"ISOLATION OF BACTERIA FROM HEAVILY RUST-CONTAMINATED SITE FOR IRON OXIDE NANOPARTICLE SYNTHESIS AND EVALUATION OF ANTIMICROBIAL AND DYE DECOLORISATION PROPERTIES"

A DISSERTATION

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BIOTECHNOLOGY

Submitted by

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CANDIDATE'S DECLARATION

I **Arif Khan,** Roll Number: **2K22/MSCBIO/13** student of M.Sc. Biotechnology hereby declares that the project dissertation titled **"Isolation of Bacteria from Heavily Rust-Contaminated Site for Iron Oxide Nanoparticle Synthesis and Evaluation of Antimicrobial and Dye Decolorization Properties"** submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science is an authentic record of my own work carried out during the period from January 2024 to May 2024, under the supervision of Prof. Jai Gopal Sharma. This work has not been previously formed as the basis for the award of any degree, Diploma Associateship, Fellowship, or other similar title or recognition.

My review paper has been accepted in a Scopus-indexed journal with the following details:

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CERTIFICATE

This is to certify that the project Dissertation titled **"Isolation of Bacteria from Heavily Rust-Contaminated Site for Iron Oxide Nanoparticle Synthesis and Evaluation of Antimicrobial and Dye Decolorization Properties"** which is submitted by **Arif Khan**, roll no. **2K22/MSCBIO/13** from Department of Biotechnology, Delhi Technological University, Delhi, is a record of the original project work carried out by the student herself under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this university or elsewhere.

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Delhi Technological University	**Delhi Technological University**

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“Isolation of Bacteria from Heavily Rust-Contaminated Site for Iron Oxide Nanoparticle Synthesis and Evaluation of Antimicrobial and Dye Decolorization Properties”

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ABSTRACT

This study presents a novel approach for the synthesis of iron oxide nanoparticles (IONPs) utilizing bacteria isolated from a heavily rust-contaminated site. The precursor for IONP synthesis was collected rust, abundant in the environment. Bacterial strains capable of mediating this synthesis were isolated and characterized. Various physicochemical techniques including UV- Vis spectroscopy, zeta-potential, solubility testing, and FTIR were employed for the characterization of the synthesized IONPs.The antimicrobial efficacy of the synthesized IONPs was assessed against some microbial strains. Results demonstrated promising antimicrobial efficacy, indicating the potential of these nanoparticles as effective antimicrobial agents. Furthermore, the ability of the IONPs to decolorize methylene blue dyes commonly found in industrial effluents was evaluated. The results revealed efficient dye decolorization properties, highlighting the potential applicability of these nanoparticles in wastewater treatment processes. Overall, this study underscores the utility of bacteria isolated from rust-contaminated environments for the eco-friendly synthesis of IONPs with multifunctional properties. The demonstrated antimicrobial activity and dye decolorization potential suggest promising applications in biomedical and environmental fields, opening avenues for further exploration and development of sustainable nanomaterials.

LIST OF PUBLICATIONS

1. A paper entitled “Phytochemistry of Aloe Vera: A Catalyst for Environment-friendly Diverse Nanoparticles with Sustained Biomedical Benefits” has been accepted in Nature Environment and Pollution Technology (NEPT).

2. A paper entitled “Bio Synergize: Microbial Synergy Driving Simultaneous Bioremediation and Nanoparticle Synthesis” has been accepted in African Journal of Biological Sciences (AJBS).

3. A paper entitled “Insights into the Inhibition Mechanism of Lipoteichoic Acid Synthase (LtaS) Enzyme by Endophytic Fungal Metabolites: A Molecular Docking and Dynamics Simulation Study for Combatting Drug Resistance” has been accepted in International Conference on Emerging Technologies in Science and Engineering (ICETSE) – 2024.

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CHAPTER 1

INTRODUCTION

The scientific community has shown significant interest in nanoscience in recent decades because of its capacity to generate a wide range of nanoparticles (NPs) using novel approaches in a sustainable manner. These nanoparticles (NPs) have been utilized in many sectors including pharmacy, medical examination and treatment, energy, electronics, chemical, agricultural, and space. Nanoparticles (NPs) are tiny particles with a diameter of less than 100 nm. They can be made from inorganic or organic materials and have unique features compared to larger materials(I. Khan et al., 2019). Nanoparticles can be produced by chemical, physical, and biological techniques. The primary physical techniques utilized in the production of nanoparticles are lithography, crushing, grinding, milling, pyrolysis, and physical vapor deposition (PVD). Conversely, the chemosynthesis of nanoparticles includes photocatalytic reduction, chemical reduction, sol-gel synthesis, CVD, electrolysis, and microwave-assisted synthesis. Physical methods are not suitable for mass manufacturing of nanomaterials due to their lower nanoparticle yield, higher energy requirements, and higher input costs. In recent years, chemical techniques have been the preferred approach for synthesizing nanoparticles (NPs) due to their ability to reduce metals and produce homogeneous NPs with great precision, while consuming less energy. Therefore, these conventional techniques for synthesizing NPs are arduous, tedious, and dangerous, and rely on the utilization of harmful substances that are unsafe (genotoxic, carcinogenic) and serve as potent pollutants in the environment(Arshad et al., 2017). Furthermore, the medical use of NPs derived from chemosynthesis has been restricted as a result of their inherent instability and hazardous properties. Hence, it is imperative to devise effective, dependable, non-toxic, and environmentally acceptable methods for the production of nanoparticles (NPs). One way to accomplish this is by creating NPs through the use of natural resources, such as the biological system, microbes and their enzymes, and biodegradable polymers. Nanoparticle production

utilizing biological systems is characterized by its rapidity, feasibility, and eco-friendliness. Furthermore, it is possible to regulate the toxicity and size properties of the NPs(Salem & Fouda, 2021).

Bacteria have become notable contributors in the field of nanoparticle synthesis, demonstrating their inherent biological machinery to create nanoparticles with exceptional accuracy and effectiveness. This emerging discipline takes advantage of the varied metabolic capacities of bacterial species, utilizing their enzymatic abilities to decrease metal ions and direct the generation of nanoparticles(Fahmy et al., 2018). nanoparticles can be synthesized either inside bacterial cells or using the supernatant of bacterial cells. These cells contain various proteins, enzymes, and polysaccharides that serve as reducing agents, responsible for converting metal ions into metal nanoparticles(Singh et al., 2015).

Bacteria possess unique biological features and biochemical machinery that make them more beneficial when compared to fungi and plants for nanoparticle manufacturing. Firstly, bacteria have a clear advantage due to their fast growth rate, which is made possible by their highly efficient cellular replication machinery(Qamar & Ahmad, 2021). The fast expansion of nanoparticles enables the efficient and quick synthesis of large quantities within a very short period. Moreover, the genetic manipulability of bacteria allows the creation of strains specifically designed for the nanoparticle's fabrication. This can be achieved by introducing genes that encode enzymes involved in nanoparticle formation or by modifying metabolic pathways to improve the efficiency of nanoparticle production(Iravani & Varma, 2022).

In addition, microorganisms have a broad range of metabolic pathways that can be utilized for the creation of nanoparticles. These pathways frequently entail the conversion of metal ions into their elemental forms, which act as the fundamental components for the creation of nanoparticles. In addition, bacteria have the ability to thrive under diverse environmental circumstances, including both aerobic and anaerobic conditions. This allows for the creation of nanoparticles in diverse physiological conditions(Marooufpour et al., 2019).

The continued presence of iron pollution in the environment is a major environmental issue that has extensive effects on ecosystems and human well-being. Iron is a crucial

micronutrient for many biological functions. However, high levels of iron can have harmful effects when it is released into the environment through natural processes or human actions. Industrial operations, such as mining, metal processing, and manufacturing, lead to the emission of iron pollutants into the atmosphere, water bodies, and soil, which promotes environmental degradation(Fazekašová & Fazekaš, 2020). Iron contamination in terrestrial ecosystems can have a detrimental impact on the quality and fertility of soil. High levels of iron can hinder the growth of plants by interfering with the mechanisms that allow them to absorb nutrients and by disrupting the critical microbial populations in the soil that help nutrient metabolism. Moreover, iron contaminants can permeate into groundwater, so polluting sources of drinking water and presenting hazards to human well-being. Extended exposure to high levels of iron in drinking water has been linked to a range of health problems, such as gastrointestinal illnesses and neurological consequences(W. Guo et al., 2015).

