IMPACT OF NICKEL(II) OXIDE NANOPARTICLE ON BREVIBACILLUS GROWTH FOR BIOREMEDIATION

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE AWARD

OF THE DEGREE

OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

Kaushlendra Kumar

2K21/MSCBIO/18

Under the supervision of:

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Bawana Road, Delhi – 110042

MAY, 2023

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CANDIDATE'S DECLARATION

I Kaushlendra Kumar, Roll Number: 2K21/MSCBIO/18, student of M.Sc. Biotechnology, hereby declare that the work which is presented in the Major Project entitled "**Impact of Nickel(II) oxide nanoparticle on brevibacillus growth for bioremediation**" in the fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, DTU, is an authentic record of my own carried out during the period from Jan to May 2022, under the supervision of **Prof Jai Gopal Sharma**.

I have not applied for any other degree at this or any other University based on the information contained in this report. The following details about the related study have been approved in the SCI/SCI Scopus Index Conference:

Title of the Paper: "Advanced Soil Remediation Technique Combining Chemical Oxidation and Biological Treatment"

Author Names: Manesh Kumar, Kaushlendra Kumar, Jai Gopal Sharma

Name of Conference: 4th International Conference On Emerging Trends in Multi-Disciplinary Research "ETMDR-2023"

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Kaushlendra Kumar (2K21/MSCBIO/18)

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CERTIFICATE

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Place: Delhi
Date: 30/05/23

Prof. Jaigopal Sharma
SUPERVISOR, In-charge,
Environmental & Industrial biotechnology laboratory
Department of Biotechnology
Delhi Technological University Delhi, India 110042

30/05/2023

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Head of Department
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"Impact of Nickel(II) oxide nanoparticle on brevibacillus growth for bioremediation"

Abstract

The utilization of nanoparticles in bioremediation has garnered considerable interest due to their potential in improving pollutant removal efficiency. In this particular study, My focus was to investigate how nickel nanoparticles affect the growth of Brevibacillus brevis bacteria, which is known for its bioremediation capabilities. We introduced nickel nanoparticles into the growth medium and carefully assessed their influence on bacterial growth. The outcomes of my experiments reveal a notable stimulatory effect of nickel nanoparticles on the growth and proliferation of Brevibacillus brevis bacteria. This observation suggests that nickel nanoparticles can be effectively employed as a valuable tool in bioremediation strategies, specifically targeting the elimination of pollutants and contaminants from the environment. However, it is crucial to conduct further research to gain a deeper understanding of the underlying mechanisms behind this stimulation and to optimize the application of nickel nanoparticles in bioremediation practices. Ultimately, these findings contribute to the advancement of innovative and sustainable approaches for efficient environmental cleanup.

Keywords: Bioremediation, Brevibacillus bravis, Nickel(II) oxide nanoparticle, bacterial growth

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Kaushlendra Kumar
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LIST OF ABBREVIATIONS

NiO: Nickel(II) oxide

NiONPs: Nickel(II) oxide nanoparticles

NPs: Nanoparticles

UV- Vis: UV- visible spectroscopy

DLS: Dynamic Light Scattering

ROS: Reactive oxygen species

OD: Optical density

TEM: Transmission electron microscope

B. Brevis: Brevibacillus Brevis

PAHs: Polycyclic Aromatic Hydrocarbons

FDA: Fluorescein diacetate

Rpm: rotations per minute

SPR: Surface Plasmon Resonance

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Chapter 1

Introduction

In recent years, there has been significant interest in Nickel(II) oxide nanoparticles (NiONPs) due to their unique physical and chemical properties, as well as their potential applications in various fields [1][2]. These nanoparticles possess notable redox activity, catalytic behavior, and a high surface area, making them attractive for use in electronics, energy storage, and environmental remediation [3][4].

However, concerns have been raised regarding the potential impact of NiONPs on biological systems and environmental ecosystems [5]. Specifically, their effect on microbial growth and ecosystem balance has come under scrutiny [6]. Brevibacillus brevis, a Gram-positive bacterium commonly found in diverse environments such as soil, water, and plants, has emerged as a model organism for studying the influence of nanoparticles on microbial growth [7]. The sensitivity of B. brevis to environmental stressors and its ecological significance make it an ideal candidate for investigating the effects of NiONPs [8].

Comprehending the interactions between NiONPs and B. brevis is crucial for evaluating the potential risks associated with nanoparticle exposure and ensuring their safe utilization in various applications [9]. By studying the impact of NiONPs on B. brevis, researchers aim to gain insights into the specific mechanisms through which these nanoparticles may affect microbial growth and ecosystem dynamics [10].

Understanding the potential risks associated with NiONPs and their effects on B. brevis will contribute to informed decision-making regarding their application in technology and environmental contexts . It will help ensure the responsible and sustainable use of NiONPs while minimizing potential adverse effects on microbial populations and ecological systems [11], [12]. By elucidating the interactions between NiONPs and B. brevis, scientists can provide valuable information for risk assessment and the development of guidelines or regulations governing the use of NiONPs in various fields[11], [12].

The impact of nanoparticles on different classes of bacteria can vary significantly, and the specific mechanisms behind their toxicity are not yet fully understood [13]. Additionally,

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factors such as the methods used for nanoparticle synthesis, the shape, size, composition, and the presence of stabilizing agents can lead to divergent conclusions, even when studying closely related nanosuspensions [14],[15]. Given these uncertainties, there is a critical need for a comprehensive study to address these gaps in knowledge. This study aims to investigate the effects of Nickel(II) oxide (NiO) nanoparticles on the growth of Brevibacillus bacteria. By examining the growth response of Brevibacillus bacteria in the presence of NiO nanoparticles, we hope to gain insights into the specific interactions and potential impacts of these nanoparticles on bacterial growth and viability. The findings of this study will contribute to a better understanding of the effects of NiO nanoparticles and help elucidate their potential implications in various applications, including bioremediation, where Brevibacillus bacteria play a significant role.

The study was initiated by conducting a thorough examination of bacterial growth patterns, meticulously observing any changes influenced by the nanoparticles. Additionally, a comprehensive microscopic analysis was performed to detect and document any alterations in the bacteria's morphology caused by the nanoparticles. By delving into these investigations, my primary objective was to utilize the knowledge acquired from this study to explore and exploit potential biological applications. The aim was to uncover novel possibilities and deepen the comprehension of the intricate realm of nanobiotechnology. By unraveling the effects of nanoparticles on bacterial growth and morphology, this research contributes to the broader field of nanobiotechnology and paves the way for innovative advancements in biomedical and biotechnological applications. The findings of this study hold the potential to open new avenues for research and development, leading to the design of improved nanoparticle-based technologies with enhanced effectiveness and safety profiles.

Thus, within this captivating journey through the realm of nanotechnology, I tirelessly explore its profound implications, navigating uncertainties, and pushing the boundaries of knowledge. Their endeavors pave the way for transformative advancements in medicine, diagnostics, and ultimately, the well-being of humanity.

In this captivating exploration of the vast domain of bioremediation, I have embarked on a tireless quest to unravel its profound implications, continually pushing the boundaries of knowledge while navigating through uncertainties. Throughout this journey, my efforts have been focused on unearthing the transformative advancements that nanotechnology holds for

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fields such as medicine and diagnostics. The ultimate goal is to improve the well-being of humanity by harnessing the potential of bioremediation and nanotechnology in addressing critical healthcare challenges. By delving deep into the intricacies of nanotechnology, my research aims to contribute to the broader scientific community's understanding and pave the way for groundbreaking innovations that have the power to revolutionize healthcare practices. The significance of these advancements lies in their ability to offer improved medical treatments, more accurate diagnostics, and ultimately, a brighter future for humanity's overall well-being.

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Chapter 2

Review of Literature

Nanoparticles have gained significance in bioremediation due to their unique properties that can enhance the efficiency and effectiveness of the process [16]. Here are some key reasons why nanoparticles are beneficial in bioremediation:

Increased surface area: Nanoparticles have a high surface area-to-volume ratio, providing a larger contact area for interactions with contaminants. This increased surface area allows for enhanced adsorption, binding, and degradation of pollutants by microorganisms, thereby improving the overall bioremediation efficiency [17].

Enhanced reactivity: Nanoparticles exhibit increased reactivity due to their small size and high surface area, allowing for more efficient interactions with contaminants. This increased reactivity facilitates the degradation of various pollutants, including organic compounds, heavy metals, and persistent organic pollutants (POPs) [18].

Carrier for nutrients and microbial cells: Nanoparticles can act as carriers for essential nutrients, such as carbon, nitrogen, and phosphorus, which are crucial for microbial growth and activity. They provide a protective environment for microorganisms, ensuring the sustained release of nutrients and creating favorable conditions for their survival and degradation capabilities [19].

