"Effects of Cerium Oxide Nanoparticles on *Brevibacillus brevis* Growth: A Comprehensive Study"

A Dissertation

submitted in partial fulfillment of the requirement
for the award of the degree
of

Master of Science

In

Biotechnology

Submitted by

Shivam Sharma

(2K21/MSCBIO/48)

Under the supervision of

Prof. JaiGopal Sharma

Department of Biotechnology
Delhi Technological University
(Formerly Delhi College of Engineering)
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CANDIDATE'S DECLARATION

I hereby certify that the work which is presented in the research work entitled "Effects of Cerium Oxide Nanoparticles on *Brevibacillus brevis* Growth: A Comprehensive Study" in fulfillment of the requirement for the award of Degree of Masters of Sciences in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 2-Jan-2023 to 28-May-2023, under the supervision of Prof. Jaigopal Sharma.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been accepted in SCI/SCI expanded /SSCI/ Scopus Indexed Journal OR peer-reviewed Scopus Index Conference with the following details.

Title of the Paper: Bioremediation of Environmental Pollutants using White Rot Fungi.

Author Names: Shivam Sharma Prachi Choudhary and Jai Gopal Sharma.

Name of the conference: 2nd International Conference on Advances in Water Treatment and Management(ICAWTM-23).

Conference Date and Venue: 11-12 March 2023 at Pandit Deendayal Energy University (PDEU), Gandhinagar, Gujarat, India.

Registration: Done

Status of Paper: In Proceedings.

Date of Paper Acceptance: 21 Feb 2023

Place: Delhi

Shivam Sharma

Certificate

This is to certify that the Project dissertation titled "**Effects of Cerium Oxide Nanoparticles on *Brevibacillus brevis* Growth: A Comprehensive Study**." which is submitted by **Shivam Sharma**, Roll no. **2k21/MSCBIO/48**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Sciences, is a record for the project work carried out by the student 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.

Place: Delhi

Date:

30/05/2023

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ACKNOWLEDGMENT

I would like to express my deepest gratitude to our research guide **Professor Jaigopal Sharma, Department of Biotechnology, Delhi Technological University** for his unstinted inspiration, invaluable guidance, encouragement, keen interest, good wishes and valuable suggestions throughout my entire research tenure.

I express my kind regards and gratitude to **Professor Pravir Kumar, Head of Department, Department of Biotechnology, Delhi Technological University** and all the faculty members for helping in my project.

I am thankful to **Neha ma'am, Megha ma'am** and all my classmates for giving me moral boost and making my hopes alive with energy and enthusiasm to carry out the present work and helping hand at every. My sincere thanks to my parents and family members for the encouragement and moral support I received during the tenure of study.

Place: Delhi

Shivam Sharma

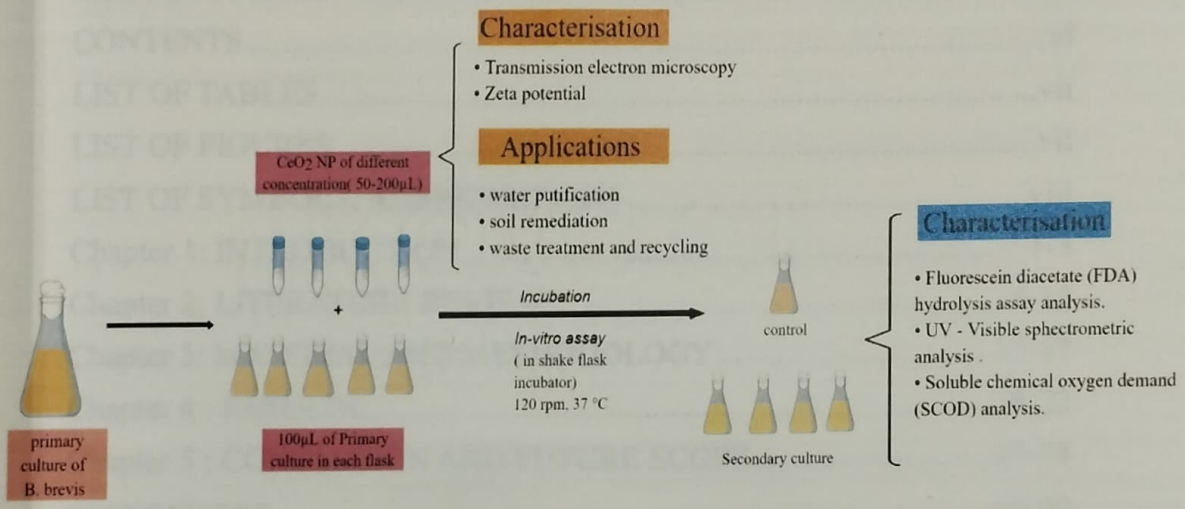
"Effects of Cerium Oxide Nanoparticles on *Brevibacillus brevis* Growth: A Comprehensive Study"