Iron rust is a widespread type of iron contamination in the environment, a common result of corrosion on iron-based products, it creates a complicated environmental problem with far-reaching consequences for aquatic ecosystems and infrastructure(Coetser & Cloete, 2005). Rust formation is a chemical process in which iron undergoes oxidation, facilitated by the presence of moisture and oxygen, resulting in the production of different iron oxide compounds(Morcillo et al., 2011). This corrosion is not limited to the surface; instead, it is an ongoing process that consistently releases iron ions into the environment, especially into water systems through runoff or direct contact(S. Guo et al., 2019). The introduction of iron into aquatic habitats can have a profound impact on the chemistry of water, resulting in disturbances in the cycling of nutrients and subsequent ecological imbalances(Rabajczyk & Namieśnik, 2014). High levels of iron can hinder the development of aquatic plants and algae, which in turn disrupts the food chain and reduces the quality of the environment for different animals, such as fish and invertebrates. Moreover, the presence of excessive iron in aquatic environments offers a direct and immediate danger to the survival and well-being of aquatic organisms, impacting their physiological functions and ability to reproduce successfully (Kimbell et al., 2020).

Microorganisms have developed defense mechanisms, such as intracellular bioaccumulation, extracellular precipitation, and alterations in efflux pumps, to survive in severe environments characterized by high exposure to a high concentration of heavy metals(Jin et al., 2018). The microorganism possesses the capacity to neutralize toxic metal ions by either converting them into a state with zero valence or forming metal nanoparticles(Iravani & Varma, 2020). This ability allows for the efficient removal of harmful chemicals from wastewater and other polluted sources, as well as the production of nanoparticles with potential applications in various biomedical fields.

Iron oxide nanoparticles (NPs) possess many potential benefits over other nanoparticles due to their unique physiochemical qualities, including low toxicity, strong catalytic activity, small sizes, and various physical features(Sun et al., 2014). iron oxide, similar to other metal oxide molecules, has been discovered to have antibacterial properties. An advantage of using inorganic oxides such as iron oxide as antibacterial agents is that they include important mineral components for humans and show strong effectiveness even in small amounts. Iron oxide nanoparticles demonstrate potent antibacterial efficacy against a broad spectrum of bacteria. The antimicrobial activity of IONPs is believed to be influenced by various mechanisms. Among these mechanisms, the production of Reactive Oxygen Species is considered to be the most significant reason for the microbicidal effect of IONPs. Therefore, using IONPs demonstrates promising antimicrobial activity(Gudkov et al., 2021).

In the 19th century, Methylene Blue became essential in a wide range of sectors, including textiles, printing, and the production of coloring agents(Schirmer et al., 2011). Its adaptability also extends to modern fields, where it plays important roles in medical diagnostics, histological staining procedures, and cutting-edge research projects. Methylene blue is a reliable and valuable tool in the field of medicine. It helps to clearly identify biological structures during microscopic examinations and is used as an additional diagnostic method in procedures such as sentinel lymph node mapping(Oz et al., 2011).

However, the widespread presence of methylene blue also gives rise to significant environmental concerns. Although it is extremely useful, its ability to dissolve in water and its resistance to breaking down make it a long-lasting pollutant in aquatic

ecosystems(Oladoye et al., 2022). The release of effluents containing methylene blue from diverse industrial operations into rivers and streams presents a substantial risk to environmental well-being. Methylene blue has the potential to disturb the natural ecological processes and pose a threat to aquatic plants and wildlife in water habitats. Furthermore, extended exposure to high levels of methylene blue has been linked to negative health consequences in both people and animals, including skin irritation and more serious physiological disruptions(Dabhane et al., 2021).

This work investigates the use of rust, a widely available and inexpensive supply of iron, as a starting material for the manufacturing of iron oxide nanoparticles using Bacterial isolates. Our objective is to utilize rust to establish a sustainable and cost-effective method for manufacturing IONPs of superior quality. This method not only tackles the environmental impact caused by rust but also utilizes the metabolic powers of bacteria to aid in the creation of nanoparticles. This project involves the use of the bacterial strains that were isolated from the highly rust-contaminated soil as a bio-reducing agent to produce iron oxide nanoparticles. The particles will then be characterized using a UV-visible spectrometer, zeta-potential, and FTIR analysis. This study also involves the inhibitory effect of iron oxide nanoparticles on gram-positive bacteria (*Brevibacillus brevis*) and gram-negative (*Escherichia coli*) and the decolorization effect on methylene blue that is the most commonly used dye for the colorization of fabrics. The utilization of iron nanoparticles to decolorize methylene blue offers a promising solution for mitigating water pollution produced by synthetic dyes. This procedure utilizes the catalytic characteristics of iron nanoparticles to effectively decompose methylene blue molecules, disintegrating their chromophore groups and eradicating their coloration. Iron nanoparticles function as efficient remediation agents by facilitating electron transfer processes and surface interactions. They provide a cost-effective and environmentally sustainable method for cleaning wastewater that is contaminated with methylene blue and other organic dyes.

CHAPTER 2

LITERATURE REVIEW

2.1 Biogenic Synthesis of Nanoparticles

The biological synthesis of Nanoparticles holds immense potential as environmentally conscious as it is sustainable, cost-effective, produces minimal waste, and has low production costs. The main disadvantage that chemical and physical methods of nanoparticle synthesis hold is that they are not eco-friendly and this disadvantage has been conquered by the biogenic approach of nanoparticle synthesis, additionally the chemically synthesized nanoparticles have poor biomedical applications as a result of their low biocompatibility and toxicity. The biosynthesis of nanoparticles can be carried out using bacteria, fungi, algae, and plants. The biosynthetic process involves the reduction of metal ions into metal nanoparticles. The compounds present in microbes act as stabilizing and reducing agents for the biogenic synthesis of nanoparticles. The microorganism provides a benefit over plant-mediated synthesis of nanoparticles i.e. they can reproduce easily when compared with plants. Microbes in the presence of metal oxides can synthesize metal nanoparticles as well as can be used for the bioremediation of heavy metals this synergy is due to the ability of microorganisms to tolerate the high concentration of heavy metals because of the presence of intracellular and extracellular proteins that mediate the adsorption and chelation of metal(Gomaa, 2022). The synthesis of metallic nanoparticles can occur either within the microbial cell or using the supernatant of the microbial cell which consists of different proteins, enzymes, and polysaccharides of the microorganism that act as reducing agents and are responsible for the reduction of metal ions into metal nanoparticles.

2.2 Synthesis of Nanoparticles Within the Cell.

The intracellular synthesis of nanoparticles involves the reduction of metal ions into metal nanoparticles or metal oxide nanoparticles within the cell. The positively charged metal ion binds to the negatively charged polysaccharide and protein of the cell wall of the microbe and subsequently enters inside the cell via the magnesium or manganese transport chain. NADPH-dependent reductase is responsible for reducing metal ions into elemental atoms. Further, the formed nanoparticles accumulate inside the cell and are stabilized within via various proteins, amino acids, and peptides, thus the intracellular synthesis of nanoparticles involves three steps; trapping of metal ion onto the cell wall of microorganism, bio-reduction by enzymes present in the cytoplasm, and capping and stabilization by organic molecules within the cell(Fariq et al., 2017). The nanoparticles produced within the microbial cell are small as compared to the nanoparticles formed extracellularly. Alfryyan et al. 2022 performed the biosynthesis of Ag nanoparticles using *Bacillus cereus.* The biosynthesis was carried out using both extracellular and intracellular approaches. Further characterization of nanoparticles synthesized by both approaches revealed that Ag nanoparticles synthesized using the intracellular approach were found to exhibit spherical morphology with a size of 45.4 nm while Ag nanoparticles synthesized using the extracellular approach were 90.8 nm in size. Thus, their results revealed that nanoparticles synthesized using the intracellular approach are smaller in size as compared to those synthesized using the extracellular approach(Alfryyan et al., 2022). Bacteria are most commonly used for the biosynthesis of nanoparticles because of their ability to grow fast, they can be easily handled and manipulated. Rajeshkumar S et al. 2013 carried out the extracellular and intracellular biogenic synthesis of Ag nanoparticles using *Vibrio alginolyticus,* which is a marine bacterium. In the intracellular synthesis approach the precursor salt, i.e. Silver Nitrate was directly added to the culture broth which further entered inside the cell where the silver ions were reduced to silver nanoparticles and a change in the color of the culture broth was observed whereas, in the extracellular synthesis, the precursor salt was added in the supernatant and color change was observed which

confirmed the bio-reduction of silver ions into silver nanoparticles by the enzymes, proteins, polysaccharides of bacteria present in the supernatant. The formed Ag nanoparticles had a size range of 50-100nm. They observed that the biosynthesis of Ag nanoparticles was completed within 4 hours in the intracellular synthesis approach while the complete reduction of silver ions took 12 hours in the extracellular synthesis approach. Further, they concluded that nanoparticles synthesized via the extracellular approach were more stable as compared to those synthesized via the intracellular approach(Rajeshkumar S et al., 2013). Jain et al. 2020 biosynthesized ZnO nanoparticles using a bacterium, *Serratia nematodiphila* which is a zinc-tolerant bacterium. The biosynthesis was carried out using the intracellular approach in which the zinc sulfate was directly added to overnight grown culture which was further reduced to ZnO nanoparticles. Further characterization of the formed ZnO nanoparticles was characterized to be spherical in shape and had a size range between 15-30 nm(D. Jain et al., 2020).

