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ORIGINAL ARTICLE **OPEN ACCESS**

Biodegradation of textile dye Acid blue 113 using iron nanoparticles synthesized by *Pseudomonas stutzeri*

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ABSTRACT
The textile sector is among the most polluting because it utilises such huge quantities of dye that pollutes the water. In the current work, textile di-azo dye acid blue 113 degradation with the help of iron nanoparticles produced using bacteria Pseudomonas stutzeri. 98.25 % degradation was observed in 15 min upon addition of nanoparticles. The optimized parameters for synthesis are time 96hrs, temperature 28^0 C ,400 rpm. The nanoparticles were synthesized at within 15 min by Pseudomonas stutzeri *using ferric chloride as a precursor. Characterization of synthesized nanoparticles using UV Visible spectrophotometry, FTIR, SEM, XRD. Iron nanoparticles' synthesis by* Pseudomonas stutzeri in eco-friendly manner *and its use in acid blue 113 degradation serve a significant work in the field in bioremediation.*
Keywords*: Pseudomonas stutzeri, iron nanoparticles, acid blue 113,biodegradation*

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INTRODUCTION

Textile sector secure a special position in the Indian economy by boosting industrial production, exports, and employment [1]. However, because it includes use of synthetic dyes that have negative impact on the environment and life forms, the effluent produced from textile industry has turned into an environmental pollution source constantly [2]. Due to their intriguing chemical, optical, and electronic capabilities, metal nanoparticles have captured the attention of scientists. It is simple, ecologically sound, and offers a quick means to meet the growing need for safe, non-toxic, and biocompatible nanoparticles around the world. Researchers have taken a particular interest in the green synthesis of nanoparticles utilizing microbes due to simple handling processes, environmentally friendly disposal, and significantly simpler downstream processing [3]. Among all microbes, bacteria have the innate capacity to reduce a given metal to produce metal nanoparticles [4]. Due to their genetic diversity and adaptable metabolic systems, microbes are a preferable choice for the biological remediation of dye pollution [5]. Additionally, the biological treatment uses fewer reagents and produces less sludge than other methods, making it a more affordable and eco-friendly option. It can result in the total mineralization of dyes and is also cost-effective [6]. The use of iron nanoparticles (FeNPs) has the potential to reduce environmental pollution. Nanoscale zero-valent iron (nZVI) is becoming more popular for environmental clean-up because of nanoscale iron's reactive nature and its high surface area to volume ratio.[7,8].

Pseudomonas stutzeri is currently acknowledged to be a member of the Gammaproteobacterial class.[9,10]. It is a widely distributed nonfluorescent denitrifying bacterium that has also been identified by humans as an opportunistic pathogen [11]. The taxonomy of such varied taxonomical group has been clarified over the past 15 years. The species has attracted a lot of attention due to its unique metabolic characters: Several strains exhibit characteristics of degradation of contaminants, heavy metals.[12].

In today's world, number of organic dyes has been extensively applied in thefood, textile, cosmetics, leather, paper and plastic companies for aesthetic reasons. Most of them could be dangerous and pose a significant damage to the climate. One of the difficult issues facing environmentalists and industry is treating and removing organic dyes from textile effluent. There are several physicochemical techniques already in use These, however, are inefficient and produce new chemicals that call for more treatments. Recent years have seen a significant increase in interest in current technologies that use nanocatalysts to remove dyes and other organic pollutants from the ecosystem [13]. The simple method that does not need the usage of organic solvents is to treat dyes in presence of a biocompatible, environmentally safe

catalyst. Acid Blue 113 is a di-azo dye that is crucial to industry and is used to colour polyamide fibre, wool, silk, leather, and paper. This dye has been degrading more quickly in recent years. In the current study, a bacterium that degrades di-azo dyes (dyes with two -N=N- azo linkages) [14], *Pseudomonas stutzeri*, was tested for its efficiency in bioremediation and degradation studies. In this context, efforts were made to study the degradation of acid blue 113 , textile dye di-azo in nature with the help of iron nanoparticles synthesized by *Pseudomonas stutzeri* bacterium.