ABSTRACT

The impact of cerium oxide nanoparticles (CeO_2 NPs) on the growth of Brevibacillus brevis, a Gram-positive bacterium with potential industrial applications, was investigated. CeO_2 NPs have gained significant attention due to their unique physicochemical properties and wide range of applications. However, their potential toxicity to microorganisms, including bacteria, raises concerns regarding their environmental and biological effects.In this study, B. brevis cultures were exposed to varying concentrations of CeO_2 NPs, ranging from 0 to 200 µl, to assess their impact on bacterial growth. The CeO_2 NPs were characterized for their size, shape, and surface charge to ensure consistent and reliable experimental conditions. The growth of B. brevis was monitored using optical density measurements and colony counting techniques.

The results revealed a concentration-dependent effect of CeO_2 NPs on B. brevis growth. At lower concentrations (50-100 µl), the CeO_2 NPs had a stimulatory effect on bacterial growth, leading to increased optical density and colony counts compared to the control group. This stimulatory effect could be attributed to the potential role of CeO_2 NPs as nanocatalysts, promoting cellular metabolism and enhancing nutrient uptake. However, as the concentration of CeO_2 NPs increased beyond 100 µl, a dose-dependent inhibitory effect on B. brevis growth was observed. Higher concentrations of CeO_2 NPs resulted in reduced optical density and colony counts compared to the control group. This inhibition could be attributed to the potential toxicity of CeO_2 NPs, causing cellular damage, oxidative stress, or disruption of metabolic pathways in B. brevis. These findings highlight the complex and concentration-dependent nature of the impact of CeO_2 NPs on B. brevis growth. While lower concentrations may promote growth, higher concentrations exhibit inhibitory effects. Understanding the mechanisms underlying these effects is crucial for assessing the potential risks and benefits associated with the use of CeO_2 NPs in various applications.Further investigations are needed to elucidate the underlying mechanisms of CeO_2 NP-induced growth stimulation or inhibition in B. brevis, including the potential involvement of oxidative stress, nanoparticle uptake, and interactions with cellular components. These findings contribute to the growing body of knowledge on the interactions between nanoparticles and bacteria, facilitating the development of safer and more sustainable nanomaterials in various fields.

GRAPHICAL ABSTRACT



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LIST OF ABBREVIATIONS

Ce-NPs	Cerium nanoparticles
NPs	Nanoparticles
SEM	Scanning electron microscopy
XPS	X-ray photoelectron spectroscopy
SAED	Selected area electron diffraction
XRD	X-ray diffraction
FE-SEM	Field emission scanning electron microscopy
HR-TEM	High-resolution TEM
DLS	Dynamic light scattering
FTIR	Fourier transform infrared spectroscopy
EDS	Energy dispersive spectroscopy
UV-Vis	UV-visible spectroscopy
TEM	Transmission electron microscopy
Rpm	rotations per minute
AFM	Atomic force microscopy
ROS	Reactive oxygen species
OD	Optical density

Chapter 01

Introduction

In recent years, there has been considerable interest in cerium oxide nanoparticles (CeO2 NPs) due to their distinct physical and chemical characteristics, which offer promising prospects for diverse fields. These nanoparticles exhibit remarkable redox activity, catalytic behavior, and high surface area, making them attractive for diverse technological applications, including electronics, energy storage, and environmental remediation. However, concerns have been raised regarding their potential impact on biological systems and environmental ecosystems, particularly with regard to microbial growth and ecosystem balance. Brevibacillus brevis, a Gram-positive bacterium commonly found in various environments such as soil, water, and plants, has been identified as a model organism for investigating the impact of nanoparticles on microbial growth. Its sensitivity to environmental stressors and its ecological significance make it an ideal candidate for studying the effects of CeO2 NPs. Understanding the interactions between CeO2 NPs and B. brevis is crucial for assessing the potential risks associated with nanoparticle exposure and ensuring their safe utilization in various applications.

This study presents a comprehensive review of the investigations conducted on the influence of cerium oxide nanoparticles on the growth of Brevibacillus brevis. It explores the physicochemical properties of CeO2 NPs, their interactions with B. brevis, and the potential mechanisms underlying their toxicity. Furthermore, it discusses the ecological considerations associated with CeO2 NP exposure and their potential effects on microbial communities and environmental ecosystems.