2.3 Synthesis of Nanoparticles Using the Supernatant of Microbial Cell

Synthesis of nanoparticles can also be done using the cell-free extract or using the supernatant of the microbial cell which consists of different enzymes, polysaccharides, and proteins of the microbial cell wall that serve as a reducing agent for the reduction of metal ions into metal nanoparticles. Some enzymes such as NADH-dependent enzymes which are released into the culture medium as extracellular enzymes serve in the reduction of metal ions. The proteins, peptides, and other organic molecules found in the supernatant which is released by the microbial cell serve as a stabilizing and capping agent for the synthesized nanoparticles(Murali et al., 2023). Bacteria can successfully be employed for the extracellular synthesis of nanoparticles. Saravanan et al. 2017 carried out the biosynthesis of Ag nanoparticles using *Leuconostoc lactis* secreted exopolysaccharide. They removed the biomass and the exopolysaccharide secreted by the bacteria was used for the biosynthesis. The EPS was used for the purpose of bio-reduction of Ag^{+} ions, stabilization, and capping of Ag nanoparticles The EPS was added to distilled water, silver nitrate was added

and the solution was incubated in a dark condition. A visible change in color from a colorless solution to a yellow color was observed which indicated the fabrication of silver nanoparticles. The silver nanoparticles underwent characterization using multiple techniques, revealing, an average size of 35nm. They concluded that exopolysaccharide-mediated synthesis of Ag nanoparticles is an easy, efficient, and environmentally friendly process and can be employed in the textile industry in the removal of dye(Saravanan et al., 2017). Wang et al. 2022 biosynthesized Ag nanoparticles using a probiotic. They used kimchi which is a fermented food product for the isolation of probiotic strains for the biosynthesis of Ag nanoparticles. *Bacillus sonorensis* MAHUQ-74 strain was isolated and was utilized for the bio-reduction of metal ions to a zero-valent state. The supernatant of the culture was separated from bacterial biomass using centrifugation and to the supernatant silver nitrate was added. Solution color changes to deep brown from yellow color indicating the reduction of Ag^{+} ions to Ag nanoparticles, further characterization of nanoparticles using various techniques revealed that the Ag nanoparticles had a size range of 13-50nm. The biosynthesized nanoparticle exhibited potent antibacterial activity against different strains of *E. coli O15:H7*(Wang et al., 2022).

In another study, *Bacillus subtilis* which was isolated from the rhizosphere soil was used for the synthesis of iron oxide nanoparticles. Isolated bacteria were then inoculated into LB broth. The supernatant of the culture was separated from bacterial biomass using centrifugation and in the supernatant aqueous solution of 2 mM Fe_2O_3 was added. The color of the solution changes from brick red to dark brown indicating the conversion of Fe_2O_3 into Fe_3O_4 nanoparticles. Characterization of nanoparticles further revealed that iron oxide nanoparticles have a size range of 60- 80 nm(Sundaram et al., 2012).

2.4 Iron Oxide Nanoparticles from the Rust

The overutilization of iron metal has resulted in the accumulation of thousands of tonnes of scrap and rusted iron in garbage yards, with no viable option for reusing or deriving any benefits from it thus far. This has created a hazardous

environmental problem in numerous places worldwide. majorly in water bodies where it accumulates in very high concentrations affecting the nutrient cycle, water chemistry, and ecological balance (Rabajczyk & Namieśnik, 2014). The utilization of rust in nanoparticle production has great potential for environmental remediation. The bio-synthesis of iron oxide nanoparticles using biological components has several advantages, including cost-effectiveness, simplicity, non-toxicity, elimination of hazardous substances, easy availability, and the ability to remove toxic chemicals.

In a study, Iron oxide nanoparticles were synthesized using a chemical reaction involving the combination of hot red pepper and waste rust iron extract at a temperature of 300°C for 1.5 hours. There were various biochemical molecules found in the pepper that helped in the conversion of rust into iron oxide nanoparticles. The synthesized nanoparticle has a size range of from 27.5 to 29.2 nm having a cubical structure. Also, these iron oxide nanoparticles were checked for antibacterial properties. The efficacy of prepared by combining chili with rust iron extract surpassed that of nanoparticles prepared just from rust iron extract. These nanoparticles are not only interacting with cell walls but also penetrate inside the bacteria. When compared to gram-negative bacteria they showed the highest rate of distraction in gram-positive bacteria(Abid & Kadhim, 2022). In another study, iron oxide nanoparticles were synthesized via a chemical method from rust using the rose plant extract. The synthesized nanoparticle has a size of 79.59 nm and also showed effective antifungal activity(Abid et al., 2021).

Utilizing bacteria to synthesize nanoparticles from rust is a novel approach. this novel technique harnesses the natural abilities of bacteria to produce nanoparticles with precision and efficiency. By leveraging the metabolic pathways of bacteria, the synthesis of nanoparticles with tailored properties, ranging from size and shape to surface chemistry. This remarkable approach not only offers a sustainable alternative to traditional synthesis methods but also unlocks new opportunities in diverse fields such as medicine, electronics, and environmental engineering.

2.5 Anti-microbial Activity of Iron Oxide Nanoparticles

Iron oxide nanoparticles (IONPs) have gained considerable interest in recent years because of their potential antibacterial capabilities. These nanoparticles possess intrinsic antibacterial properties, which make them promising candidates for a range of medicinal and environmental applications.

The primary reason for the antibacterial effectiveness of IONPs is their capacity to produce reactive oxygen species (ROS) upon contact with biological systems. ROS, such as hydroxyl radicals (•OH) and superoxide radicals (O_2•$^-$), are extremely reactive chemicals that are recognized for their harmful impact on microbial organisms(AlMatar et al., 2017). ROS, when they come into contact with bacterial cell membranes, cause lipid peroxidation, protein denaturation, and DNA damage, which ultimately results in the death of the cell(Kohanski et al., 2010).

Moreover, the compact dimensions and expansive surface area of IONPs intensify their interaction with microbial cells, promoting the infiltration of nanoparticles into the cell membrane and subsequent interference with cellular functions. IONPs possess a distinctive characteristic that allows them to effectively combat various types of bacteria, fungi, and even viruses (Gudkov et al., 2021). In a study, iron oxide nanoparticles were synthesized using *Lagenaria Siceraria*. Synthesized nanoparticles were cubical in shape, stabilized, and 30-100nm in size range. The antimicrobial activity of these nanoparticles was investigated against *Staphylococcus aureus* and *E. coli*. The zone of inhibition for *S. aureus* was 8 mm and for *E. coli* it was 10mm, iron oxide nanoparticles showed effective anti-bacterial properties(Kanagasubbulakshmi & Kadirvelu, 2017). In another study, iron oxide nanoparticles were synthesized via *Laurus nobilis*. The synthesized nanoparticles were 8.03 ± 8.99 nm in size and hexagonal in shape. The anti-microbial activity of nanoparticles was evaluated against bacteria and two fungi. The synthesized nanoparticles were assessed for their antibacterial efficacy against three bacteria and two fungi. The findings indicated that the nanoparticles had a modest level of effectiveness against the Gram-positive bacterium *Listeria monocytogenes*, as well as the fungus *Penicillium spinulosum* and *Aspergillus flavus*(Jamzad & Kamari Bidkorpeh, 2020)

2.6 Decolorization of Methylene Blue

Methylene blue is a type of dye that is commonly employed in biological and chemical processes. It belongs to the category of heterocyclic aromatic dyes(Nazir et al., 2020). Iron oxide-mediated degradation of the dye is linked to the decomposition of the chromophoric group in methylene blue, resulting in the conversion of the dye into smaller by products. IONPs were synthesized via *Syzygium aromaticum* and were utilized for the decolorization of methylene blue from the aqueous solution. These nanoparticles were effective in decolourizing 92% of methylene blue within 15 minutes(A. Jain et al., 2021).