MATERIAL AND METHODS

Media and chemicals used

For the current study, pure 99%FeCl3, nutrient agar, and nutrient broth media and Acid Blue 113, ethyl acetate, $FeCl_3$ and methanol were purchased from Sigma Aldrich, India. Analytical reagent grade chemicals were utilised throughout.

Iron nanoparticles biosynthesis

Bacteria *Pseudomonas stutzeri* was isolated from a soil sample of Kaas pathar at Satara, Maharashtra, India. Molecular identification of isolated bacteria was performed by 16 S rRNA sequencing technique at NCMR, Pune.

The sterile nutritional broth was inoculated with the pure culture of *Pseudomonas stutzeri* and incubated for 24 hours at 28^0 Cat 120 rpm. Additionally bacterial biomass was gathered, cleaned and added to distilled water. Cell-free extract (CFE) was mixed with equal volume of solution$FeCl_3$ having concentration 1 mM, and then the mixture was incubated on rotatory shaker (at 120 rpm) in the dark at 28^0 C. In parallel, Ferric chloride solution having concentration 1 mM kept as a control under the same circumstances **[15]**.The ability of the isolated bacterial strain *P. stutzeri* to produce iron nanoparticles was further investigated primarily using visual colour change.

Iron nanoparticle characterization

A Biosynthesized nanoparticle was examined with the help of UV-Visible spectrum analyzer (Systronics Au-270I) at periodic intervals in the 400–800 nm wavelength range. By using a Bruker D8 ADVANCE diffractometer, X-ray diffraction analysis carried out for determining the crystallographic information of nanoparticles. Iron nanoparticles' size and shape determined using SEM, Functional biomolecules involved in nanoparticle synthesis was examined using Fourier transform infrared spectroscopy(FTIR; Shimadzu FTIR 8400) run from 400- 4000 cm^{-1}

Degradation of Acid Blue 113 via iron nanoparticle catalysis

The catalytic activity of biosynthesized NPs was evaluated by biodegradation of reactive acid blue 113 dye. One mM iron NPs was mixed in 100 mL of 1mg/100ml of acid blue 113 dye, and stirred at 120 rpm in the dark for 15 minutes **[16].** After a specific time interval, the sample was withdrawn (2 mL), A UV-vis spectrophotometer was used to record UV-Visible absorbance spectra at 595 nm, and the percentage of degradation was calculated as given in Eq.

Degradation (%) = [D0-D1/D0] x 100

where D=decolorization in percentage,D0=initial absorbance, D1=final absorbance

Study of Degraded Dye

UV Visible spectroscopy

In the study, the biodegradation of dye, absorbance of degraded dye, and control dye were all analyzed spectrophotometrically and percent decolorization was determined. Changes in its absorption spectra (400-800 nm) were noted. [17].

RESULT AND DISCUSSION

Iron nanoparticles synthesis by bacteria.

This study investigated the capability of isolated bacteria *Pseudomonas stutzeri* to produce iron nanoparticles. Iron nanoparticles' extracellular synthesis was detected using the gradual color change from yellowish to reddish brown. Within 24 hours, the colour began to manifest, and its intensity increased to 48 hours later. SPR surface plasmon resonance was responsible for colour change indicating the nanoparticle synthesis.

Iron Nanoparticle Characterization

UV visible spectroscopy

In UV-visible spectroscopy, Fe nanoparticles displayed their maximum absorption at 233 nm (Fig. 1). Previous research work shows similar type of results as described by Awwad and Salem [18].