Characteristics related to the physical and chemical attributes of cerium oxide nanoparticles:

Cerium oxide(CeO_2) nanoparticles possess unique physicochemical properties that contribute to their biological interactions and potential toxicity. One key aspect is their size, which often falls within the nanoscale range (1-100 nm).[1] The small size allows for increased surface area, leading to enhanced reactivity and potential interactions with biological systems. Additionally, the shape of CeO2 NPs, such as spherical or rod-like structures, can influence their cellular uptake and toxicity.[2]

The surface characteristics of CeO2 NPs, including surface charge and stability, also play a crucial role in their interactions with microbial cells.[3] [4]The surface charge affects the electrostatic interactions between nanoparticles and the bacterial cell membrane, influencing their uptake and internalization mechanisms. Furthermore, the stability of CeO2 NPs in various media, such as water or growth media, affects their aggregation and subsequent interactions with microbial cells.

Interaction of Cerium Oxide Nanoparticles with Brevibacillus brevis:

The interaction of CeO2 NPs with Brevibacillus brevis involves a series of processes, including nanoparticle uptake, internalization, and intracellular distribution. Several mechanisms have been proposed for the uptake of nanoparticles by bacterial cells, including passive diffusion, endocytosis, and active transport. The mode of uptake can depend on the characteristics of both the nanoparticles and the microbial cells. Internalized CeO2 NPs can be localized in various cellular compartments, such as the cytoplasm, cell membrane, or organelles. The distribution of nanoparticles within the bacterial cells can impact their potential toxicity and subsequent cellular responses.[5] Understanding the internalization mechanisms and intracellular fate of CeO2 NPs in B. brevis is essential for comprehending their impact on microbial growth.

Impact on Brevibacillus brevis Growth:

Assessing the impact of CeO2 NPs on Brevibacillus brevis growth is crucial for understanding their potential toxicity. Numerous studies have demonstrated that CeO2 NPs can exert adverse effects on bacterial growth, leading to growth inhibition and altered physiological responses.[6] The extent of growth inhibition can depend on various factors, including nanoparticle concentration, exposure time, and growth conditions.

Oxidative Stress Induction:

One significant mechanism underlying the toxicity of CeO_2 NPs is the generation of reactive oxygen species (ROS). CeO_2 NPs possess redox-active properties that enable them to switch between Ce3+ and Ce4+ oxidation states.[2] This redox cycling can lead to the generation of ROS, including superoxide radicals (O2•-) and hydrogen peroxide (H2O2), which can induce oxidative stress in bacterial cells.

Brevibacillus brevis possesses antioxidant defense mechanisms to counteract oxidative stress. These defense mechanisms include enzymatic antioxidants such as superoxide

dismutase (SOD) and catalase, as well as non-enzymatic antioxidants like glutathione. However, excessive ROS production resulting from CeO2 NP exposure can overwhelm these defense systems, leading to oxidative damage and growth inhibition.

Mechanisms of CeO2 NP Toxicity:

The mechanisms underlying CeO2 NP toxicity in Brevibacillus brevis are multifaceted and may involve various cellular processes. One proposed mechanism is the direct interaction between CeO2 NPs and cellular components, leading to structural and functional damage. The small size and high surface area of CeO2 NPs facilitate their penetration into bacterial cells, allowing them to interact with intracellular components such as proteins, DNA, and membranes.Another proposed mechanism is the oxidative stress induced by CeO2 NPs. ROS generated by the redox cycling of CeO2 NPs can cause oxidative damage to cellular components, including lipids, proteins, and DNA. [1]Additionally, CeO2 NPs may interfere with essential cellular processes and signaling pathways, further contributing to growth inhibition and altered physiological responses in B. brevis.

Ecotoxicological Considerations:

Understanding the potential ecological consequences of CeO2 NP exposure is crucial for assessing their overall impact on microbial communities and environmental ecosystems. Microorganisms play vital roles in maintaining ecosystem balance, nutrient cycling, and overall ecosystem health. Therefore, the effects of CeO2 NPs on the broader microbial community should be considered.CeO2 NPs released into the environment can interact with soil and water microbial communities, potentially altering their composition, diversity, and ecological functions.[7] The specific effects can depend on various factors, including nanoparticle concentration, exposure duration, and environmental conditions. Assessing the ecotoxicological effects of CeO2 NPs on microbial communities can provide insights into their potential risks and broader environmental implications.

By exploring the physicochemical properties of CeO2 NPs, their interactions with B. brevis, and the potential mechanisms underlying their toxicity, we can gain insights into their potential risks and develop strategies to ensure their safe application.[8] Furthermore, considering the ecological implications of CeO2 NP exposure is crucial for assessing their overall impact on microbial communities and environmental ecosystems. Further research is needed to unravel the complexities of CeO2 NP toxicity and their implications for environmental safety.