In a study, iron nanoparticles synthesized via endophytic fungi, *Penicillium oxalicum* were used for the decolorization of methylene blue in the existence of hydrogen peroxide. Free radicals were generated by the nanoparticle in the existence of hydrogen peroxide, these free radicals broke down the azo bonds that are present in methylene blue. These nanoparticles were able to decolorize 99.17% of methylene blue within 6 hours of treatment(Mathur et al., 2021). Iron nanoparticles function as efficient remediation agents by facilitating electron transfer processes and surface interactions. They provide a cost-effective and environmentally sustainable method for cleaning wastewater that is contaminated with methylene blue and other organic dyes.

CHAPTER 3

EXPERIMENTAL

3.1 Material and Method

- The soil sample was randomly collected from the contaminated site and then mixed to create a single composite sample.
- The sterile plastic bag was used for the transportation of soil samples to the laboratory.

  All the visible debris, rocks, and roots were removed from the sample.
- Bacteria employed for the synthesis of IONPs were isolated from the contaminated soil sample.
- Scrapes for the iron rust were collected from a site at Delhi Technological University. Distilled water was utilized for the washing of glassware and solution preparation.

3.2 Isolation of Bacteria

To isolate bacteria from the soil sample, serial dilution was conducted. Serial dilution was performed to get separated colonies on nutrient agar plates.

- Ten test tubes were obtained and 9 ml of saline solution was added to every tube. These tubes were then sealed with cotton plugs and subjected to autoclaving.
- The dilution tubes were labeled with numbers representing the dilution factor, ranging from 10^{-1} to 10^{-10}, and were organized in increasing order of dilution.
- Under sterile conditions, 1g of soil was mixed aseptically transferred to the tube labeled as 10^{-1}.
- The tube containing the 10^{-1} dilution was vigorously mixed using vortexing. 1 milliliter of solution was transferred from a tube with a dilution ratio of 10^{-1} to

a tube with a dilution ratio of 10^{-2}. The original culture has been diluted at a ratio of 1:100.

- Similarly, a sequence of dilutions was created, spanning from 10^{-1} to 10^{-10}.

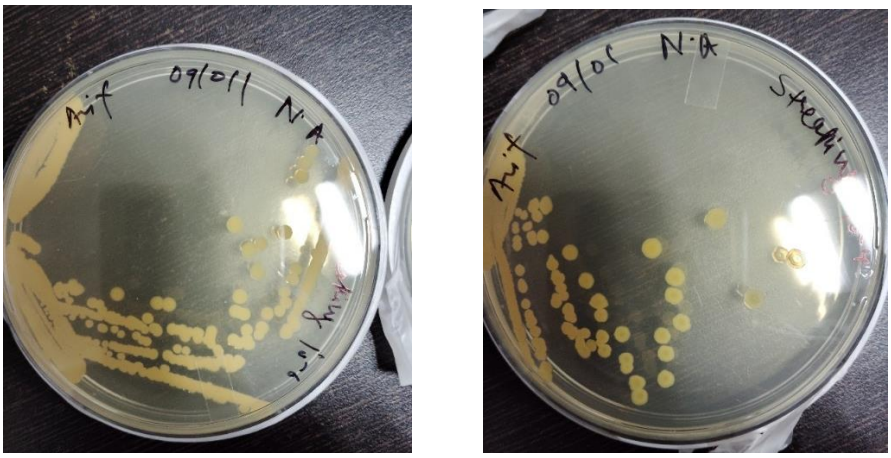
Fig. 3.1 Serial dilution test tubes consisting of a sequence of Dilutions ranging from 10^{-1} to 10^{-10}.

3.2.1 Media Preparation

- Nutrient agar media was prepared as it promotes the growth of a wide spectrum of bacteria (non-fastidious).

- 2.8 gm nutrient agar media was mixed with 100 ml distilled water in a sterile conical flask. And after that media was sterilized via autoclaving for 15- 20 minutes.
- Once the media was autoclaved it was cooled down (45-50°C) and poured into a sterile petri plate.
- from the serially diluted test tubes, 0.1ml of the sample was transferred to the nutrient agar plate with the pipette.
- Nutrient agar plates were then incubated for a period of 24- 48 hours at 37°C, and the plates were inspected for the presence of any type of growth on the media.

- Two types of distinct colonies were visualized in the nutrient agar plates. From the nutrient plates, these colonies were streaked into sterile nutrient plates and then further incubated at 37 °C.



Strain 1 Strain 2

Fig. 3.2 Two Bacterial isolates, were isolated from the collected soil sample from the iron-contaminated site.

3.3 Checking the Ability of Bacteria to Synthesize Iron Nanoparticles.

- 150 ml of LB broth was prepared by adding 3gm of LB broth powder into 150 ml of sterile distilled water and then autoclaved for sterilization.
- Once the broth was cooled down it was divided into 2 equal parts in sterile conical flasks. 2 bacteria that were isolated from the contaminated sites were inoculated in the LB broth. Then broth was incubated for 24 hours at a temperature of 37°C.

Fig. 3.3 Inoculation of 2 bacterial isolates to the Lysogeny Broth.

- Centrifugation of Bacterial broth at 10,000 rpm was done after the completion of the incubation period for 10 minutes, the pellet was discarded and the supernatant was transferred into a sterile conical flask.
- 50 ml of broth was used for the synthesis of nanoparticles from $Fecl_3$. 0.5g $FeCl_3$ was added as a metal salt in 50 ml broth containing the supernatant. 15 ml of broth without the addition of salt was taken as the control.

Fig. 3.4 Conical flask containing (a) Control containing only supernatant, (b) and (c) supernatant of two different bacteria containing $FeCl_3$ as a metal salt, (d) Control containing supernatant of another bacterial strain.

- Subsequently, the control and salt-containing supernatants were incubated overnight at a temperature of 37°C. The next day color of the supernatant was changed. Color changing is an indication of nanoparticle synthesis. Thus, the color change clearly indicates a clear sign of nanoparticle fabrication.
- Then centrifugation was carried out for the isolation of nanoparticles from the supernatant, supernatant was centrifuged at 10000 rpm for 5 minutes. The supernatant was removed and the pellet was washed with distilled water twice.

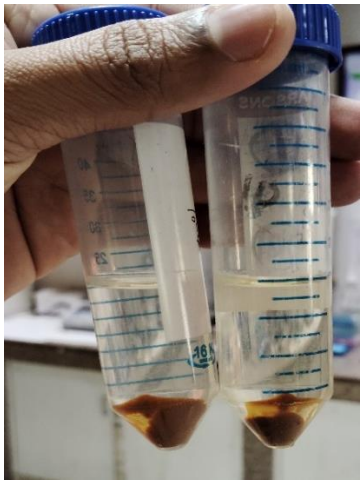


Fig. 3.5 Pellet containing Iron oxide nanoparticles.

- After the washing pellet was shifted into a glass plate and dried in a hot air oven for 2 hours at 80°C. fine powder was produced which was stored in the fridge.

3.4 Utilization of Rust for the Synthesis of Nanoparticles

3.4.1 Iron Rust Preparation

- Scrap iron that was heavily rusted was collected from a site at Delhi Technological University. Iron scraps were washed with distilled water to eliminate impurities such as dust particles, soil particles, and water-soluble impurities.
- Scraps were then kept for drying. After drying these iron scraps were broken down into smaller pieces with the help of a mortar pester. All the bigger particles of irons were removed and then the powdered part was added to the beaker containing hot water (100 °C).

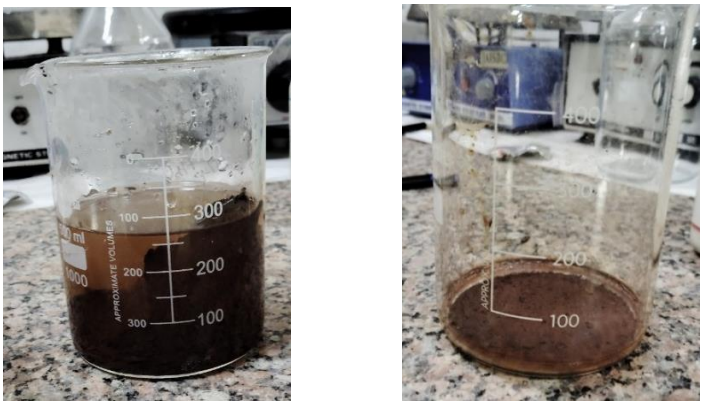


Fig. 3.6 Addition of rust into the Hot Distilled water and then settling of rust at the bottom.