Targeted delivery of enzymes and biomolecules: Nanoparticles can be functionalized with specific enzymes or biomolecules involved in pollutant degradation pathways. This functionalization enables targeted delivery of these biocatalysts to contaminated sites, increasing their effectiveness in breaking down pollutants [20].

Contaminant immobilization: Nanoparticles can immobilize contaminants by adsorption or precipitation, preventing their migration and minimizing their bioavailability. This immobilization reduces the risks associated with contaminant exposure and allows for subsequent controlled degradation or removal [21].

Synergistic effects: Nanoparticles can exhibit synergistic effects when combined with

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microbial activity. For example, nanoparticles can enhance the growth and activity of specific microbial strains or consortia, promoting the degradation of pollutants. Conversely, microorganisms can modify nanoparticles, increasing their reactivity and effectiveness in pollutant transformation [22].

Remediation of recalcitrant pollutants: Nanoparticles have demonstrated potential in the remediation of recalcitrant pollutants that are resistant to conventional bioremediation approaches. They can facilitate the breakdown of complex organic compounds, such as polychlorinated biphenyls (PCBs) and chlorinated solvents, which are typically challenging to degrade using traditional methods [23].

Minimization of environmental impacts: The use of nanoparticles in bioremediation has the potential to minimize the release of harmful pollutants into the environment. By improving the efficiency of pollutant degradation, nanoparticles can accelerate the remediation process, reducing the duration and extent of environmental contamination.

While nanoparticles offer numerous advantages in bioremediation, it is important to consider potential risks and environmental implications associated with their use. Further research is necessary to better understand nanoparticle behavior, fate, and potential toxicity to ensure their safe and responsible application in bioremediation practices [24].

2.1 Importance of Brevibacillus bacteria in bioremediation

Brevibacillus bravis bacteria, which are gram-positive, rod-shaped bacteria belonging to the Firmicutes phylum, can be found in various habitats such as soil, water, plants, and the gastrointestinal tracts of animals [8]. One notable characteristic of Brevibacillus species is their capacity to form resilient endospores, enabling them to survive in challenging environmental conditions [25]. Their significance in bioremediation stems from their versatile metabolic abilities and their capability to break down diverse contaminants. Here are several key aspects underscoring the relevance of Brevibacillus bacteria in bioremediation:

a) **Versatility in contaminant degradation:** Brevibacillus bacteria exhibit an impressive range of enzymatic capabilities that equip them with the ability to degrade a diverse array of pollutants. These microorganisms have been extensively studied and have shown remarkable efficiency in breaking down various classes of pollutants, including organic compounds and heavy metals [26], [27].

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In terms of organic compounds, Brevibacillus bacteria have demonstrated their proficiency in degrading hydrocarbons, pesticides, polycyclic aromatic hydrocarbons (PAHs), and pharmaceuticals [28]. Hydrocarbons, which are a common component of petroleum and oil spills, can be efficiently broken down by these bacteria. Pesticides, which pose a significant threat to the environment and human health, can also be effectively degraded by Brevibacillus bacteria. Furthermore, the complex structure of polycyclic aromatic hydrocarbons (PAHs) is no challenge for these versatile bacteria, as they possess the necessary enzymatic machinery to break them down. Even pharmaceutical compounds, which often find their way into wastewater and natural ecosystems, can be successfully degraded by Brevibacillus bacteria [28].

In addition to their proficiency in degrading organic compounds, Brevibacillus bacteria also play a role in the degradation of heavy metals. Heavy metals, such as chromium and arsenic, are persistent environmental pollutants that can cause severe harm to ecosystems and human health [29]. Brevibacillus bacteria employ multiple mechanisms to participate in the degradation of these metals. Reduction, where the bacteria convert the toxic forms of the metals into less harmful forms, is one such mechanism. Precipitation, where the metals are transformed into insoluble forms that can be easily removed from the environment, is another strategy employed by these bacteria. Additionally, volatilization, in which the bacteria facilitate the release of heavy metals into the atmosphere, aids in reducing their environmental impact [29].

The versatility of Brevibacillus bacteria in pollutant degradation can be attributed to their diverse enzymatic machinery. These bacteria possess a wide range of enzymes that target specific pollutants, allowing them to efficiently break down different classes of contaminants [28]. The enzymatic machinery enables the bacteria to metabolize and transform the pollutants into less harmful substances or completely mineralize them. Overall, Brevibacillus bacteria have proven to be valuable organisms in the field of bioremediation due to their ability to degrade various classes of pollutants. Their enzymatic diversity and efficiency in degrading organic compounds, such as hydrocarbons, pesticides, PAHs, and pharmaceuticals, highlight their potential in cleaning up contaminated environments. Moreover, their participation in the degradation of heavy metals like chromium and arsenic through reduction, precipitation, and volatilization further underscores their significance in addressing metal pollution. Continued research and exploration of the enzymatic machinery of Brevibacillus bacteria hold promise for future applications in environmental

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remediation efforts [28], [29].

b) **Adaptability to harsh environmental conditions:** Brevibacillus bacteria are known for their remarkable adaptability to various environmental conditions, making them highly suitable for bioremediation in a wide range of contaminated sites [30]. These bacteria can thrive and function effectively in extreme temperatures, pH levels, and salinity, providing them with the versatility needed to address diverse environmental challenges [31].

One of the key advantages of Brevibacillus bacteria is their ability to tolerate and flourish in extreme temperatures. They have been found in environments with both high and low temperatures, including hot springs, cold soils, and even permafrost. This thermal tolerance enables them to survive and carry out bioremediation processes in contaminated sites where temperature fluctuations are prevalent, such as industrial wastewaters and oil spills. Their capability to thrive across a wide temperature range allows them to adapt and degrade pollutants effectively in various thermal conditions. Furthermore, Brevibacillus bacteria exhibit a notable ability to withstand extreme pH levels. They have been isolated from environments with both acidic and alkaline conditions, such as acid mine drainage sites and alkaline soils. This pH tolerance is advantageous for bioremediation efforts in environments with highly acidic or alkaline pollutants, such as acidic mine tailings or alkaline industrial effluents. Brevibacillus bacteria's resilience in the face of extreme pH levels enhances their potential as effective bioremediation agents.

In addition to temperature and pH, Brevibacillus bacteria have demonstrated the capacity to tolerate varying salinity levels. They have been found in saline environments, including salt lakes and coastal areas. This adaptability to salinity enables them to thrive in saline-contaminated sites, such as brackish water bodies or saltwater-impacted soils. Their ability to function under different salinity conditions makes them suitable candidates for bioremediation efforts in coastal or industrial sites where salinity can pose a challenge to other organisms.

The adaptability of Brevibacillus bacteria to extreme temperatures, pH levels, and salinity greatly expands their potential applications in bioremediation. Their ability to thrive in diverse environmental conditions allows them to be deployed in contaminated sites that exhibit a wide range of physical and chemical characteristics. Industrial wastewaters, which can vary in temperature, pH, and salinity, can be effectively treated

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by these bacteria. Likewise, oil spills, where fluctuating temperatures and salinity levels are common, can benefit from the resilience of Brevibacillus bacteria. Even polluted soils, which often exhibit varying pH and salinity, can be remediated with the help of these adaptable microorganisms. The adaptability of Brevibacillus bacteria stems from their genetic and physiological flexibility, enabling them to adjust their metabolism and enzymatic activity to suit the specific conditions of their environment. This adaptability is a result of their evolutionary history in diverse habitats and their ability to acquire and exchange genetic material through mechanisms like horizontal gene transfer. Such genetic plasticity allows Brevibacillus bacteria to survive and thrive in challenging environments, making them valuable tools for bioremediation strategies.

The versatility of Brevibacillus bacteria in adapting to extreme temperatures, pH levels, and salinity positions them as promising candidates for bioremediation in a wide range of contaminated sites. Their ability to tolerate and function effectively under such challenging conditions expands the scope of their application, enabling them to address diverse environmental pollutants. Further research and exploration of the unique adaptability mechanisms of Brevibacillus bacteria can unlock their full potential in bioremediation efforts worldwide [30], [31].

c) **Production of specialized enzymes:** Brevibacillus bacteria are renowned for their production of a diverse array of extracellular enzymes, such as lipases, proteases, esterases, and oxidases. These enzymes play a crucial role in breaking down complex contaminants, transforming them into simpler and less toxic compounds that can be easily metabolized by the bacteria themselves or other microorganisms in the environment [32].

Lipases hydrolyze lipids and ester bonds, aiding in the degradation of hydrocarbons and pesticides. Proteases break down proteins into smaller peptides and amino acids, facilitating the mineralization of organic contaminants.

Esterases catalyze the hydrolysis of ester bonds found in pesticides and other organic compounds.

Oxidases participate in oxidation reactions, converting complex organic molecules into less toxic forms. The production of these enzymes reflects the adaptability of Brevibacillus bacteria to respond to diverse pollutants.