Chapter 02.

Literature Review

Nanoparticles have gained significance in bioremediation due to their unique properties that can enhance the efficiency and effectiveness of the process. 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.[9]

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).

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.[10]

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

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.[9]

Synergistic effects: Nanoparticles can exhibit synergistic effects when combined with 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.

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.

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.[11]

Cerium oxide nanoparticles (CeO2) have gained significant attention in the field of bioremediation due to their unique properties and potential applications. Here are some key points highlighting the importance of cerium oxide nanoparticles in bioremediation:

Catalytic Activity: CeO2 nanoparticles possess excellent catalytic properties, including redox activity and oxygen storage capacity. These properties make them effective catalysts for various bioremediation processes, such as the degradation of organic pollutants and the removal of toxic metals from contaminated environments.[12]

Reactive Oxygen Species (ROS) Generation: CeO2 nanoparticles can generate reactive oxygen species (ROS) when exposed to certain environmental conditions. ROS, such as hydroxyl radicals, superoxide radicals, and singlet oxygen, have strong oxidative capabilities and can degrade organic pollutants and inhibit the growth of harmful microorganisms.

Adsorption Capacity: CeO2 nanoparticles exhibit a high surface area and can adsorb contaminants onto their surfaces. This adsorption ability enables CeO2 nanoparticles to effectively remove pollutants from aqueous solutions and soil matrices, thereby assisting in the remediation of contaminated sites.

Metal Ion Stabilization: CeO2 nanoparticles have been found to stabilize metal ions, such as arsenic and chromium, by converting them into less toxic forms. This stabilization prevents the release of hazardous metal ions into the environment and reduces their bioavailability, thereby minimizing their detrimental effects on ecosystems.

Environmental Compatibility: CeO2 nanoparticles are considered environmentally compatible due to their low toxicity compared to other nanoparticles. However, it is essential to conduct thorough risk assessments to ensure their safe application in bioremediation

practices.

Overall, the unique properties of cerium oxide nanoparticles make them valuable tools in bioremediation processes. Their catalytic activity, ROS generation, adsorption capacity, metal ion stabilization, antibacterial properties, and environmental compatibility contribute to their significance in addressing environmental pollution and promoting sustainable remediation strategies.[13] Continued research and development in this field hold promise for harnessing the full potential of cerium oxide nanoparticles in bioremediation applications.

2.1 Importance of Brevibacillus bacteria in bioremediation

Brevibacillus bacteria are a group of gram-positive, rod-shaped bacteria belonging to the phylum Firmicutes. They are widely distributed in diverse habitats, including soil, water, plants, and the gastrointestinal tracts of animals. Brevibacillus species are characterized by their ability to form endospores, which are highly resistant structures that allow them to survive harsh environmental conditions.[14] Brevibacillus bacteria play a crucial role in bioremediation due to their diverse metabolic capabilities and ability to degrade a wide range of contaminants.

Here are some key points highlighting the importance of Brevibacillus bacteria in bioremediation:

a) Versatility in contaminant degradation: Brevibacillus bacteria are highly valuable in bioremediation due to their remarkable versatility in degrading different types of contaminants. These bacteria possess a diverse range of enzymatic systems that allow them to break down various classes of pollutants commonly found in contaminated environments. For instance, they have demonstrated proficiency in degrading organic compounds like hydrocarbons, which are commonly found in petroleum and oil spills. Brevibacillus bacteria can efficiently metabolize these hydrocarbons, including both simple and complex compounds, contributing to the cleanup of polluted sites.

Moreover, Brevibacillus bacteria have been shown to be effective in degrading pesticides, which are widely used in agricultural practices and can contaminate soil and water systems. These bacteria possess enzymes capable of breaking down pesticide molecules, thus reducing their persistence and potential harm to ecosystems.[15]

In addition to organic compounds, Brevibacillus bacteria also exhibit the ability to participate in the bioremediation of heavy metals. They possess mechanisms for the reduction, precipitation, and volatilization of heavy metal ions, such as chromium and arsenic. Through

these processes, Brevibacillus bacteria can transform and immobilize these toxic metals, reducing their availability and potential for environmental harm.