- After 10 minutes supernatant was transferred into another beaker. All the rust was settled down after some time.
- Carefully, supernatant will be discarded, and collected rust at the bottom of the beaker will be transferred into a glass plate and kept for 2-3 hours at 60°C in a hot air oven. after the drying of rust, fine powder was produced.

Fig. 3.7 Dried powder of rust obtained after drying in a Hot air oven for 2h.

3.4.2 Preparation of Nanoparticles

- 200 ml of LB broth was prepared by mixing 4 gm of LB broth powder in 200 ml distilled water in a sterile conical flask.
- LB broth was then autoclaved for sterilization and once it got cooled down it was inoculated with bacteria that have higher efficacy for nanoparticle synthesis.

- Then inoculated LB broth was kept at a temperature of 37°C for 24 hours in the incubator. After 24 hours LB broth becomes translucent(cloudy) as an indication of bacteria growth.
- LB broth was centrifuged for the separation of bacterial cells from the broth and a supernatant was used for the nanoparticle synthesis.
- Bacterial supernatant contains the various components that are secreted by the bacteria such as proteins, enzymes, and polysaccharides that reduce spent iron into iron oxide nanoparticles.
- Broth was centrifuged at 10000 rpm for a period of 10 minutes; the pellet was discarded and the supernatant was transferred into a sterile conical flask. 2 gm rust powder was added into the supernatant and then incubated overnight at a temperature of 37°C.

Fig. 3.8 Conical flask containing (a) Control containing only supernatant, (b) and (c) supernatant of two different bacteria containing rust, (d) Control containing supernatant of another bacterial strain

- During the incubation periods the color of the solution changes, which is a clear indication of nanoparticle synthesis.
- It was then centrifuged for the separation of iron nanoparticles. The solution was centrifuged at 10000 rpm for 5 minutes, the supernatant was removed and the pellet was washed with distilled water twice.
- After the washing step, the pellet was then transferred to a glass plate and kept for drying in a hot air oven at 90°C for 2 hours. Once nanoparticles were dried it was then transferred into Eppendroff for storage and kept in the fridge.

3.5 Characterization of Bacteria

3.5.1 Gram Staining

Gram staining is a key technique employed in microbiology to categorize bacteria into two primary categories according to variations in their cell wall composition. Hans Christian Gram created the procedure in 1882, which continues to be extensively employed in microbiology laboratories across the globe as one of the most prevalent staining techniques(D. Badar et al., 2022).

3.6 Characterization of IONPs

3.6.1 Colour Change of the Solution

The change in color of a solution is a fundamental characteristic of nanoparticles created through biogenic synthesis. Bacterial supernatants used as bioreactors for nanoparticle synthesis reduce metal ions, leading to the formation of nanoparticles. The process of reduction often results in a discernible change in the color of the reaction mixture. The addition of rust and $FeCl_3$ to the flask containing supernatant causes a noticeable change in the color of the reaction mixture, indicating the formation of iron oxide nanoparticles.

3.6.2 UV-Vis Spectroscopy

UV-Vis spectroscopy is a widely used and useful method for characterizing nanoparticles, particularly for investigating their optical characteristics. UV-Vis spectroscopy determines a sample's electronic structure and composition by measuring how much ultraviolet and visible light it absorbs(Karami et al., 2016). When nanoparticles are exposed to UV or visible light, they engage with electromagnetic radiation in a manner that depends upon their size, shape, and composition. The contact may lead to the production of absorption peaks or

bands in the UV-Vis spectrum, which can be examined to derive significant information about the nanoparticles.

To get an absorption spectrum, we dissolved the iron oxide nanoparticles in a solution of weak sulfuric acid and measured the level of absorbance over a range of wavelengths from 300 to 800 nm using the Eppendorf Bio-Spectrometer.

3.6.3 Zeta- Potential

The zeta potential is an essential feature for studying the stability and behavior of nanoparticles in colloidal environments. It denotes the electrostatic potential at the boundary between a nanoparticle and a liquid medium. It quantifies the strength of the repulsion or attraction between nanoparticles, which affects their dispersion, aggregation, and interaction with nearby molecules or surfaces(Ji, 2014).

Nanoparticles having a zeta potential exceeding +30 mV or falling below -30 mV exhibit significant cationic or anionic properties, respectively. As a result, they experience sufficient repulsion to prevent aggregation and maintain stability. When the value falls between the range of -10 mV to +10 mV, it is classified as neutral and indicates that the particles have a high tendency to agglomerate. When the results fall between the range of ±10 mV and ±30 mV, it suggests intermediate stability and there is a possibility of aggregation under certain circumstances(Clogston & Patri, 2011). The stability of iron oxide nanoparticles was evaluated using a zeta potential (ZP) analyzer, namely the Malvern Zetasizer.

3.6.4 FTIR

FTIR spectroscopy is employed to determine the specific functional groups present in biomolecules, hence providing insight into their composition. The spectral region typically utilized is the Mid-Infrared range, which spans from

4000 cm^{-1} to 200 cm^{-1}. Infrared radiation induces various forms of atomic vibration, including stretching and bending of chemical bonds. Therefore, these vibrational signals are identified and transformed into an FTIR spectrum, which represents the percentage of transmittance vs the wavenumber in cm^{-1}.

The FTIR spectrum consists of two primary sections, namely the fingerprint region, which spans from 1200 cm^{-1} to 400 cm^{-1}. This area is unique to the biomolecule and plays a significant role in characterizing and identifying the molecule. The spectral range between 4000 cm^{-1} and 1200 cm^{-1} has well-defined peaks corresponding to specific functional groups. An example of this is a wide peak centered about 3400 cm^{-1}, which is a distinct signal indicating the O-H stretching mode of alcohols, hence verifying the existence of an alcohol group. The width of a peak is indicative of the polarity and hydrogen bonding between the atoms. FTIR spectroscopy can be employed for nanoparticle characterization due to the unique optical properties shown by their surface(Eid, 2021).

The characterization of iron oxide nanoparticles was conducted using a PerkinElmer Fourier transform infrared (FT-IR) spectrometer with a wavelength range of 400 to 4500 cm^{-1}.

3.7 Evaluating the Antimicrobial Activity of IONPs

The antibacterial potential of IONPs manufactured using Bacterial Culture was evaluated against two bacteria, *Brevibacillus brevis*, and Escherichia coli. A simple disc diffusion experiment was conducted to evaluate the antibacterial activity of synthesized iron nanoparticles. The agar disc diffusion method is the main technique used to assess the antimicrobial properties of nanomaterials. It is crucial to note that this is only appropriate for materials that may undergo diffusion. The agar disc diffusion test is a quantitative, simple, and straightforward procedure(Borcherding et al., 2014).

Procedure:

- Nutrient Agar plates have been prepared. Two plates were streaked with *E. coli* (a gram-negative bacterium), while the other two plates were streaked with *Brevibacillus brevis* (a gram-positive bacterium).
- A disc was made containing a 3% solution of (IONPs) and a 400ug/ml solution of Rifaximin. Positioned the disc on each plate and subjected it to incubation at a temperature of 37°C for a time period of 24 hours.
- The scale was used to measure the diameter of the zones of inhibition. The zone of inhibition was recorded in mm.

3.8 Nano Decolorization of Methylene Blue

The photocatalytic degradation activity of iron nanoparticles has been evaluated by observing the degradation of methylene blue (MB) when exposed to sunlight. 25mg of nanoparticles were dispersed in 5 ml of distilled water. 2mg methylene blue was dissolved in 100ml of distilled water and 1ml prepared nanoparticle solution was dissolved in the dye solution.

The experimental setup was exposed to sunlight for 2 hours in order to observe the dye's color change. The resulting solution was observed using a UV- Vis spectrometer at the wavelength of 680 nm, and the percentage of dye removal was determined by the formula:

$$\textbf{Dye removal (\%)} = (C_0 - C) / C_0 \times 100 \qquad \textbf{(3.1)}$$

Here, C_0 is the beginning absorbance of dye when the dye solution was prepared, and C is the end absorbance when the solution of dye was treated with nanoparticles and exposed to the sunlight(Dharshini et al., 2023).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Evaluating the Ability of Bacteria to Synthesize Iron Nanoparticles Using $FeCl_3$ as a Metal Salt

While evaluating whether the bacteria isolated from a highly contaminated site containing rust and iron are able to synthesize the iron oxide nanoparticles or not were evaluated. The first sign that indicates a clear sign that nanoparticle synthesis has taken place is the change in the color of the solution. The flask containing the supernatant of the two bacterial isolates was taken and in both the flasks FeCl3 metal salt was added to manufacture iron oxide nanoparticles. Following the incubation period, there was a significant change in the colour of the solution indicating the supernatants of the bacterial isolates are suitable to be used as a reducing agent for the synthesis of nanoparticles. Fig.4.1 represents the change in the color of the solution after the incubation period. Centrifugation was done to isolate the formed nanoparticles which were subsequently dried in a hot air oven for 2-3h.