The enzymatic diversity enables the bacteria to target various organic compounds, promoting their detoxification and removal from the environment. The production of

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extracellular enzymes is often regulated by the presence of specific pollutants, allowing Brevibacillus bacteria to optimize enzyme production based on the contaminants present. Overall, the enzymatic capabilities of Brevibacillus bacteria make them valuable bioremediation agents in combating environmental pollution [31], [32].

d) **Biofilm formation and attachment:** Brevibacillus bacteria have the remarkable ability to form biofilms, which are complex and organized communities of microorganisms that adhere to surfaces. These biofilms play a critical role in the survival and persistence of the bacteria in polluted environments. By forming biofilms, Brevibacillus bacteria create a protected microenvironment that offers numerous advantages for bacterial growth and the colonization of contaminated surfaces[33].

Biofilm formation begins when individual bacteria attach to a surface and multiply, gradually forming a structured community. The bacteria within the biofilm are embedded in a self-produced matrix composed of polysaccharides, proteins, and DNA, which provides structural support and protection. This matrix acts as a shield, safeguarding the bacteria from various environmental stresses, including fluctuations in temperature, pH levels, and nutrient availability. The protective nature of the biofilm matrix enhances the survival of Brevibacillus bacteria in polluted environments that may pose challenges to their growth and persistence [34].

The biofilm structure also facilitates the colonization of contaminated surfaces. The matrix allows the bacteria to firmly adhere to surfaces such as soil particles, sediments, and industrial equipment, preventing their washout or dispersion. This attachment mechanism promotes the long-term presence of Brevibacillus bacteria in contaminated areas, enabling them to establish and maintain a population capable of degrading pollutants over an extended period.

Furthermore, biofilms enable close proximity and interactions between bacterial cells, promoting cooperative behavior and the exchange of genetic material. Within the biofilm, Brevibacillus bacteria can communicate through a process known as quorum sensing, where they release and detect signaling molecules. This communication system allows the bacteria to coordinate their activities, such as the production of enzymes for pollutant degradation, optimizing their overall effectiveness.

Moreover, the proximity of bacterial cells within the biofilm facilitates the transfer of genetic material, including the exchange of genes involved in pollutant degradation. This horizontal gene transfer contributes to the adaptation and evolution of

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Brevibacillus bacteria, enhancing their ability to degrade a wide range of pollutants. The formation of biofilms by Brevibacillus bacteria is a significant attribute that enhances their survival, colonization, and pollutant degradation capabilities in contaminated environments. These biofilms create a protected microenvironment, shielding the bacteria from environmental stresses and providing a platform for close interactions and cooperative behaviors. The attachment of the biofilm to surfaces ensures the long-term presence of the bacteria, enabling them to persist and effectively degrade pollutants over an extended period. The formation and maintenance of biofilms contribute to the success of Brevibacillus bacteria as valuable agents in bioremediation strategies for contaminated sites [35], [36].

2.2 Physiochemical properties of NiO nanoparticles

Nickel(II) oxide (NiO) nanoparticles exhibit several unique physicochemical properties that make them of interest in various applications. Here are some key physicochemical properties of NiO nanoparticles:

(a) Particle Size: NiO nanoparticles typically have a size range of a few nanometers to tens of nanometers. The small size imparts a large surface area-to-volume ratio, enhancing their reactivity and potential interactions with other substances [37].

(b) Crystal Structure: NiO nanoparticles possess a crystal structure similar to bulk NiO, which is a face-centered cubic lattice. The crystalline nature of the nanoparticles contributes to their stability and influences their physical and chemical properties [38].

(c) Surface Chemistry: The surface of NiO nanoparticles plays a critical role in their reactivity and interactions. The presence of surface defects, vacancies, and different chemical species on the nanoparticle surface can influence their catalytic activity, adsorption properties, and potential toxicity [39].

(d) Optical Properties: NiO nanoparticles exhibit interesting optical properties. They typically appear as a dark brown or black color due to their strong absorption in the visible range. The absorption and emission properties of NiO nanoparticles can be tuned by controlling their size and surface modifications, making them suitable for optoelectronic applications [40].

(e) Magnetic Behavior: NiO nanoparticles exhibit antiferromagnetic behavior at low temperatures, meaning their magnetic moments align in an antiparallel manner.

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However, the magnetic properties of NiO nanoparticles can be influenced by factors such as size, shape, and surface modifications [41].

(f) Redox Activity: NiO nanoparticles are known for their redox activity. They can undergo reversible oxidation and reduction processes, making them useful as catalysts or catalyst supports in various chemical reactions, such as hydrogenation and oxidation reactions [37].

(g) Thermal Stability: NiO nanoparticles exhibit high thermal stability, making them suitable for applications that involve elevated temperatures, such as catalysis or thermal barrier coatings [42].

(h) Electrical Conductivity: NiO nanoparticles are generally considered to be an insulator in their pure form. However, their conductivity can be modified by doping or introducing defects, making them potential candidates for electronic and sensor applications [43].

(i) Surface Plasmon Resonance: NiO nanoparticles also exhibit plasmonic properties, with a characteristic surface plasmon resonance (SPR) peak in the ultraviolet to visible range. The SPR behavior can be utilized in sensing and optical applications [44].

2.3 Importance of Nickel oxide nanoparticle on positive bacterial growth

In certain cases, nickel oxide nanoparticles have the potential to promote the growth of bacteria. This positive effect can occur through several mechanisms. Firstly, nickel oxide nanoparticles can act as a source of nutrients for bacteria. Over time, these nanoparticles can undergo slow degradation or release ions that serve as essential nutrients for bacterial growth. By providing a supplementary nutrient source, the nanoparticles support the proliferation of bacteria.

Additionally, bacteria have the ability to form biofilms, which are complex communities of microorganisms attached to surfaces. Nickel oxide nanoparticles can serve as a substrate for biofilm formation, providing a surface for bacteria to adhere to and grow on. This phenomenon can lead to increased bacterial growth and the formation of dense bacterial colonies. Furthermore, nickel oxide nanoparticles may interact with bacteria in a synergistic manner, enhancing their growth. For example, these nanoparticles can facilitate the transport of certain nutrients or mediate the exchange of genetic material among bacteria. Such interactions can contribute to increased bacterial growth and adaptation.

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However, it is crucial to note that the effects of nickel oxide nanoparticles on bacterial growth can vary significantly depending on specific conditions. Factors such as the bacterial strain, nanoparticle concentration, and environmental conditions can influence the outcome. Therefore, a careful evaluation is necessary to fully understand the precise impact of nickel oxide nanoparticles on bacterial growth in a given context.

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Chapter 03

Material and Methodology

3.1 Source of microorganism

The microorganism Brevibacillus brevis used in this study was sourced from the Environmental and Industrial Biotechnology Laboratory at Delhi Technological University (DTU), Delhi. The pure culture obtained was cultivated on nutrient agar and regularly sub-cultured to ensure its viability throughout the experiment.

To ensure the reliability and reproducibility of the experimental results, a pure culture of Brevibacillus brevis was obtained and consistently subcultured. This approach minimized the presence of contaminants and genetic variations, providing a controlled environment for studying the effects of cerium oxide nanoparticles on Brevibacillus brevis growth. By maintaining a pure culture and avoiding interference from other microorganisms or internal variations within the bacterial strain, the experimental conditions remained consistent and the observed outcomes could be attributed to the specific interaction between Brevibacillus brevis and nickel(II) oxide nanoparticles.

3.2. Preparation of CeO2 Nanoparticle Dispersion

a. Accurately weigh 1.7 grams of NiO nanoparticles using a precision balance.
b. Achieve a homogeneous dispersion of the NiO nanoparticles by adding them to 10 ml of water. It is essential to prevent the nanoparticles from aggregating, and this can be accomplished by utilizing sonication. Sonication involves subjecting the dispersion to high-frequency sound waves, which disintegrate aggregates and promote even distribution.
c. Once the NiO nanoparticles are dispersed, it is crucial to evaluate the properties of the dispersion. Techniques like dynamic light scattering (DLS) can be employed to analyze the size distribution of the nanoparticles in the dispersion. Additionally, zeta potential measurements can offer insights into the surface charge and stability of the nanoparticles within the dispersion. These characterization techniques are valuable for understanding the behavior of the NiO nanoparticles and ensuring the consistency and quality of the dispersion used in the study.

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3.3 Bacterial Culture Preparation:

a. Start the B. brevis culture by transferring a small portion of the pure culture into 50 ml of nutrient broth medium. The nutrient broth medium contains necessary nutrients and creates a favorable environment for bacterial growth.

b. Place the culture under suitable conditions, including the appropriate temperature, to facilitate the growth and multiplication of the bacteria. The length of the incubation period depends on the specific growth characteristics of Brevibacillus brevis and the desired growth phase for the experiment.