The versatility of Brevibacillus bacteria in degrading a wide range of contaminants makes them highly valuable in bioremediation efforts. Their enzymatic capabilities enable them to target different classes of pollutants, allowing for a comprehensive approach to pollutant removal and environmental cleanup. By harnessing the degradation potential of these bacteria, bioremediation strategies can be tailored to specific pollutants and contaminated sites, leading to more efficient and sustainable remediation processes.

b) Adaptability to harsh environmental conditions: Brevibacillus bacteria are known for their remarkable adaptability to thrive in various challenging environmental conditions, making them highly valuable for bioremediation purposes. [16]These bacteria have demonstrated the ability to survive and grow in a wide range of conditions, including extreme temperatures, pH levels, and salinity. One significant aspect of Brevibacillus bacteria is their tolerance to extreme temperatures. They have been found in environments with both high and low temperatures, such as hot springs and cold regions. This adaptability to different temperature ranges enables them to function effectively in environments where temperature fluctuations are common, including industrial sites, where elevated temperatures can be encountered.

In addition to temperature, Brevibacillus bacteria can also withstand extreme pH levels. They have been isolated from acidic and alkaline environments, highlighting their capability to adapt to a wide pH range. This adaptability is particularly advantageous in bioremediation scenarios where the pH of the contaminated site may vary significantly.

Furthermore, Brevibacillus bacteria have shown tolerance to high salinity conditions. They have been found in environments with high salt concentrations, such as saline soils and marine sediments. [17], [18]This ability to thrive in saline conditions makes them well-suited for bioremediation efforts in areas affected by saltwater intrusion or industrial sites where high salinity is a factor.[18]

Overall, the adaptability of Brevibacillus bacteria to extreme temperatures, pH levels, and salinity expand their potential applications in bioremediation. Their ability to survive and function in diverse environmental conditions makes them suitable candidates for addressing contamination issues in a wide range of settings, including industrial wastewater, oil spills, and polluted soils.

c) Production of specialized enzymes: Brevibacillus bacteria play a crucial role in bioremediation due to their ability to produce a wide array of specialized enzymes. These

extracellular enzymes are instrumental in the degradation of complex contaminants, including organic pollutants, found in contaminated sites.

One of the important enzymes produced by Brevibacillus bacteria is lipase. Lipases are responsible for the breakdown of complex lipid compounds, such as fats and oils, into simpler components like fatty acids and glycerol. This enzymatic activity aids in the remediation of environments contaminated with hydrocarbon-based pollutants, such as oil spills or industrial effluents.

Proteases are another class of enzymes produced by Brevibacillus bacteria. These enzymes play a crucial role in the degradation of proteins, breaking them down into amino acids. This enzymatic activity is vital for the efficient breakdown of organic matter, including protein-based pollutants like animal waste or protein-rich industrial byproducts.

Esterases, produced by Brevibacillus bacteria, are involved in the hydrolysis of ester bonds. These enzymes help in the degradation of ester-containing compounds, such as certain pesticides, herbicides, and plasticizers. By breaking down these complex esters, Brevibacillus bacteria contribute to the detoxification and transformation of these contaminants into less harmful substances.

Oxidases are enzymes produced by Brevibacillus bacteria that catalyze oxidation reactions. These enzymes are involved in the breakdown of various organic compounds, including aromatic pollutants like polycyclic aromatic hydrocarbons (PAHs). The oxidases aid in the conversion of these complex compounds into simpler and more manageable forms.

The production of these specialized enzymes by Brevibacillus bacteria demonstrates their capacity to enzymatically degrade a wide range of complex contaminants. By releasing these enzymes into their surrounding environment, Brevibacillus bacteria facilitate the breakdown of pollutants, transforming them into less toxic and more biodegradable compounds. This enzymatic activity is crucial for the success of bioremediation efforts, as it enables the bacteria to effectively target and metabolize specific pollutants, contributing to the restoration of contaminated ecosystems.[18]

d) Biofilm formation and attachment: Brevibacillus bacteria are recognized for their capability to form biofilms, which are complex and organized communities of microorganisms that adhere to surfaces. Biofilms offer several advantages to Brevibacillus bacteria, particularly in the context of bioremediation.

Biofilm formation enables Brevibacillus bacteria to establish a protected microenvironment that promotes their growth and survival. Within the biofilm, bacterial cells are embedded in a matrix composed of extracellular polymeric substances (EPS), which provide structural

support and protect the bacteria from adverse environmental conditions, such as fluctuations in nutrient availability, pH levels, and temperature. This protective matrix also helps to shield the bacteria from the toxic effects of pollutants present in the contaminated environment.

The attachment of Brevibacillus bacteria to surfaces through biofilm formation is of significant importance in bioremediation scenarios. Contaminated surfaces, such as those found in industrial wastewater treatment systems, oil pipelines, or polluted soils, can serve as a source of nutrients and contaminants for bacterial growth. By forming biofilms, Brevibacillus bacteria effectively colonize these surfaces, establishing a stable and persistent presence.