Fig. 4.1 Color change of the supernatant containing metal salt observed after 24 hr of the incubation period.

Fig. 4.2 Powder of Iron oxide nanoparticles obtained after drying in a Hot air oven for 2h.

4.2 Utilization of Rust for the Synthesis of IONPs

The rust powder was utilized as a metal salt for the manufacturing of IONPs. After evaluating the ability of bacterial supernatant to synthesize IONPs from $FeCl_3$ as a metal salt. The bacterial supernatant was employed for the synthesis of IONPs utilizing the supernatant of the bacterial strain. The rust was introduced to the supernatant and was incubated overnight. After several rounds of washing the pellet of IONPs was obtained and was allowed to dry in a hot air oven. Fig represents the powder of iron oxide nanoparticles after drying.

Fig. 4.3 Manufacturing of IONPs by rust and utilizing the bacterial supernatant for reducing the metal ion.

4.3 Characterization of Bacteria

Gram staining revealed the presence of rod-shaped bacillus in chains, which were stained purple. This confirmed that the colonies produced on the nutrient agar were composed of a gram-positive bacterium.

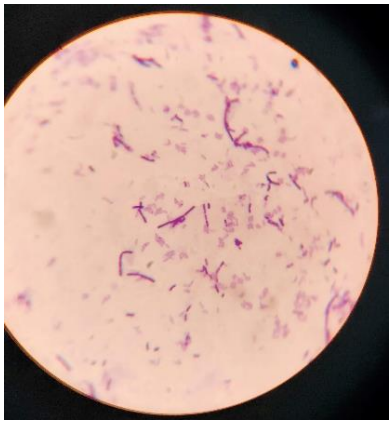


Fig. 4.4 Gram Staining of isolated Bacteria.

4.4 Characterization of Iron Oxide Nanoparticles

Identification and evaluation of IONPs were done by various chemical methods such as solubility studies and characterization of nanoparticles was done by UV-Vis Spectrophotometry, solubility test, FTIR, and Zeta potential(Devi et al., 2019).

4.4.1 Color Change

When the rust powder was added to the supernatant of bacteria and was incubated overnight a significant color change was observed which is a clear sign indicating the synthesis of iron oxide nanoparticles by the enzymes and proteins of bacteria present in the supernatant.

Fig. 4.5 A visible color change of the solution following the incubation period.

4.4.2 Solubility Studies

Observed the solubility of iron nanoparticles in water, alcohol, acid, and alkali by introducing a small quantity of the sample into a test tube and adding a few ml of the respective solvent. The mixture was then heated. The iron nanoparticles demonstrated significant solubility in acid (HCl). It exhibited limited solubility in alcohol and NaOH. It exhibited water insolubility.

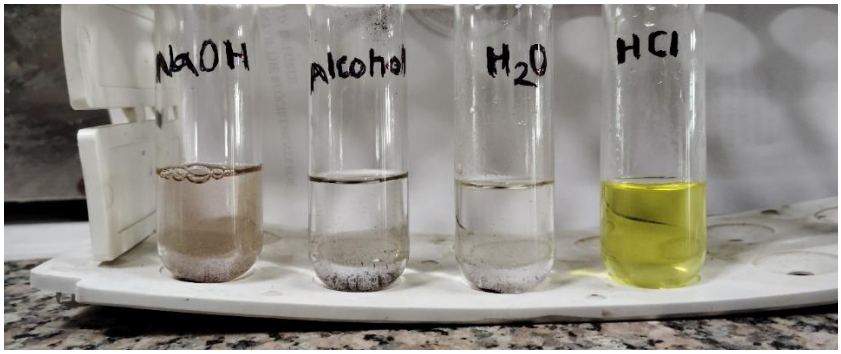


Fig. 4.6 Solubility of iron oxide nanoparticles in (a) Sodium Hydroxide, (b) Alcohol, (c) Water, (d) Hydrochloric acid.

4.4.3 UV- Vis Spectrophotometry

The formation of iron oxide nanoparticles was determined with the help of UV-Vis Spectrophotometry. Bacterial supernatants have a significant amount of various biological compounds. These components help in the formation of iron oxide nanoparticles. The peak absorption was detected at 260nm(S. Khan et al., 2022)(Naz et al., 2019).

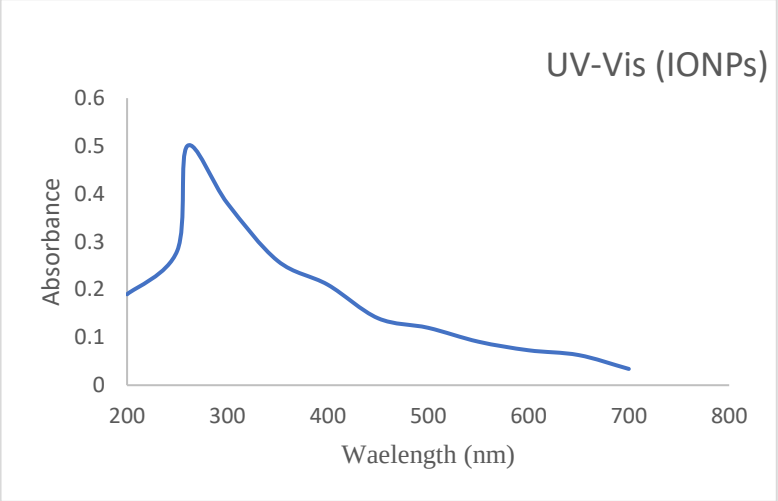


Fig. 4.7 Absorbance spectrum of iron oxide NPs.

The obtained absorption spectrum is displayed in Figure. The presence of IONPs is indicated by the peak around 260nm.

4.4.4 Zeta- Potential

Based on the Zeta potential of – 23.3mV. it can be concluded that Synthetic nanoparticles have a net negative charge on their surface and are moderately stable. Nanoparticles will have electrostatic repulsion between them that hinders immediate aggregation, it is not stable for the long term and tends to aggregate under certain optimal conditions(Iqbal et al., 2020)(Bhuiyan et al., 2020).

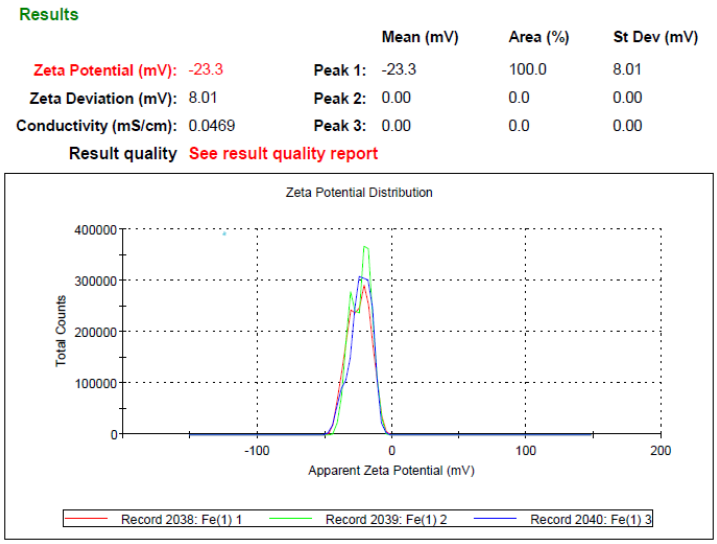


Fig. 4.8 Zeta- potential of (IONPs)

4.4.5 FTIR analysis

The FTIR spectrum is often divided into two regions: the fingerprint region, ranging from 1400 cm^{-1} to 400 cm^{-1}, and the functional group region, ranging from 4400 cm^{-1} to 1400 cm^{-1}. There are two bands observed at 3277 cm^{-1} and 1,625 cm^{-1}, which correspond to the stretching and blending vibrations of water molecules or hydroxyl groups. This signifies the existence of a minimal quantity of water molecules or specific hydroxide groups on nanoparticle surfaces. The discovery was made as a result of synthesizing the product in a water-based solution.