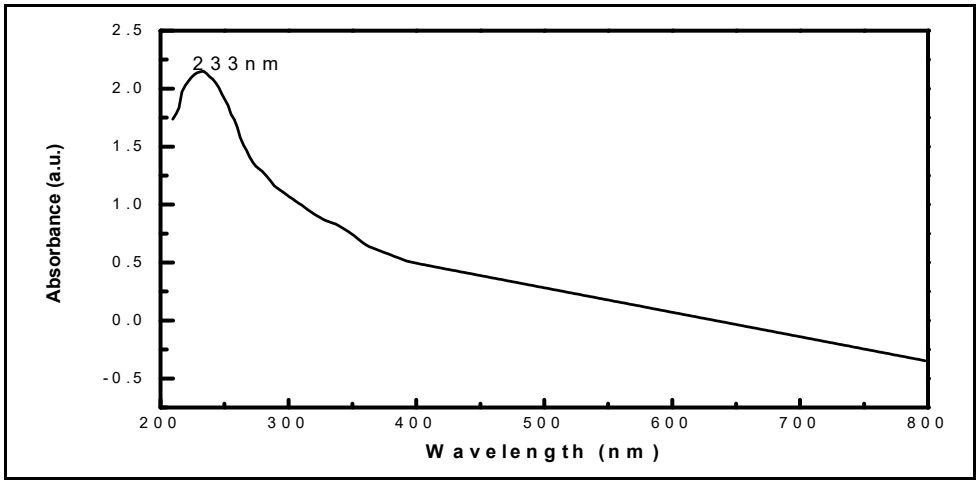

Figure 1: UV-visible spectrum showing a peak at 233 nm.

FTIR

FTIR measurements of iron nanoparticles used to investigate functional group involved in iron nanoparticle synthesis[19].Here, Fig. 2 shows four different peak functional groups at 3343 cm-1, 2922 cm-1, 1632 cm-1, and 640 cm-1 indicating the formation of Fe NPs. These peaks correspond to stretching of O-H vibrations (phenolic or alcoholic), asymmetric stretching of C-H, and CO stretching. The other peak 640 cm^{-1} might be due to the N-H stretching present in primary amine and alkyne. This shows that functional groups significantly aid in the stabilizing and capping of iron nanoparticle production.The results are according to previous characterization studies of iron nanoparticle explained by Niraimathee et al [20].