The biofilm lifestyle of Brevibacillus bacteria enhances their ability to degrade pollutants over an extended period. Within the biofilm, bacterial cells can interact and communicate with each other, leading to coordinated metabolic activities and gene expression patterns. This communication, known as quorum sensing, allows the bacteria to optimize their degradation capabilities by synchronizing the production of enzymes and other essential factors involved in pollutant breakdown. The biofilm structure also provides a surface area for the accumulation of pollutants, facilitating their direct contact with the bacterial cells and promoting efficient degradation.Furthermore, biofilm-associated Brevibacillus bacteria exhibit increased resistance to antimicrobial agents and environmental stresses compared to their planktonic counterparts. This resilience is attributed to the protective nature of the biofilm matrix, which can act as a barrier against toxins and antibiotics. By persisting and remaining active within the biofilm, Brevibacillus bacteria can continue their bioremediation activities even under challenging conditions.

In summary, the ability of Brevibacillus bacteria to form biofilms and attach to surfaces plays a crucial role in bioremediation. Biofilm formation provides a protective niche for bacterial growth, enhances colonization of contaminated surfaces, promotes long-term pollutant degradation, and increases resistance to adverse conditions. Understanding and harnessing the biofilm-forming capabilities of Brevibacillus bacteria can contribute to the development of effective bioremediation strategies for the cleanup of polluted environments.

2.2 Importance of cerium oxide nanoparticle on positive bacterial growth

Cerium oxide nanoparticles, also known as ceria nanoparticles or nanoceria, have been widely studied for their various applications, including their potential antimicrobial properties. While cerium oxide nanoparticles are primarily known for their catalytic and antioxidative properties, there is limited research on their specific role in promoting positive bacterial growth.

In general, nanoparticles can interact with bacteria in different ways depending on their surface properties, size, and concentration. Some nanoparticles, including certain metal and metal oxide nanoparticles, have been reported to exhibit antimicrobial activity by damaging the cell membranes, disrupting cellular processes, or generating reactive oxygen species (ROS) that can be toxic to bacteria.

However, the effect of cerium oxide nanoparticles on bacterial growth appears to be more complex. Research suggests that cerium oxide nanoparticles can exhibit both antimicrobial and pro-growth effects depending on the specific conditions.[19] Here are a few potential mechanisms through which cerium oxide nanoparticles may contribute to positive bacterial growth:

Antioxidant properties: Cerium oxide nanoparticles possess a unique property known as the Ce3+/Ce4+ redox cycle, which enables them to scavenge and neutralize ROS. Excessive ROS production can lead to oxidative stress and damage bacterial cells. By reducing oxidative stress, cerium oxide nanoparticles may create a more favorable environment for bacterial growth.[20]

Enhanced cell viability: Studies have shown that cerium oxide nanoparticles can improve cell viability and protect against cell death in certain situations. Bacterial cells exposed to oxidative stress or toxic compounds may benefit from the antioxidative properties of cerium oxide nanoparticles, allowing them to survive and proliferate under challenging conditions.

Modulation of signaling pathways: Cerium oxide nanoparticles have been reported to interact with cellular signaling pathways involved in various biological processes. It is possible that these nanoparticles could influence signaling pathways in bacteria, leading to positive growth responses. However, more research is needed to understand the specific mechanisms and conditions under which this effect occurs.[21]

It is important to note that the impact of cerium oxide nanoparticles on bacterial growth can vary depending on several factors, including nanoparticle concentration, size, surface functionalization, bacterial species, and environmental conditions.[22], [23] The specific outcomes may also differ between different studies, making it challenging to draw definitive conclusions.

Overall, while cerium oxide nanoparticles have shown potential for positively influencing bacterial growth through their antioxidative properties and other mechanisms, further research is needed to better understand their precise effects and optimize their applications in this context.The cerium oxide nanoparticles are characterized using various methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray

diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) .[23] Various other methods used for characterization are enlisted in **table 1**.

2.3 Applications of cerium oxide nanoparticle

Cerium oxide nanoparticles (nanoceria) have several environmental applications due to their unique properties. Cerium oxide nanoparticles can be used in water treatment processes to remove contaminants and pollutants. They have been found to be effective in the removal of heavy metals, organic pollutants, and bacteria from water. The nanoparticles can adsorb or catalytically degrade these pollutants, helping to purify water sources.Cerium oxide nanoparticles have the ability to catalytically convert harmful gases and pollutants into less harmful compounds. They can be employed in catalytic converters and exhaust systems of vehicles and industrial processes to reduce emissions of pollutants such as nitrogen oxides (NOx), carbon monoxide (CO), and volatile organic compounds (VOCs). This helps in mitigating air pollution and improving air quality.[24]

