, iron, platinum, gold, etc can be used for the said purpose (Katheresan, 2018). Under stressed condition, microbes reduce metal ions to its metal oxides and produce NP (Jain, 2020).

This study includes the biosynthesis of ZnO nanoparticles by bacterial isolates. NP synthesis relies on the capacity of isolates to tolerate the toxicity levels of heavy metals. Isolates were then used to synthesize NP. These NP were allowed to react with the reactive red dye (one of the azo dye) under sun light and its effect was observed.

2. MATERIALS AND METHODS

2.1 Chemicals, reagents, and instruments

Zinc sulfate, sodium hydroxide, sodium nitrate, monopotassium phosphate, dipotassium phosphate, magnesium sulfate, ferrous sulfate, sodium chloride, reactive red dye, potassium dichromate, concentrated sulfuric acid, silver sulfate, mercuric sulfate, ferroin indicator, ferrous ammonium sulphate was of analytical grade. All the culture media were purchased from Hi-media. All reagents, media, and solution were prepared in double distilled water when required. All the requirement were sterilized at 121 ^{0}C, 15 lbs. pressure for 15 minutes when and where needed. The following instruments were used during the whole study: Autoclave, hot air oven, laminar air flow, rotary shaker, weighing balance, magnetic stirrer, vortex, refrigerator, light microscope, uv-vis spectroscope, centrifuge, COD digester.

2.2 Enrichment and Isolation dye degraders

The effluent sample was collected and allow for enrichment process and isolation. Effluent Sample is collected from local industry. Enrichment in special media promotes the growth of dye degraders and increases their number. The minimal salt media (MSM) broth with 2 ppm dye (reactive red) as a carbon source was used as the enrichment media. Then, 1 mL effluent sample was added aseptically into 100 mL MSM broth and kept on shaker for 24 h. For isolation, 1 mL culture was diluted to 10^{-6} times. 0.1 mL amount from all the dilution tube was spread on separate MSM plates and incubated overnight at 37 ^{0}C. After incubation, isolated colonies were transferred to nutrient agar (NA) plates and slants for preservation. Cultural characteristics and morphological characteristics were observed (Aneja, 2018).

2.3 Screening

Isolated bacterial strains were screened for heavy metal tolerance. Iron and zinc are the important salts used in NP mediated dye degradation so selected for the screening. The agar diffusion technique was performed with varying concentration of zinc (zn), and ferrous (Fe). Briefly, massive culturing of strain was spread out on NA plates and equidistant wells of 9 mm were made. Heavy metal sulphate salts ($FeSO_4$ and $ZnSO_4$) were filled in the wells at concentrations of 0.015 M, 0.025 M, 0.035 M, 0.050 M. Inoculated plates were incubated at 37 ^{0}C for 24 h (Alvarado-Campo, 2023). All experiments were carried out in triplicate and compared to control where sterile distilled water was added in well. Microorganisms exhibiting growth at

maximum heavy metal concentration, that metal and that strain was used further.

2.4 Characterization and identification of strains

The selected bacterial strains were first identified by their cultural characters on NA plate as well as morphological characters. Bacterial strains were further analyzed for biochemical characterization. Sugar fermentation, methyl red (MR) and voges-proskauer (VP) tests, nitrate reduction test, indole production test, triple sugar iron (TSI) test, citrate utilization test, urea hydrolysis tests, lipid hydrolysis test, gelatine hydrolysis test were conducted for biochemical characterization in suitable culture media (Aneja, 2018).

2.5 Bio-synthesis of Nanoparticle

1 mL inoculum was aseptically added in 100 mL nutrient broth (NB) and incubated on rotary shaker for 24 h. The culture broth, after incubation, was centrifuged to separate biomass and supernatant. Supernatant was used separately for extracellular synthesis of NP. Supernatant was added in 0.07 M zinc sulphate and again incubated overnight on rotary shaker (Koul, 2021). Here, microbial enzymes present in supernatant were acted as capping agent and play crucial role in the synthesis of NP. Synthesized nanoparticles can be observed as white precipitates at the bottom of flask. Absorbance spectra of NP were taken at 300 to 500 nm to check its purity (Siddique, 2021). Here, biomolecules secreted by isolate may acts as capping and stabilizing agent for the NP. So, there is no need for the additional of surfactants or capping agents from outside.