The presence of a distinct peak at 1521 cm^{-1} may be attributable to the stretching of aromatic ring C−C or C−N bonds. The presence of bands at approximately 1022 cm^{-1} indicated the stretching of C−O bonds in alcohols, ethers, or esters. The detection of these groups in the spectra of the nanoparticles suggests that these organic groups are exhibited on nanoparticles surface, potentially as a capping layer on the iron oxide matrix(Bilesky-José et al., 2021).

The presence of prominent absorption peaks at 524 and 435 cm^{-1} can be associated with the vibrations of the Fe–O bond. as stretching vibrations of the metal-oxygen bond can occur in the lower frequency range(Lassoued et al., 2017).

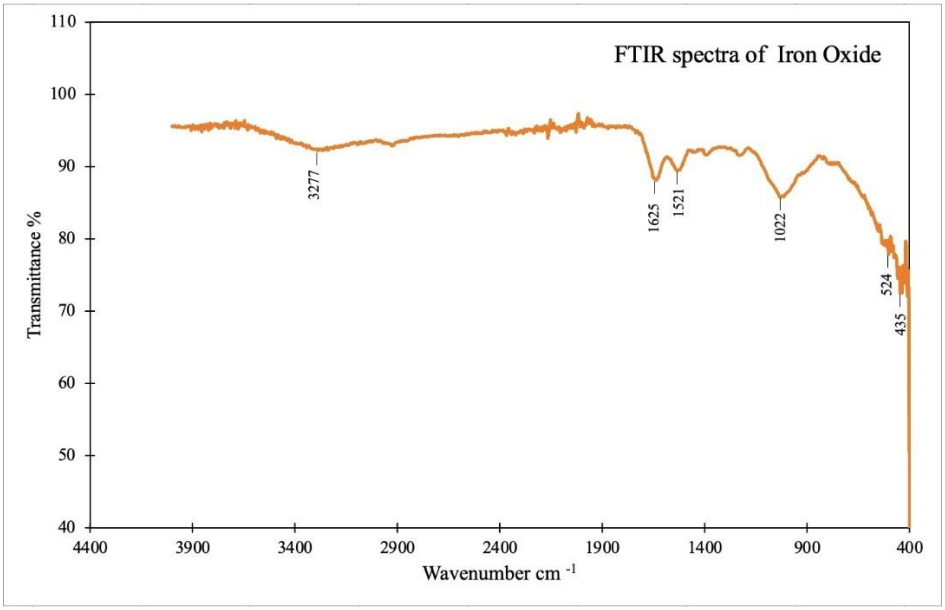
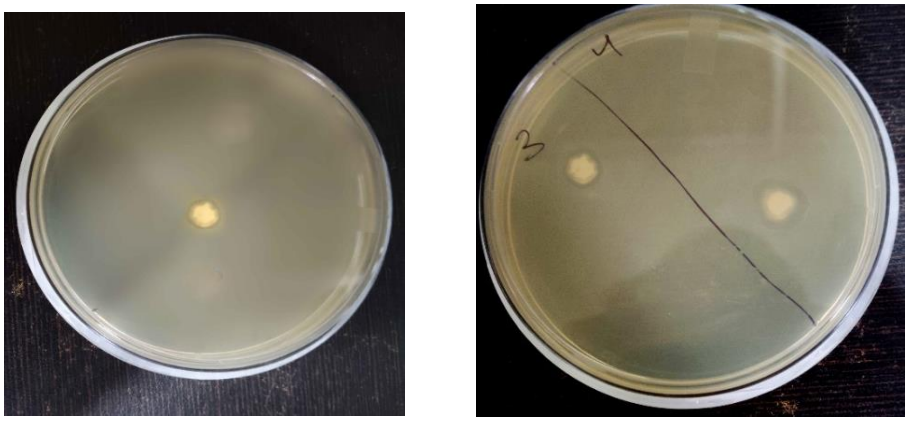


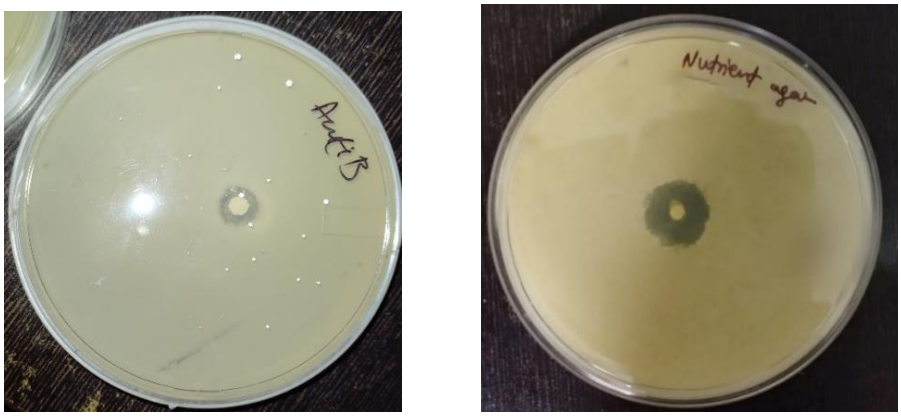
Fig.4.9 FTIR Spectrum of iron oxide nanoparticle.

4.5 Antibacterial Properties of Iron Oxide Nanoparticles

The zone of inhibition (ZOI) for Rifaximin against *E. coli* and *Brevibacillus brevis* were measured at 13mm and 9.5mm, respectively. In contrast, iron oxide nanoparticles (IONPs) exhibited broader inhibition zones, with ZOI of 16.8mm for *E. coli* and 11.5mm for B. brevis. These results indicate that IONPs possess a greater antibacterial effect compared to *Brevibacillus brevis*, particularly evident against *E. coli*.



(a) **(b)**



(c) (d)

Fig. 4.10 Evaluation of antibacterial activity**.**

growth of *Brevibacillus brevis* in the presence of **(a)** Rifaximin **(b)** (IONPs)

E. coli growth in **(c)** Rifaximin **(d)** (IONPs)

Table 4.1 Diameter of zone of inhibition of IONPs measured in the plate of gram-negative & gram-positive bacteria.

	E. coli	*Brevibacillus brevis*
Rifaximin	13mm	9.5mm
(IONPs)	16.8mm	11.5mm

4.6 Nanoparticles-mediated Decolorization of Methylene Blue

The decolorization experiment was carried out according to the procedures mentioned in Chapter 3. After keeping the setup in 2 hours in the sunlight absorbance was measured at 680 nm.

Fig 4.11 Decolorization effect of IONPs

Dye Decolorization (%) = (2.07 – C0.71)⁄2.07 × 100

Dye Decolorization = 65.7%

In this study, the decolonization efficiency of iron oxide nanoparticles was 65.7%. The degradation of the dye is linked to the decomposition of the chromophoric group in methylene blue, resulting in the conversion of the dye into smaller by-products.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

This research highlights the significant potential of using bacteria isolated from contaminated sites to produce nanoparticles from rust in an effective manner. By conducting careful research and analysis, we have proven that these bacterial strains have the natural capability to facilitate the creation of iron nanoparticles. This provides a sustainable and environmentally friendly route for producing nanomaterials. Furthermore, our research has uncovered that the nanoparticles produced by bacterial interaction demonstrate strong antibacterial properties and exceptional effectiveness in degrading methylene blue, a prevalent water contaminant. The adaptability and practical relevance of the synthesized nanoparticles make them interesting candidates for many applications in healthcare, environmental remediation, and industrial processes.

The utilization of bacteria to produce iron nanoparticles from rust is an innovative method with substantial implications for both the scientific and industrial fields. Through harnessing the innate skills of microbes, we may convert rust, a widely found ubiquitous waste product, into valuable nanomaterials that have numerous potential uses. This environmentally conscious synthesis method not only tackles the issue of rust deposition but also provides a sustainable and economically efficient approach to manufacturing nanoparticles in huge quantities. Considering the future, the potential for this technology is exceedingly encouraging. Additional research efforts have the potential to uncover the complexities of bacterial-mediated nanoparticle synthesis, enabling the improvement and streamlining of the process. Through an enhanced comprehension of the molecular mechanisms at play, scientists may optimize several factors, such as bacterial strains, growth conditions, and reaction kinetics, to improve the effectiveness and output of nanoparticle synthesis. Furthermore, investigating the

extensive range of bacteria and their distinct capacities to manipulate metal ions offers a thrilling opportunity for creativity and advancement. Customized methods for synthesizing nanoparticles can be created by utilizing the unique characteristics of distinct bacterial species, in order to fulfill the requirements of diverse applications. This technique not only expands the range of possible nanoparticle products but also encourages interdisciplinary collaboration among microbiology, materials science, and nanotechnology.