Cerium oxide nanoparticles can be used for soil remediation, particularly for the removal of heavy metals from contaminated soils. The nanoparticles can bind with the heavy metal ions and facilitate their extraction from the soil matrix. This remediation process helps in restoring soil health and reducing the potential for contamination of groundwater and plants. Cerium oxide nanoparticles can be employed as sensors for environmental monitoring. They can detect and quantify various environmental pollutants and contaminants. By functionalizing the nanoparticles with specific receptors or indicators, they can be used to monitor parameters such as pH, temperature, heavy metal concentrations, and pollutant levels in air and water. Cerium oxide nanoparticles have shown potential in renewable energy applications. They can be used as catalysts in fuel cells and electrolyzers to enhance their efficiency and reduce the required operating temperatures. The nanoparticles facilitate the electrochemical reactions involved in energy conversion and storage processes.

It's important to note that while cerium oxide nanoparticles offer potential environmental benefits, their long-term environmental impact and potential risks are still being studied. Care must be taken in their production, use, and disposal to minimize any adverse effects on ecosystems and human health.

Techniques	Characteristics identified	References
SEM(Scanning electron microscopy)	Elucidate morphology and size	[23]
AFM(Atomic force microscopy)		[25]
HR -TEM (High-resolution TEM)		[26]
TEM (Transmission electron microscopy)		[27]
FTIR(Fourier transform infrared spectroscopy)	Analyze structure,composition and crystallinity	[19], [25]
XPS (X-ray photo-electron spectroscopy)		[23]
DLS (Dynamic light scattering)		[27]
XRD (X- Ray diffraction)		[23]
UV- visible sphectrometric analysis		[27]

Table 1: Common techniques used for characterization of nanoparticle

Chapter 03

Material and Methodology

3.1. Source of microorganism

The bacterial strain Brevibacillus brevis used in the study was sourced from the Environmental and Industrial Biotechnology Laboratory in the Department of Biotechnology at Delhi Technological University (DTU) in Delhi. A pure culture of Brevibacillus brevis was obtained from the laboratory and subsequently maintained on a nutrient agar medium. Regular subculturing of the strain was performed to ensure its viability and purity throughout the course of the experiment.

By obtaining a pure culture and consistently subculturing the strain, any potential contaminants or genetic variations were minimized, ensuring the reliability and reproducibility of the experimental results. This process allows for the controlled study of the impact of cerium oxide nanoparticles on Brevibacillus brevis growth without interference from other microorganisms or variations within the bacterial strain itself.

3.2. Preparation of CeO2 Nanoparticle Dispersion

a. Measure 1.7 grams of CeO2 nanoparticles accurately using a balance.

b. Disperse the CeO2 nanoparticles by adding them to 10 ml of water. It is important to achieve a uniform dispersion to prevent aggregation of nanoparticles. This can be achieved by using sonication, which involves subjecting the dispersion to high-frequency sound waves to break up aggregates and promote uniform distribution.

c. After dispersing the CeO2 nanoparticles, it is crucial to characterize the dispersion to assess its properties. Techniques such as dynamic light scattering (DLS) can be employed to determine the size distribution of the nanoparticles in the dispersion. Additionally, zeta potential measurements can provide insights into the surface charge and stability of the nanoparticles in the dispersion. These characterization techniques help in understanding the behavior of the CeO2 nanoparticles and ensure the quality and consistency of the dispersion used in the study.

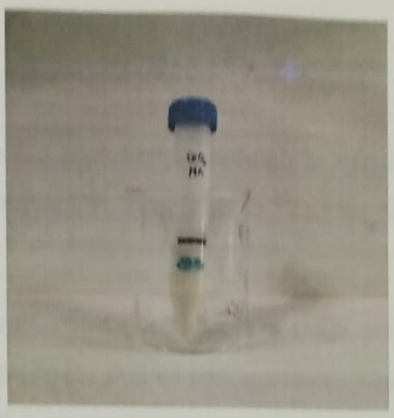

Figure 1: CeO_2 nanoparticle dispersion

3.3 Bacterial Culture Preparation:

a.) Inoculate the B. brevis culture by transferring a small amount of the pure culture into 50 ml of nutrient broth medium. The nutrient broth provides essential nutrients and a favorable growth environment for the bacteria.

b.) Incubate the culture under suitable conditions, such as appropriate temperature to allow the bacteria to grow and multiply. The duration of incubation depends on the specific growth characteristics of Brevibacillus brevis and the growth phase desired for the experiment.