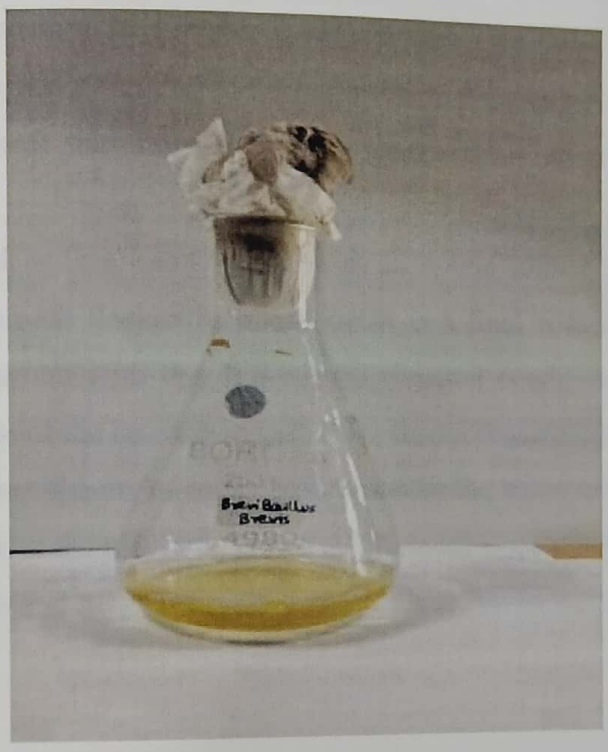

Figure 1: Primary culture of Brevibacillus brevis after incubation (OD_{600}: 0.78)

3.4 Experimental Setup:

a. Obtain sterile Falcon tubes and appropriately label them to indicate the different concentrations of NiO nanoparticles. For instance, assign labels such as 25 µg, 50 µg, 75 µg, and 100 µg to the tubes.

b. Using a pipette, carefully transfer the corresponding volumes of the NiO nanoparticle dispersion into each Falcon tube that matches the desired concentrations. Ensure that the nanoparticles are uniformly dispersed within the solution.

c. Alongside the tubes containing NiO nanoparticles, prepare control tubes that do not contain any NiO nanoparticles. These control tubes will serve as a reference for

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comparison purposes.

3.5. Bacterial Exposure:

a. Prepare five nutrient broth flasks, each containing 50 ml of medium, and label them accordingly: control, 25 µg, 50 µg, 75 µg, and 100 µg. These labels indicate the different concentrations of NiO nanoparticles that will be added to each flask.

b. Add the appropriate concentrations of NiO nanoparticles to each labeled flask. For the control flask, no nanoparticles are added, while the other flasks will receive 25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml of the nanoparticle dispersion, respectively. Ensure accurate measurement and transfer of the nanoparticles to achieve the desired concentrations.

c. Inoculate each flask with a known volume of B. brevis culture. Add 100 µl of the bacterial culture to each flask, which will result in a final volume and nanoparticle concentration consistent with the desired experimental conditions. The inoculation ensures the presence of the bacteria in each flask for subsequent growth.

d. Thoroughly mix the contents of each flask to ensure the nanoparticles are uniformly distributed throughout the medium. This step promotes even exposure of the bacteria to the nanoparticles and helps maintain consistent conditions across the experimental setup.

3.6. Growth Measurement:

a) Place the conical flasks containing the bacterial suspensions in a shaker flask incubator set to 37°C and 120 rpm. This controlled environment provides the necessary conditions for Brevibacillus brevis to grow, including temperature and agitation.

b) Regularly assess the growth of Brevibacillus brevis in the conical flasks by periodically measuring the optical density (OD). Use a spectrophotometer to determine the absorbance of the bacterial suspension at a specific wavelength, typically 600 nm. The OD at 600 nm serves as an indicator of the bacterial population density, reflecting the growth of Brevibacillus brevis over time.

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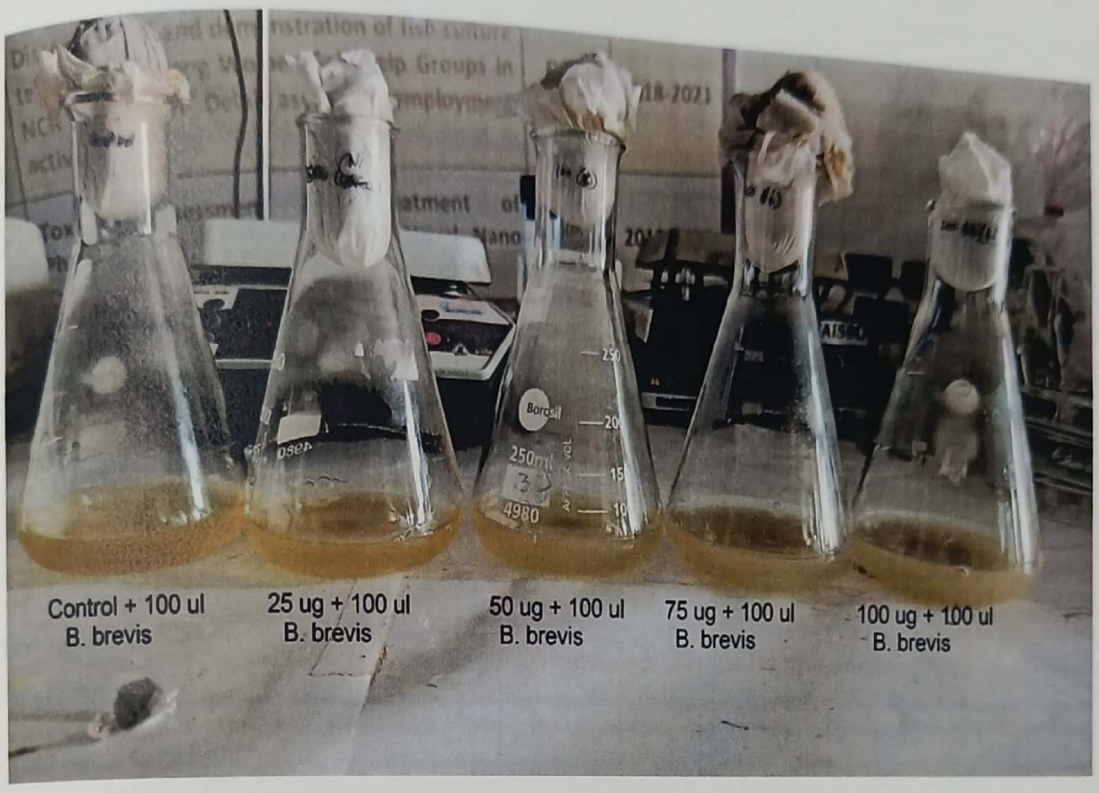

Figure 2: Secondary culture with Nickel(II) oxide nanoparticle treated bacterial suspension

3.7. Data Analysis:

a) Generate growth curves for each concentration of nanoparticles and the control group by plotting the optical density (OD) values against the duration of incubation.

b) Examine the growth patterns and determine growth parameters, including growth rate, duration of the lag phase, and duration of the log phase, through analysis of the growth profiles.

3.8 Total enzyme activity

The FDA hydrolase activity assay was conducted to evaluate the breakdown of FDA into fluorescein. The procedure involved the following steps:

a. Preparation of Culture Samples:

* Transfer 2 ml of the B. brevis culture to an appropriate container.
* Transfer 2 ml of the B. brevis_NiONPs culture to a separate container.
* Prepare positive and negative controls:
    * Positive control: Place 2 ml of the B. brevis culture in a separate container.

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* Negative control: Take a separate container containing FDA solution without any culture.

b. Incubation:

- Add 20 ml of phosphate buffer (pH = 7.4) to each culture sample (B. brevis and B. brevis_NiONPs).
- Introduce 0.2 ml of FDA stock solution (1000 µg mL-1) to each culture sample.
- Place the containers in a water bath shaker set at 30°C and a shaking speed of 150 rpm.
- Allow the samples to incubate for 40 minutes, enabling the hydrolytic cleavage of FDA by the enzyme activity present in the cultures.

c. Extraction:

- Pour 20 milliliters of a solution consisting of chloroform and methanol in a 2:1 ratio into each sample.
- Ensure thorough mixing of the suspension.
- Use a centrifuge to spin the samples at 6000 revolutions per minute for a duration of 7 minutes.
- Once the centrifugation is complete, the extracted fluorescein product will be present in the liquid above the sediment.

d. Determining Fluorescence Intensity:

- Collect the liquid above the sediment from each sample and move it to individual cuvettes.
- Employ a fluorometer to gauge the fluorescence intensity of the liquid.
- Configure the fluorometer to excite at a wavelength of 490 nm and emit at 519 nm.
- Document the fluorescence intensity readings for each sample, encompassing both the positive and negative controls.