The practical applications of iron nanoparticles synthesized from rust through bacterial intervention are extensive and varied, in addition to their contribution to furthering fundamental scientific understanding. Within the field of biomedicine, nanoparticles demonstrate potential for medication transport, imaging, and therapies, providing precise and effective solutions for healthcare obstacles. these nanoparticles have the capacity to reduce pollutants, aid in wastewater treatment, and promote sustainable industrial operations.

As research advances in this area, it becomes evident that the process of synthesizing iron nanoparticles from rust with the help of bacteria has the potential to transform the field of materials science and engineering. By adopting nature-inspired methods, we can discover new possibilities in producing sustainable nanomaterials, leading to a future where innovation aligns with environmental responsibility for the improvement of society.

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Date: 27/5/2024

Final Acceptance Letter

Paper No. B-4182

To,

S. Yadav, A. Khan and J. G. Sharma
Department of Biotechnology, Delhi Technological University,
Delhi-110042, India

Dear Sir/Madam

We are glad to inform you that your paper entitled **"Phytochemistry of Aloe Vera: A Catalyst for Environmentfriendly Diverse Nanoparticles with Sustained Biomedical Benefits"** has been accepted for publication in the scientific research journal *Nature Environment and Pollution Technology* after thorough reviewing, and revision. The paper is likely to come in Vol. 24, No. 1 (March), Year 2025.

Thanking you,

Yours sincerely,

P. K. Goel
Chief Editor

AFJBS African Journal of Biological Sciences

ACADEMIC PAPER ACCEPTANCE LETTER

Dear Author (s),

Arif Khan, Suman Yadav, Jai Gopal Sharma.

Date: 27, April,2024
Paper Id: AFJBS-2024-HR-279

We Respect Your Research Contribution in the ***African Journal of Biological Sciences (ISSN:2663-2187),*** We are pleased to inform you that your article has been Reviewed by respective Editorial experts and standard papers with grammar reports and similarities reports,

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Regards

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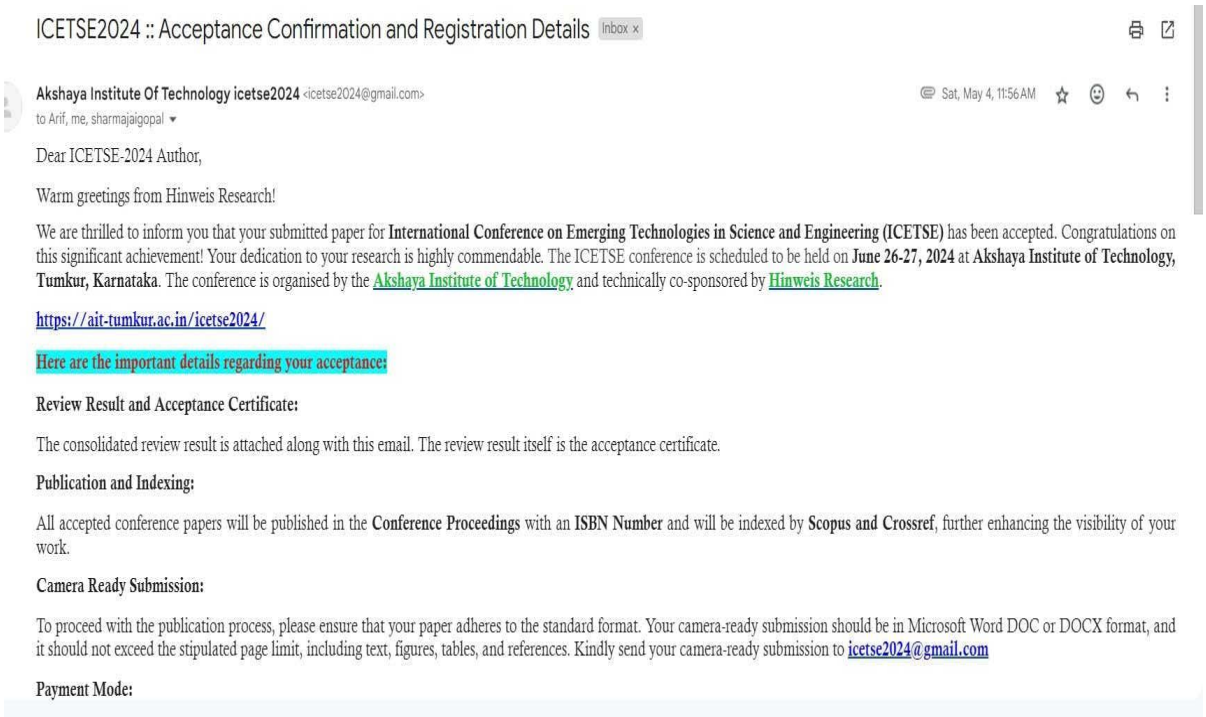

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Akshaya Institute Of Technology icetse2024 <icetse2024@gmail.com> Sat, May 4, 11:56 AM

to Arif, me, sharmajaigopal

Dear ICETSE-2024 Author,

Warm greetings from Hinweis Research!

We are thrilled to inform you that your submitted paper for **International Conference on Emerging Technologies in Science and Engineering (ICETSE)** has been accepted. Congratulations on this significant achievement! Your dedication to your research is highly commendable. The ICETSE conference is scheduled to be held on **June 26-27, 2024** at **Akshaya Institute of Technology, Tumkur, Karnataka**. The conference is organised by the Akshaya Institute of Technology and technically co-sponsored by Hinweis Research.

https://ait-tumkur.ac.in/icetse2024/

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Academic Qualification:

Class	University/ Board	Institution	Percentage/ CGPA	Year of Passing	Remark
Msc	**Delhi Technological University (DTU)**	**DTU Department of Biotechnology**	**Result awaited**	**2024**	
B.Sc	**University of Delhi**	**Bhaskarachary a College of applied Sciences**	**7.703**	**2022**	**passed**
12th	**CBSE**	**UCSKM public school, Bhiwadi, Rajasthan**	**88.4**	**2019**	**Passed**
10th	**CBSE**	**Suraj public school, Bhiwadi, Rajasthan**	**9.2**	**2017**	**Passed**

Accepted review paper

1. A paper entitled “Phytochemistry of Aloe Vera: A Catalyst for Environment-friendly Diverse Nanoparticles with Sustained Biomedical Benefits” has been accepted in Nature Environment and Pollution Technology (NEPT).

2. A paper entitled “Bio Synergize: Microbial Synergy Driving Simultaneous Bioremediation and Nanoparticle Synthesis” has been accepted in African Journal of Biological Sciences (AJBS).

Conference

1. A paper entitled “Insights into the Inhibition Mechanism of Lipoteichoic Acid Synthase (LtaS) Enzyme by Endophytic Fungal Metabolites: A Molecular Docking and Dynamics Simulation Study for Combatting Drug Resistance” has been accepted in International Conference on Emerging Technologies in Science and Engineering (ICETSE) – 2024

Hands-on training/Internship done;

- Instrumentation training done from Ganesh Foundation, kirti Nagar, New Delhi
- Laboratory associate role in Molecular biology laboratory from Biolim Centre for science and Technology.
- Completed practical training for 1 month (June 2023- July 2023) in HPLC.
- Undergone practical training in the microbiology department from Auriga research private limited.

Short-term course done :

- Online Short-Term Certificate Course on "Immunological Techniques and their Applications" organized by the Department of Zoology of Bhaskaracharya College of Applied Sciences (under the aegis of IQAC) in collaboration with the Department of Zoology, University of Delhi from October 22 – November 09, 2021

Workshop attended;

- National Online Workshop on "Astrobiology organized by the Department of Microbiology, Bhaskaracharya College of Applied Sciences under the aegis of IQAC and DBT Star College Scheme in collaboration with Spaceonova, held on October 29-30, 2021.
- participated workshop titled "Analysing Spectrophotometric Data through MS Excel for Quantitative Estimation of Biomolecules' organized by Sukshmjeev Society, Department of Microbiology in association with the Department of Biochemistry on July 5, 2021.
- Participated in the workshop; principles and Application of Spectrophotometry organised by the Department of Biochemistry, Bhaskaracharya College of Applied Sciences on 16th September 2020

Curricular/Extra-Curricular Activity:

- Participated in HPV Kavach – a social awareness campaign against cancers caused by human papillomavirus.
- Participated in Sir Alexander Flemming's birthday quiz organized by PG Department of microbiology, sacred heart college, Tirupati, Tamil Nadu held on 6 august 2021