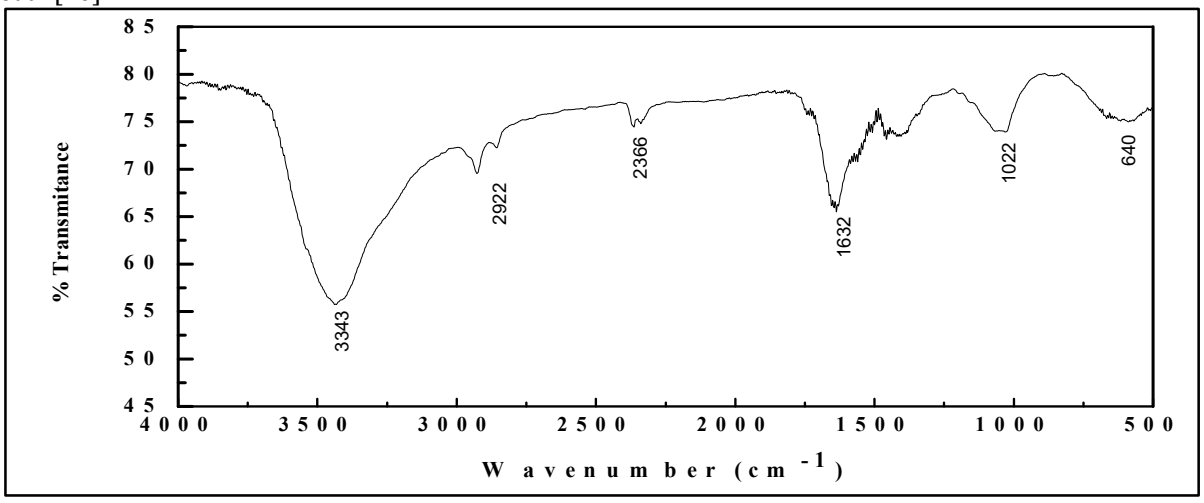

Figure 2: FTI R of iron nanoparticles

XRD

Iron nanoparticles' XRD pattern displayed in **Fig. 3**, which showed distinctive diffraction peaks at positions 30.35^0, 35.70^0, 43.47^0, 57.48^0, 63.08^0 respectively, with matching hill plane values of 220,311, 400, 333, and 440 matched the JCPDS card 01-086-1352. The crystalline structure and phase of iron nanoparticles are explained by the strong diffraction peak which indicates the synthesis of Fe_3O_4 nanoparticles. The nanoparticles' estimated size via the Debye-Scherer equation is 9 nm, which may indicate that they have large surface area. The size of a nanoparticle's crystallite is calculated using the Debye-Scherer formula **[21],** which is as follows:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D = size of the particle, λ =X-ray wavelength, K =crystalline form constant, θ =Braggs diffraction angle, β =wavelength of X-ray (1.54), and at 2θ diffraction angle, FWHM (angular full width at half maximum) of XRD peaks recorded.

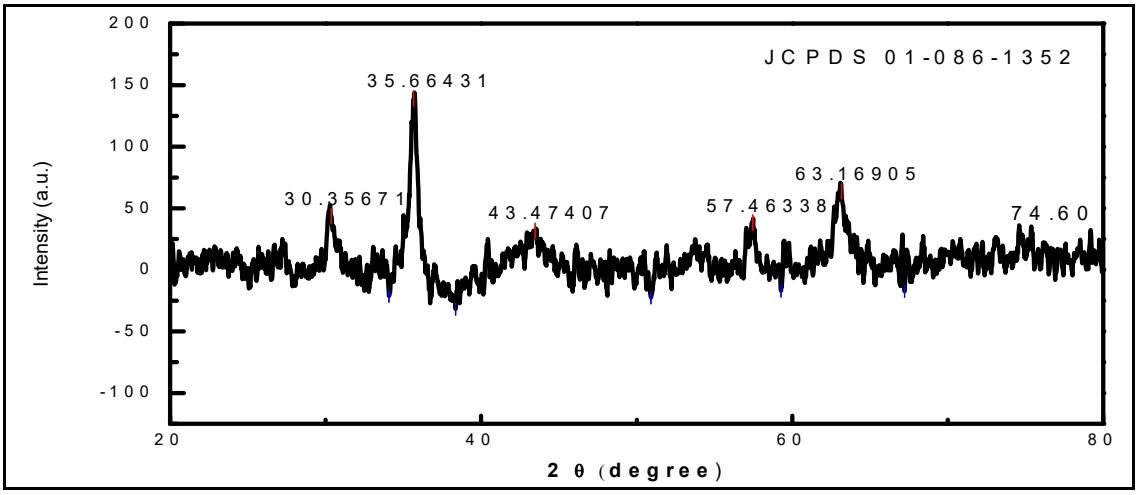

Figure 3: XRD pattern of iron nanoparticles

SEM

Using scanning electron microscopy, the iron nanoparticle's surface morphology was examined [22] as shown in Fig. 4.Monodispersed Fe NPs of size 20-50 nm was visualized from the SEM micrograph. According to previous studies combined agglomerated and monodispersed particles were reported by Siddique et al [23].

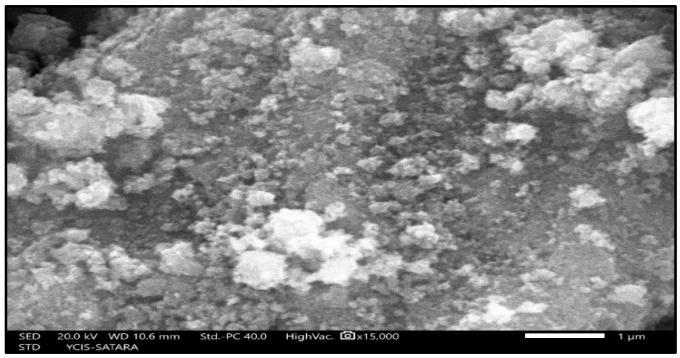

Figure 4: SEM images of iron nanoparticles

Acid blue 113degradation using biosynthesised iron nanoparticles as catalysts

In a UV-visible spectrophotometer, the typical peak of absorption at 526 nm was obtained to determine iron nanoparticles 'catalytic activity which are produced through biological means. (Fig. 5). Within 15 min, the peak intensity decreased, which suggests the degradation of Acid blue 113.