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Chapter 04

Result

4.1 Characterization of nanoparticles

The laboratory synthesized nanoparticles made of Nickel(II) oxide were subjected to characterization using two techniques: Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). Through TEM imaging, the size of the nickel(II) oxide nanoparticles was determined to be 18 nm and through DLS analysis, the average size of the nickel(II) oxide nanoparticle was 788 nm.

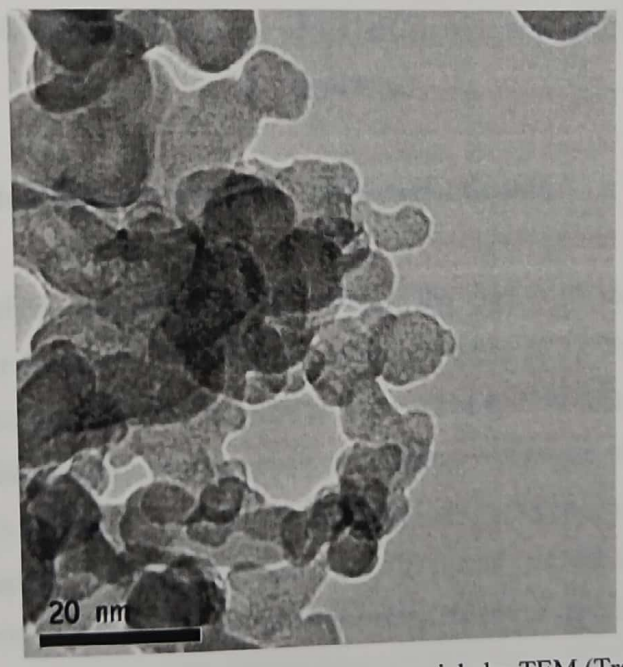

Figure 3: Characterization of Nickel (II) oxide nanoparticle by TEM (Transmission Electron Microscope)

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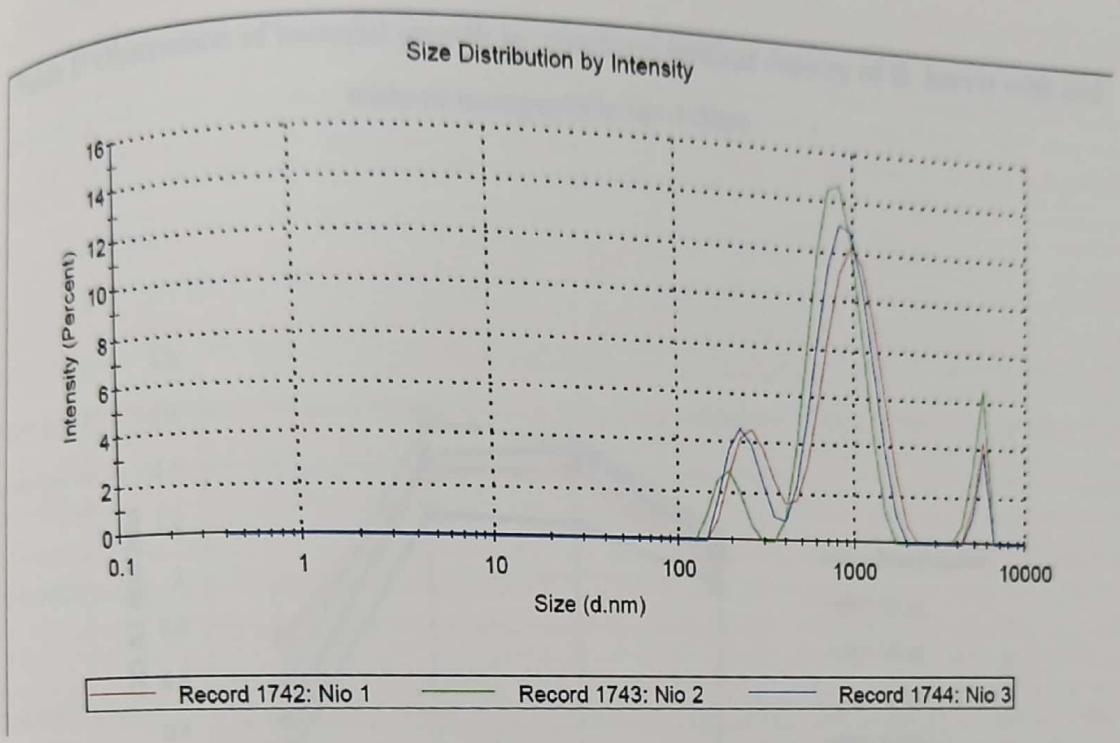

Figure 4: Size distribution intensity graph of Nickel(II) oxide nanoparticle by DLS (dynamic light scattering)

4.2 Effect of Nickel(II) Oxide nanoparticle on bacterial Growth

A comparative analysis was conducted to examine the growth of bacteria under normal conditions and when exposed to Nickel(II) oxide nanoparticles (NiO). The study revealed the impact of Nickel nanoparticles on bacterial growth. When Brevibacillus Brevis was grown under normal conditions, the growth curve clearly displayed the phases of lag, log, stationary, and death. However, when exposed to different concentrations of Nickel(II) oxide nanoparticles (25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL), a gradual reduction in the log phase was observed, indicating the microbiostatic effect of Nickel nanoparticles on Brevibacillus in a concentration-dependent manner.

Sample	1st day	2nd day	3rd day	4th day
B. brevis	0.45	1.44	1.48	1.12
25 ug	0.76	1.38	1.41	1.08
50 ug	0.58	1.56	1.51	1.18
75 ug	0.38	1.4	1.37	1.02
100 ug	0.3	1.23	1.2	0.98

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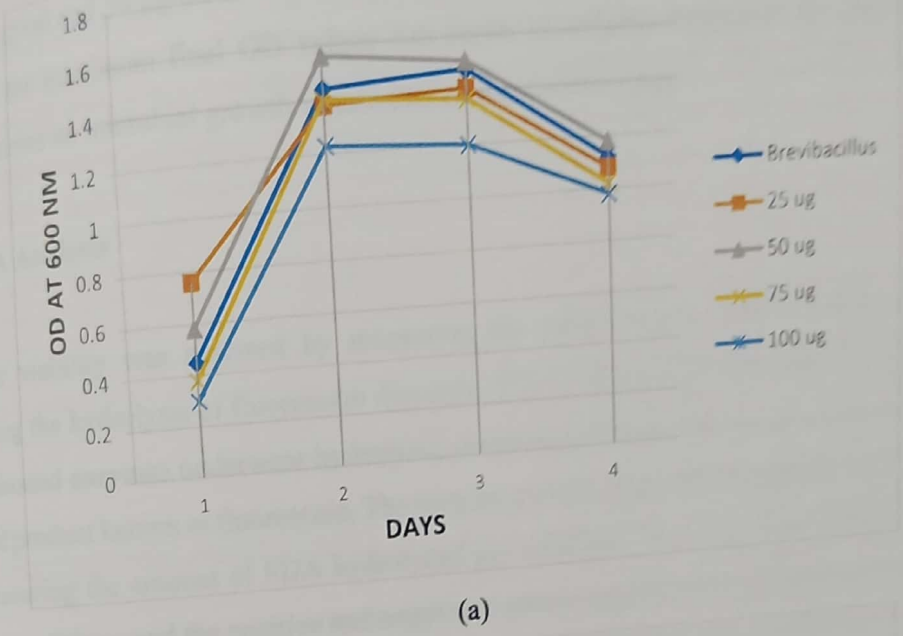
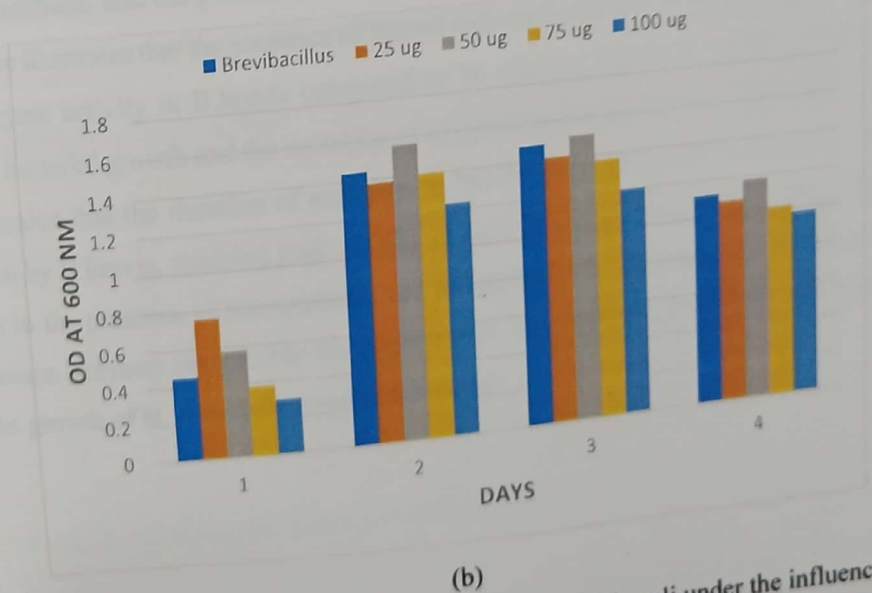
Table 1: Observation of bacterial growth by checking Optical density of B. brevis with and without nanoparticle for 4 days



(a)



(b)

Figure 5: Comparison of the growth trajectory of Escherichia coli under the influence of NiO nanoparticles versus regular growth.