2.6 Dye degradation by nanoparticle

To check the efficiency of the NP, two sets (in triplicate) were run i.e. B and Control. First, B set, used biologically synthesized NP and second set was control i.e., no NP. 50 mL dye of 50 ppm concentration was mixed with 5 mL NP in set B. Control flasks contain only 50 mL dye solution; no NP were added. Then all the flasks were kept in dark for 30 min to achieve adsorption-desorption equilibrium and immediately the photocatalytic degradation experiments were carried out under sun light. Dye degradation was checked at every 1 h interval by spectrophotometric analysis and by COD (Harinee Subramanian, 2022).

2.6.1 Spectrophotometric analysis

Dye sample was collected from the set B and control at 1 h interval. Optical density (OD) at 540 nm was checked against distilled water as blank. Decrease in OD was considered as degradation of dye (Nitin A. Mirgane, 2020).

2.6.2 Chemical oxygen demand (COD)

COD is the indication of presence of oxidizable material i.e. organic materials or pollutants. Higher the COD value, higher the pollutants. Open reflux method was performed where organic matter is completely oxidized by $K_2Cr_2O_7$ in the presence of concentrated H_2SO_4. The remaining $K_2Cr_2O_7$ is estimated by titration with ferrous ammonium sulphate (FAS) and COD value can be calculated (APHA Standard Methods for the Examination of Water and Wastewater (23rd ed.), 2017). This method is suitable for COD values ranging from 50 to 2500 mg/L.

Take 10 mL sample and dilute to 50 mL in reflux flask. Add 1 g $HgSO_4$ and glass beads in it. Very slowly add 5.0 mL sulfuric acid reagent (5.5 g Ag_2SO_4/kg H_2SO_4) with mixing to dissolve $HgSO_4$ and allow to cool the reaction mixture. Then add 25 mL 0.04167 M $K_2Cr_2O_7$. Assembled the digester assembly and precaution must be taken. Add remaining 70 mL sulfuric acid reagent (5.5 g Ag_2SO_4/kg H_2SO_4). Place the reaction mixture into COD digester at 150 ^{0}C for 2 h. After digestion, add 2-3 drops of ferroin indicator to cooled reaction mixture. Titrate the mixture against 0.25 M Ferrous Ammonium Sulphate (FAS) until color changes from blue-green to reddish-brown. Run a blank with distilled water instead of sample and follow all the steps above. Calculate the COD value from below given formula.

COD (mg/L) = [(mL of FAS for blank - mL of FAS for sample) × Molarity of FAS × 8000]/ mL sample

3. RESULTS AND DISCUSSION

3.1 Isolation and screening of dye degraders

Enrichment of soil sample was done to increase the number of dye degraders. It provides a favorable environment to the organisms present in the sample. 10 well isolated bacterial colonies were got from enriched media by dilution technique. Strains were named as S1 to S10.

Isolated bacterial strains were screened by agar diffusion method against heavy metal salts of zinc and ferrous. Zone of inhibition around well in ferrous salt containing plate is observed for all isolates. It indicates that ferrous can inhibit the bacterial growth and hence not suitable to produce ferrous NP. No zone observed in case of zinc salt as in control (Figure 1). It indicates S1 and S2 show heavy metal tolerance and hence shows the possibility to produce NP of zinc salts and selected for further study.

Figure 1 Metal tolerance of S1, S2, and control against ZnO

Screening is the selection of strains of our interest by using selective procedure. An efficient screening strategy is a key to isolate interested microbes or their variants. In this study, author's one of the objectives was to isolate NP of heavy metal. So, the purpose of the screening was to find bacterial species which can tolerate heavy metal.

3.2 Characterization and identification of isolates

Conventional methods based on the colony and morphological characterization can be used to identify some routine species. Table 1 shows colony characteristics and Table 2 shows morphological and biochemical characterization of isolates. Irregular large colony with dry and wrinkled skin like elevation of S1 suggest it as *Bacillus* strain (Branda SS, 2001). Colony and biochemical characteristics were not sufficient to identify the S2 and remain unidentified.