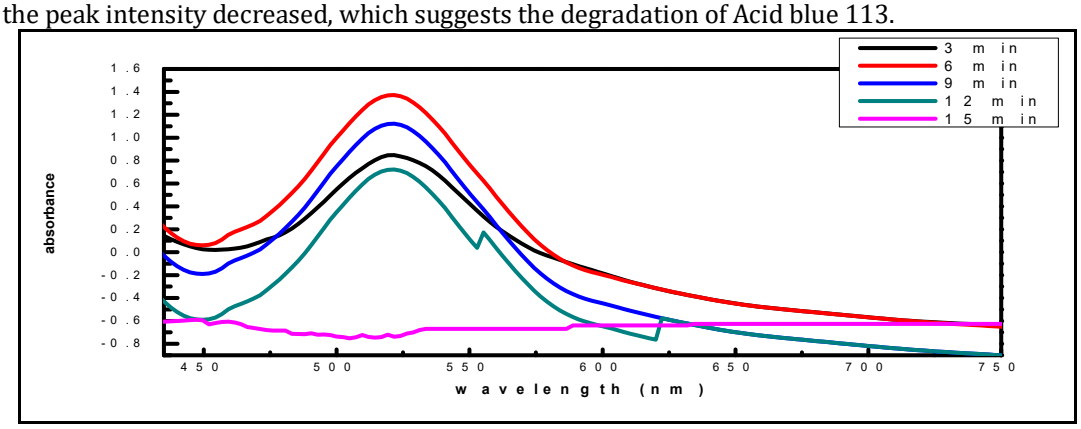

Figure 5: Acid blue 113 dye degradation upon iron nanoparticles addition as measured by UV-visible absorbance spectra.

Interestingly, upon addition of iron nanoparticles into solution of Acid blue 113 dye (concentration 1mg/100ml), gradual degradation of dye took place under dark conditions. The colour of the solution changes from blue to colourless after 15 min**(Fig. 6).**

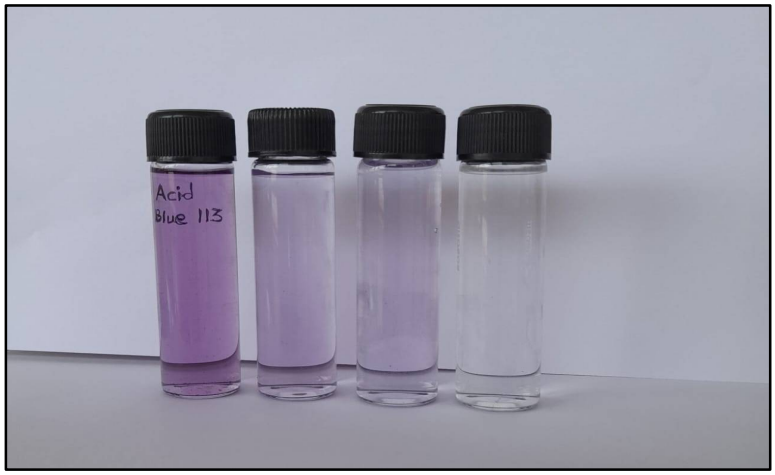

Figure 6: Gradual colour change from blue to colourless after addition of iron nanoparticles

CONCLUSION

In current research, synthesis of iron nanoparticles using cell free extract of *Pseudomonas stutzeri* in eco-friendly manner is reported. Characterization of biosynthesized Fe Nps were carried out using UV- visible spectroscopy, FTIR, SEM, XRD which shows iron nanoparticles of spherical shape having size of nearly 30-50 nm. Degradation potential of nanoparticles in acid blue 113 dye degradation was confirmed. Iron nanoparticles synthesis by *Pseudomonas stutzeri* in eco-friendly manner and its use in acid blue 113 biodegradation responsible for adding significant work in field of bioremediation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with this research.

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