In the absence of NiO nanoparticles, Brevibacillus exhibited rapid growth, reaching optical density values up to OD600nm = 1.44 within 2 days of cultivation. The stationary phase was

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reached after approximately 48 hours. The growth curves of cultures with 50 µg/ml of NiO showed significantly steeper slopes during the exponential phase compared to the control samples without added NiO. These cultures achieved the highest OD values of 1.56 within 2 days. Conversely, when Brevibacillus was grown in the presence of 75 µg/ml of NiO, the growth curve exhibited a shallower slope and lower OD600nm values compared to samples with 25 µg/ml and 50 µg/ml of NiO. This suggests that neither the maximum specific growth rate nor the maximum final OD values can serve as reliable indicators for the effects of nanoparticles on microbial growth within this concentration range.

4.3 FDA Analysis

Cellular viability was assessed by measuring the total enzyme activity using a method involving the hydrolysis of fluorescein diacetate (FDA). When FDA was applied, both bound and unbound enzymes underwent hydrolysis, resulting in the production of a yellowish-green colored product known as fluorescein. The enzyme activity in the MSM samples was quantified by measuring the amount of FDA hydrolyzed per milliliter. The study was conducted under sterile conditions, and the positive and negative controls showed minimal FDA hydrolysis. The figure illustrates that the presence of Nickel nanoparticles (B.brevis_NiNPs) led to a higher total enzyme activity in B.brevis compared to its absence (B.brevis_MSM). These findings indicate bacterial growth and the secretion of enzymes necessary for bioremediation. The study also revealed that the duration of exposure to NiNPs correlated with an increase in enzyme secretion by B.brevis, reaching peak activity at day 5 before declining. The enhanced enzyme activity in the presence of nanoparticles can be attributed to the release of protease, esterase, and laccase enzymes responsible for degradation. This pattern of enzyme secretion aligned with the growth of B. brevis observed in the study.

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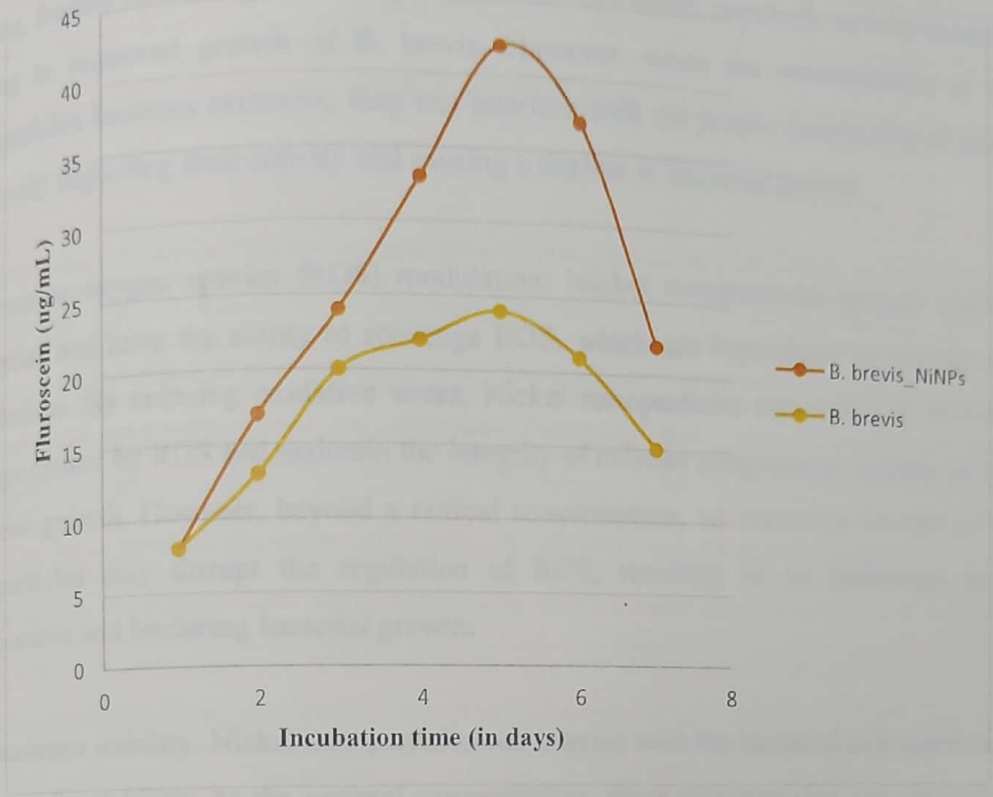

Figure 6: Total enzyme activity of B. brevis with and without NiO nanoparticle

	FLUROSCEIN (UG/ML)	
Incubation time in hrs	**B. brevis_NiNPs**	**B. brevis**
1	8	8
2	17	13
3	24	20
4	33	22
5	42	24
6	37	21
7	22	15

Table 2: Total enzyme activity data of B. brevis with and without nanoparticle

4.4 Mechanism of Nickel Oxide Nanoparticle to support bacterial growth in Brevibacillus

a) Enzyme activity enhancement: When Nickel nanoparticles are present at their optimal concentration, they can function as catalysts and augment the activity of specific enzymes within B. brevis. These nanoparticles create a suitable microenvironment or surface for enzyme

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binding, thereby facilitating their catalytic function. As a result, metabolic activity is increased, leading to improved growth of B. brevis. However, when the concentration of Nickel nanoparticles becomes excessive, they can interfere with the proper functioning of enzymes, negatively impacting their activity and causing a decline in bacterial growth.

b) Reactive oxygen species (ROS) modulation: Nickel nanoparticles possess antioxidant properties and have the ability to scavenge ROS, which are byproducts of normal cellular metabolism. By reducing oxidative stress, Nickel nanoparticles can shield B. brevis from damage caused by ROS and maintain the integrity of cellular components, thereby promoting bacterial growth. However, beyond a critical concentration, an excessive amount of Nickel nanoparticles may disrupt the regulation of ROS, resulting in an imbalance in redox homeostasis and hindering bacterial growth.

c) Membrane stability: Nickel nanoparticles can interact with the bacterial cell membrane and influence its stability. At the optimal concentration, these nanoparticles can strengthen the structure of the membrane, enhancing its integrity and flexibility. This, in turn, facilitates the uptake of nutrients and elimination of waste, promoting the growth of B. brevis. However, higher concentrations of Nickel nanoparticles can disrupt the structure of the membrane, compromising its functionality and adversely affecting the growth of B. brevis.

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Chapter 5

Discussion

The utilization of engineered nanoparticles with biocidal properties, such as silver (Ag), zinc (Zn), copper (Cu), cerium (Ce), and nickel (Ni), through nanotechnology approaches, offers innovative applications in various fields [45]. These applications include controlling the growth of undesired microorganisms on different surfaces, preventing biofouling, improving wastewater treatment and drinking water purification, and serving as prophylaxis and topical treatment for infectious diseases. However, the widespread use of these nanoparticles with antimicrobial properties and their increasing release into the environment have raised significant concerns regarding their potential (eco)toxicological effects. Moreover, there is a lack of standardized methodologies for evaluating the effects of nanoparticle exposure on microbial communities, regardless of whether the effects are beneficial (e.g., against pathogens) or detrimental to the environment[45].

Commonly used techniques like agar diffusion tests are often hindered by issues such as nanoparticle agglomeration or aggregation and reduced nanoparticle transport caused by interactions with media components or the solidified agar matrix [46]–[48]. Consequently, these tests tend to yield inconclusive results or may underestimate nanoparticle toxicity due to nanoparticle re-aggregation. Traditional growth experiments in liquid media using culture bottles are time-consuming and require frequent sampling and offline measurements. As a result, these tests are usually limited to a small number of replicates, endpoint growth determination, or analysis at specific time points. Such growth analyses lack temporal resolution and fail to capture comprehensive information on the entire cultivation process, including potential long-term effects or subtle shifts in microbial growth affected by nanoparticles. Therefore, they are inadequate for accurately monitoring the nuanced temporal changes in microbial growth caused by nanoparticles, which are crucial for a thorough evaluation of nanoparticle-mediated effect [49]–[51].