Table 1 Colony characteristics of isolates

Colony characteristics	Isolate S1	Isolate S2
Shape	irregular	round
Size	large	medium
Margin	wavy	entire
Surface	rough	smooth
Elevation	flat	raised
Consistency	dry	moist

Opacity	opaque	opaque
Pigment	nil	nil

Table 2 Morphological and Biochemical characteristics

Test	**Isolate S1**	**Isolate S2**
Morphological characteristics		
Gram stain	positive	positive
Cell shape	short rod	short rod
Endospore	present	absent
Capsule	present	absent
Motility	present	absent
Biochemical characteristics		
Carbohydrate fermentation		
• **Sucrose**	+	-
• **Glucose**	+	-
• **Mannitol**	+	+
• **Xylose**	-	-
• **Lactose**	+	-
• **Maltose**	+	-
Methyl red test	-	-
Vouge Proskauer test	+	-
Nitrate reduction test	+	-
Indole production test	-	-
Citrate utilization test	+	-
Urea hydrolysis test	-	-
Lipid hydrolysis test	+	-
Gelatinase hydrolysis test	+	-
Dehydrogenase enzyme	present	absent
Oxidase test	present	absent
Catalase test	present	absent

(+) able to consume substrate, (-) unable to consume substrate

3.3 Microbial synthesis of ZnO nanoparticles

A prominent white color at the bottom of flask indicates the presence of NP. The confirmation of NP was done by spectrophotometer. Peak around 370 nm indicates the presence of NP. In the extracellular synthesis of NP, both isolates have shown off-white color at the bottom of flask (Figure 2a). Both the isolates have shown peak near 380 nm under UV spectrophotometer (Figure 2b). There are few studies which supports the result (V. Sai Saraswathi, 2017). Further study was continue with S1 because of its higher value as in Figure 2b.

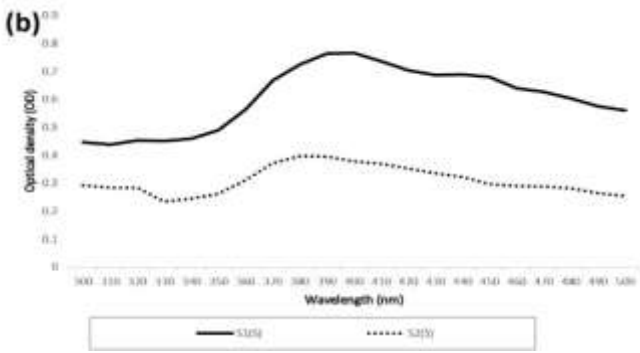

Figure 2 (a) Microbially synthesized ZnO NP, (b) Spectra of ZnO NP

3.4 Dye degradation

3.4.1 Spectrophotometric analysis

The photocatalytic degradation of reactive red dye using microbially synthesized ZnO NP by S1 was examined. Figure 3 shows the color change observed after the incubation period. It shows color change from dark red to light pink. The same trend was reflected in OD. Decrease in color intensity also matches with decrease in OD. 0.7 OD was observed after 1 h and gradually decrease to 0.5 after 7 h (Figure 4). It is evident that the dye is degrading with time. The similar observation was made by (Fatemeh Biglar, 2021).

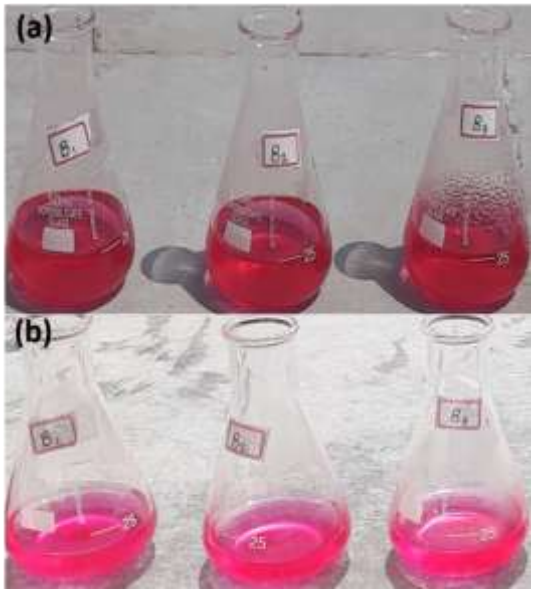

Figure 3 Color change (a) before and, (b) after photocatalytic degradation by ZnO NP