The study began by carefully examining the growth patterns of bacteria and meticulously observing any changes induced by the nanoparticles. Additionally, a comprehensive microscopic analysis was conducted to identify and document any modifications in the

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bacteria's physical structure caused by the nanoparticles. By undertaking these investigations, my main objective was to utilize the knowledge gained from this study to explore and capitalize on potential applications in the field of biology. My aim was to uncover new possibilities and deepen our understanding of the intricate domain of nanobiotechnology. By elucidating the impacts of nanoparticles on bacterial growth and morphology, this research contributes to the broader field of nanobiotechnology and lays the groundwork for innovative advancements in biomedical and biotechnological applications. The findings of this study have the potential to stimulate further research and development, ultimately leading to the design of improved nanoparticle-based technologies that offer enhanced effectiveness and safety profiles.

The findings of my study revealed a noteworthy phenomenon: the capability of nickel nanoparticles to enhance the growth of Brevibacillus brevis bacteria, which holds particular significance in the field of bioremediation. This phenomenon has the potential to expedite the breakdown and removal of pollutants and contaminants present in the environment. Brevibacillus brevis bacteria possess a wide range of enzymes that enable them to degrade various types of pollutants, including organic compounds and heavy metals. By promoting the growth of these bacteria, nickel nanoparticles can potentially augment their enzymatic activities, leading to improved efficiency and effectiveness in pollutant degradation.

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Chapter 6

Conclusion

The utilization of nickel nanoparticles has emerged as a promising approach for promoting the positive growth of Brevibacillus brevis bacteria in the context of bioremediation. The presence of nickel nanoparticles in the growth medium has been observed to have a stimulatory effect on the growth and proliferation of Brevibacillus brevis, thereby enhancing the efficiency of bioremediation processes. These findings suggest that nickel nanoparticles hold potential as a valuable tool in strategies aimed at addressing environmental issues by facilitating the removal of pollutants and contaminants.

The observed stimulatory effect of nickel nanoparticles on Brevibacillus brevis growth signifies their capability to act as growth promoters in bioremediation applications. The nanoparticles likely interact with the bacterial cells, potentially influencing cellular processes such as metabolism, enzyme activity, and gene expression. These interactions may result in improved nutrient uptake, enhanced cellular respiration, or activation of specific pathways that promote bacterial growth.

The ability of nickel nanoparticles to promote the growth of Brevibacillus brevis bacteria is particularly significant in the context of bioremediation, as it can lead to an accelerated degradation of pollutants and contaminants in the environment. Brevibacillus brevis bacteria are known for their diverse enzymatic machinery that enables them to degrade various classes of pollutants, including organic compounds and heavy metals. By stimulating the growth of these bacteria, nickel nanoparticles can potentially enhance their enzymatic activities, thereby increasing the efficiency and effectiveness of pollutant degradation.

However, it is important to note that further in-depth investigations are required to gain a comprehensive understanding of the precise mechanisms underlying the interaction between nickel nanoparticles and Brevibacillus brevis bacteria. The specific molecular and cellular mechanisms involved in the stimulatory effect of nickel nanoparticles on bacterial growth need to be elucidated. Additionally, it is crucial to assess the potential long-term effects and ecological implications of utilizing nickel nanoparticles in bioremediation processes.

Such research efforts are essential in order to optimize the application of nickel nanoparticles in bioremediation techniques and fully harness their potential in addressing environmental challenges. By gaining a comprehensive understanding of the underlying mechanisms, it will be possible to develop targeted strategies that maximize the benefits of nickel nanoparticles while minimizing any potential risks.

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In conclusion, the utilization of nickel nanoparticles has shown promising results in promoting the positive growth of Brevibacillus brevis bacteria for bioremediation purposes. The stimulatory effect of nickel nanoparticles on bacterial growth highlights their potential to enhance the efficiency of bioremediation processes and facilitate the removal of pollutants and contaminants. However, further research is necessary to unravel the precise mechanisms involved and ensure the safe and effective application of nickel nanoparticles in bioremediation strategies.

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The development of methods for treating contaminated soil and garbage is a subject of growing importance on a global scale. Fenton procedures and biological treatments have been widely utilized for a long time as conventional methods for cleaning up polluted soil and wastewater, however they still require modification due to several flaws (High Fenton oxidation process costs and protracted biotreatment remediation times). The Fenton technique and biological treatment were briefly introduced in this study, followed by a ...

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Advanced Soil Remediation Technique Combining Chemical Oxidation and Biological Treatment

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Abstract— The development of methods for treating contaminated soil and garbage is a subject of growing importance on a global scale. Fenton procedures and biological treatments have been widely utilized for a long time as conventional methods for cleaning up polluted soil and wastewater, however they still require modification due to several flaws (High Fenton oxidation process costs and protracted biotreatment remediation times). The Fenton technique and biological treatment were briefly introduced in this study, followed by a discussion of the key aspects to consider while designing a combined approach. This paper gives a summary of recent studies that have combined bioremediation and Fenton process (as pretreatment or post-treatment) to remediate wastewater or polluted soil. In this review we focus more on remediation of different types of contaminants present in soil. So that our understanding of the potential of Fenton/biotreatment improved.

Keywords— ***Fenton process, biotreatment, soil remediation, combination method, soil contaminants***

I. INTRODUCTION

High concerns have been raised about the pollution of the water and soil brought on by unauthorized discharge and inadequate waste treatment [1]. Pesticides, dyes, polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbons (PAH), and toxic metals are just a few of the pollutants that are persistent in the environment [2]. The development of treatment methods for removing these pollutants from the environment is essential since the both human health and ecosystems are directly challenged by all of these pollutants that are released into the environment [3]. Pollutants can be oxidised by Fenton processes (Fe2+/H2O2) by generating hydroxyl radicals (•OH) [4]. According to some important papers, •OH is the main reactive intermediate that causes the oxidation of organic substances and is thought to be the dominating oxidant [5]. In the Fenton oxidation process, hydrogen peroxide serves as the oxidant while Fe2+ serves as the catalyst. Biotreatment is thought of as an environmentally beneficial way for treating contaminants, but the parameters of the reaction must be carefully controlled [6]. In addition, because microorganisms have a limited ability to resist toxicity, biotreatment systems may not be successful under high level of pollution [7].

To address the drawbacks of Fenton technology (such as reagent consumption and harsh reaction processes, etc.) or the restrictions of biotreatment, a combined biotreatment and Fenton process has been created (e.g., time-consuming and condition with strict reaction, etc.). Treatment of polluted soil and waste water have both used the combined system [8]. While biotreatment in the same combined systems are able to stabilized waste materials and utilize less Fenton chemicals, in the combined systems Fenton process can increase the wastewater biodegradability, which is advantageous to biotreatment. Since the process of treatment would likely not be finished until the contaminants were completely eliminated, the biotreatment or Fenton oxidation process is not merely a pre-therapy or post-therapy [9].

II. FENTON OXIDATION PROCESSES

A potential and different approach to wastewater or soil cleanup is Fenton technology. Numerous researchers have carried out laboratory, plant-scale, and real-world applications of Fenton technology. Hydrogen peroxide is the oxidant and ferrous ions are the catalyst in Fenton reaction [10]. When Fe2+ is present , H2O2 is catalytically decomposed in a complicated chain reaction. Fe2+ acts as a catalyst as H2O2 breaks down, producing •OH in the process. Recalcitrant chemicals are then removed by the active oxidant •OH. The pH of the chemical reaction must be kept between 2.8 and 3.0 so that the Fe3+/Fe2+ couple can behave in a catalytic manner [11]. The catalytic activity might decrease as a result of the pH shift. Fe2+ can regenerate, therefore only a small quantity is needed to start the Fenton reaction. With its great performance and lack of toxicity, the Fenton process could be carried out at ambient temperature

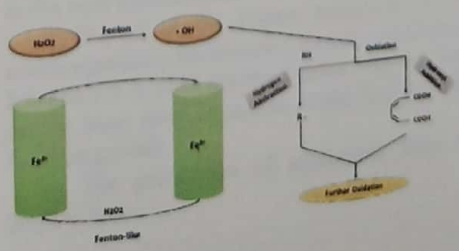

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Fig. 1. Fenton and Fenton-like process characteristics

and at normal pressure (H2O2 is transformed into H2O and O2). Because of this, the Fenton method has been widely used to treat a variety of pollutants, including wastewater from fermentation, insecticides, and pharmaceuticals [12]. Fenton technique has several drawbacks as well, such as relatively expensive reagent costs and a significant amount of iron sludge. The Fe2+ was replaced with other homo-/hero-geneous catalysts, such as Cu2+/Cu+, Fe3+, and nano iron with zero valent and pyrite to increase efficiency. Furthermore, by integrating conventional Fenton process with physical techniques like ultrasonic, UV, microwave, or electric, improved Fenton processes were created. Because of their high adaptability and degrading potential, modified Fenton technologies have recently gained popularity, as discussed by certain authors [10], [12].

Biological Treatment

The removal of environmental contaminants by living things or extracellular enzymes is a function of biological treatment. In biological treatment microorganism played a very important role. organisms utilizes substrates as sources of energy and carbon during organic metabolism. Additionally, during a redox reaction during the growth of an organism, the substrate material can function as an electron donor. In biotreatment technologies, the organism like bacteria, yeast, algae, filamentous fungi or plants, is employed to eliminate contaminants [13]. Composting, bioreactors, biofilters, bio-stimulation, and other biological treatments have the advantages of low costs, high removal rates, absence of secondary contamination, and others [14]. According to research on the anaerobic bacterial breakdown of crude methanol, microbes in sediment entirely broke down the substance. Environmental factors, the type and number of microorganisms present, and the properties of the contaminants all have an impact on the effectiveness of biological treatment [15]. Study by Aziz and Aziz (2011) came to the concllusion that during the biotreatment of landfill leachate, Aeration rate growth led to a rise in chemical oxygen demand (COD) concentration [16]. According to Joss et al. (2009), poisonous chemicals prevent microbes from ammonifying things [17]. Additionally, Lu et al. (2010) discovered that soil contaminated with petroleum still requires treatment after composting [18]. As a result, the use of biological treatment in conjunction with other technologies like Fenton, photo-Fenton, electron-Fenton, etc. has raised significant concerns [6].

III. FENTON/BIOTREATMENT METHOD

Since wastewater has a complex composition, it is always necessary to do preliminary analyses of the wastewater before selecting the best treatment options. Analysis is done on the variables biochemical oxygen demand (BOD), chemical oxygen demand (COD), toxicity and total organic carbon (TOC) Highly hazardous wastewater needs to go through the Fenton process first. After the Fenton process, wastewater could be disposed of biologically if the COD value of highly biodegradable wastewater cannot comply with discharge limitations. Fenton pretreatment should be used on partially hazardous wastewater that has a limited biodegradability and a reasonably high TOC value. The application of the combined Fenton and biological process approach required the consideration of the toxicity and biodegradability evaluations. Acute toxicity testing is typically used for toxicity studies and involves the use of microorganisms including D. magna, Vibrio fischeri [19], Pseudomonas (fluorescens or putida), Selenastrum capricornutum, and Escherichia coli, etc. [20].

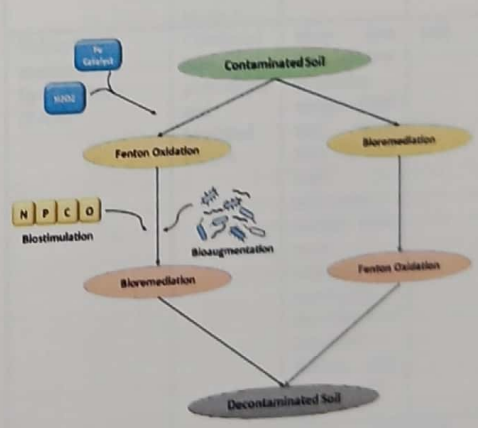

Fig. 2. Fenton and biotreatment for contaminated soil

When utilising a combined Fenton oxidation method with biological treatment, there are a number of factors to take into account.

a) A chemical oxidant's recommended dosage. For instance, using too many oxidizers would result in excessive effluent mineralization, which would result in a lack of carbon supply for microorganims in the next biotreatment.

b) Impact of the Fenton treatment on local microorganisms. For instance, the pH range between 2.8 and 3.0 is ideal for the Fenton reaction, which certain local microorganisms could find beneficial for their growth..

c) The prevention of substances from one process from

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entering another. Organic material might be created during the biotreatment process, which would then react with catalysts in the subsequent Fenton treatment to minimise the hydroxyl radicals formation.

d) Competitive environment for hydroxyl radicals. Some environmental elements, like SOM (soil organic matter) in soil, might compete with target contaminants for hydroxyl radicals. Numerous researches discovered that the competition resulted in a decrease in remedial effectiveness.

e) Various parameters such pH, temperature, volatile solids, total suspended solids, TOC, and nutritional components are analysed. The nutrients that microbes consume have an impact on their growth.

f) Because hydroxyl radicals are nonselective, there is another practical factor to consider when remediating polluted soil: how soil humus may react to hydroxyl radicals. When determining the reaction processes, route, and kinetics in this instance, the constraints must be consider [6].

For implementing Fenton/biotreatment, investigations of the various parameters mentioned above could, in general, offer useful information. Additionally, the integrated approach has been effectively used in emediation of wastewater and contaminated soil.

A. Soil remediation using Fenton/Biotreatment

Numerous pollutants affect both the water and the soil. PAHs (polycyclic aromatic hydrocarbons), heavy metal, and fertilisers, pesticides from industrial operations and agricultural practises, among other things, are the principal contaminants of soil. Unacceptable environmental dangers are produced by the harmful effects of soil contamination on biota. Because of the possible risk that contaminants posed to the surrounding environment, remediating polluted soil is a serious issue. For soil remediation, common techniques included soil washing, burning, filling, chemical oxidation, and phytoremediation. Biotreatment or Fenton oxidation were also regarded as effective remediation techniques [21]. However, the Fenton reaction had chemical impacts on the environment of the soil, and biotreatment was frequently unable to effectively remove very harmful contaminants. As time went on, researchers created a combined Fenton and biotreatment method for remediating contaminated soil, which demonstrated more treatment effectiveness than a solitary method. Jho et al. (2014) successfully remedied TPH (total petroleum hydrocarbon)-contaminated soil using the Fenton method and bioaugmentation [22]. Bioaugmentation describes the process of increasing microbial activity in soil by adding microorganisms. Another method of biotreatment called "bio-stimulation" aims to improve the naturally existing microorganisms in soil by altering nutrients or providing electron donors and electron acceptors. Goi et al. (2006) discovered that bio-stimulation and the Fenton-like process worked well to remediate oil-contaminated soil, with a 74% oil removal rate. It was also shown in another investigation that the combined bio-stimulation and Fenton process resulted in an 88.9% clearance rate of TPH in weathered oil-contaminated soil [23]. Direct electro-Fenton was utilised by Xu et al. (2015) prior to biotreatment. Pyrene's elimination rate increased from 50% to to 91.0% [24]. It has been demonstrated that the combination treatment is an effective technique for cleaning up polluted soil [6].

Targeting pollutants	Treatment techniques	Result	References
Linear alkyl benzene (LAB)	Fenton/bio-stimulation (Acinetobacter, Rhodococcus etc.)	65% of the LAB is deteriorated	[25]
Polycyclic aromatic hydrocarbons (PAH)	Combined bio-stimulation and Modified Fenton	More than 98% of low molecular weight PAH and more than 70% of high molecular weight PAH were removed by combining the effects of Modified Fenton and biodegradation.	[26]
Diesel	Bioremediation, Fenton, and modified Fenton	59% and 57% TPH removed	[27]
Crude oil	Combining Fenton process and biological treatment	After 5 and 7 days, respectively, a single Fenton at pH 3 degraded by 36 and 57%. Fenton and biological processing worked together to degrade soil crude oil by roughly 75%. A biological process eliminated 61%.	[28]

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Tank oil	Bioremediation and modified Fenton	Oil fraction (C10-C40) became more degradable and 93% of tank oil was removed after applying modified oxidation	[29]
Petroleum	Fenton-like biodegradation-based pretreatment (inoculums isolated from oil polluted soil)	Destroyed 50.6% of the total dichlorometh ane organics (TEO) and significantly decreased soil toxicity	[30]
Benzo [a] pyrene (BaP)	Fusarium solani with cyclodextrins in combination with Fenton oxidation and biotreatment	When Fenton oxidation and biotreatment were used together, 25% more BaP was destroyed than when they were used separately, which produced results of 8% and 16%, respectively	[31]
Manufactured gas Plant	Surfactants, biodegradation, and modified Fenton	98% of 2- or 3-ring hydrocarbons are removed, and 70%–85% of 4- or 5-ring hydrocarbons are removed.	[32]
Tetrachlorodibenzodioxin (TCDD)	Treatment with Fenton and aerobic biological treatment	2,3,7,8-tetrachlarodibenzo-p-dioxin (TCD-D) was 99% destroyed.	[33]
Polychlorinated biphenyl (PCB)	Combined Fenton/ biotreatment	2-chlorinated biphenyl degraded 95%	[33]

Conclusion

This review paper begins by providing a brief overview of the Fenton processes and biological treatment, followed by a discussion of the factors to be considered while building a combined system, such as toxicity and biodegradability testing. Additionally, the focus of this review is on significant Fenton/biotreatment applications in soil remediation. For time, money, and labour savings, authors in these applications put a lot of effort into changing the circumstances that apply to chemical and biological processes. Numerous attempts have been performed so far for the combined system, and the study findings have achieved significant advancements, encouraging the use of these attractive combined treatments.

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