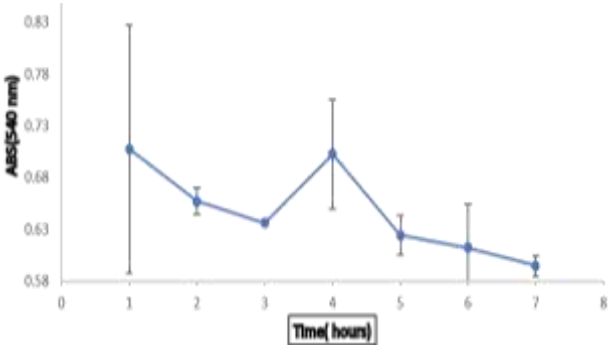

Figure 4 Dye degradation by microbially fabricated ZnO NP

3.4.2 Chemical oxygen demand

COD measures the amount of oxygen necessary to break down the organic substances that are pollutants in water. The graph shows COD of set B and control of 6th day and 12th day (Figure 5). COD of set B decrease compared to control set on 12th and 6th day. A higher COD in a sample indicates that it contains higher levels of oxidizable material. This graph visually shows that ZnO nanoparticle treatment reduces total COD content. 62% dye reduction was observed on 6th day and around 84% on 12th day. It is too high than control set i.e. 12.33%. Noorimotlagh et al. (2013) observed 75% COD removal using UV-activated ZnO NPs in just 2 hours while biogenically produced ZnO under sunlight settings achieved ~84–87% COD removal over comparable durations (Zahra Noorimotlagh, 2013). This strongly supports the high efficacy of ZnO NP–based photocatalysis for dye-laden effluents.

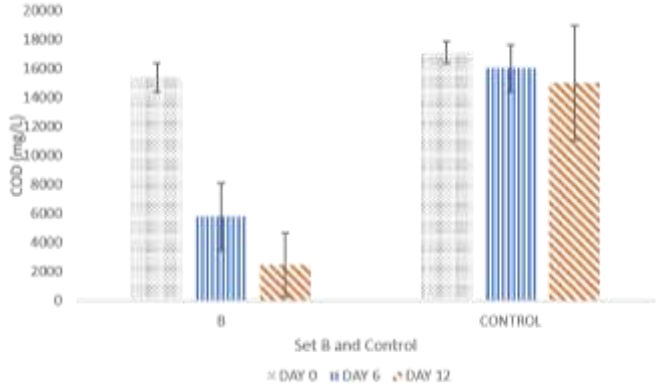

Figure 5 Change in chemical oxygen demand.

4. CONCLUSIONS

The microbial synthesis of zinc oxide nanoparticles for the photocatalytic degradation of reactive red dye presents a promising avenue for sustainable wastewater treatment. These nanoparticles demonstrate excellent photocatalytic properties, effectively degrading reactive red dye molecules under sun light irradiation. This eco-friendly approach not only offers an efficient method for dye degradation but also contributes to the mitigation of environmental pollution. Further research and development in this field hold potential for scalable applications in wastewater treatment plants, paving the way for cleaner and healthier ecosystems. Emphasis on efficiency enhancement of the photocatalytic process by optimizing the synthesis parameters of ZnO nanoparticles can be an effective approach. This could involve exploring different microbial species, growth conditions, and reaction parameters to achieve higher yields of nanoparticles with superior photocatalytic properties. As the technology matures, efforts may be directed towards scaling up the production of ZnO nanoparticles through microbial synthesis for industrial applications. Besides dye degradation, microbial-synthesized ZnO nanoparticles may find applications in other areas such as antimicrobial coatings, solar cells, sensors, and photocatalytic hydrogen production.

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