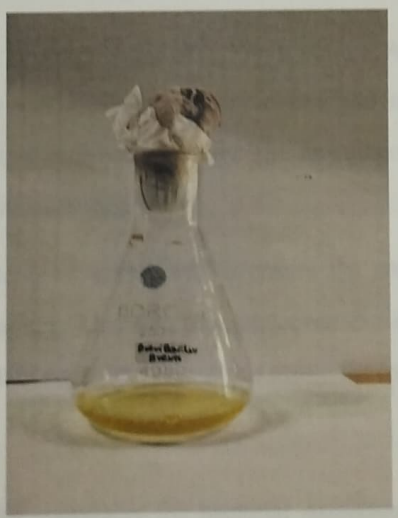

Figure 2: Brevibacillus brevis culture after incubation of 4 hours at 120 rpm/ 37 °C (OD=0.78)

3.4. Experimental Setup:

a. Take sterile falcon tubes and label them accordingly to indicate the different concentrations of CeO2 nanoparticles. For example, label tubes as 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml.

b. Using a pipette, transfer the respective volumes of CeO2 nanoparticle dispersion into each labeled Falcon tube to achieve the desired concentrations. Ensure that the nanoparticles are well-dispersed in the solution.

c. In addition to the tubes containing CeO2 nanoparticles, prepare control tubes without the addition of CeO2 nanoparticles. These control tubes will serve as a baseline for comparison.

3.5. Bacterial Exposure:

a. Prepare five nutrient broth flasks, each containing 50 ml of medium, and label them accordingly: control, 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml. These labels indicate the different concentrations of CeO2 nanoparticles that will be added to each flask.

b. Add the appropriate concentrations of CeO2 nanoparticles to each labeled flask. For the control flask, no nanoparticles are added, while the other flasks will 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 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.) Incubate the conical flasks containing the bacterial suspensions under suitable growth conditions for Brevibacillus brevis. Place the flasks in a shaker flask incubator set at 120 rpm and a temperature of 37°C. The shaker flask incubator provides the necessary agitation and controlled temperature to support bacterial growth.

b.) Monitor the growth of Brevibacillus brevis in the conical flasks by measuring the optical density (OD) at regular intervals. Use a spectrophotometer to measure 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.

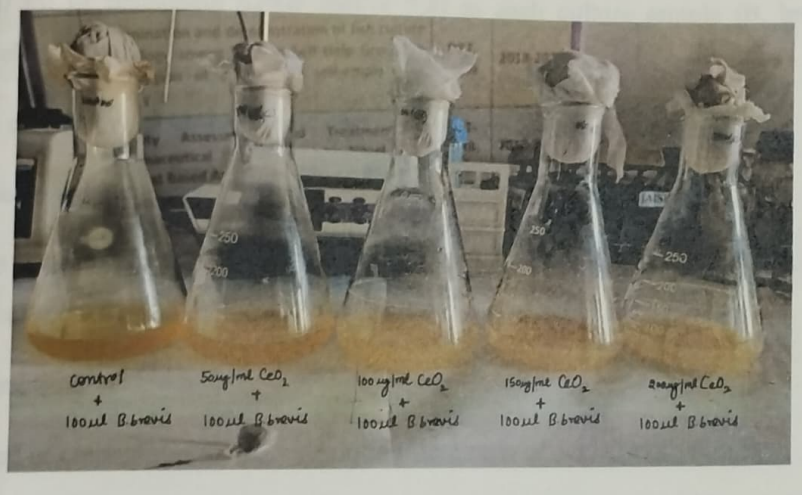

Figure 3: Nanoparticle-treated bacterial suspension

3.7. Data Analysis:

a. Plot growth curves for each nanoparticle concentration and the control group using OD values against incubation time.

b. Analyze the growth profiles and calculate growth parameters such as growth rate, lag phase duration,

3.8. Total enzyme activity

The FDA hydrolase activity assay was conducted to evaluate the ability of Brevibacillus brevis and Brevibacillus brevis_CeNPs cultures to cleave FDA into fluorescein. The assay involved several steps:

3.8.1. Preparation of Culture Samples:

- Take 2 ml of the B. brevis culture and transfer it to a suitable container.

- Take 2 ml of the B. brevis_CeNPs culture and transfer it to a separate suitable container.

- Prepare positive and negative controls:

- Positive control: Take a separate container with 2 ml of B. brevis culture.

- Negative control: Take a separate container with only FDA solution and no culture.

3.8.2. Incubation:

- Add 20 ml of phosphate buffer (pH = 7.4) to each culture sample (B. brevis and B. brevis_CeNPs).

- Add 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 shaking at 150 rpm.

- Incubate the samples for 40 minutes to allow the enzyme activity present in the cultures to hydrolytically cleave FDA.

3.8.3. Extraction:

- Add 20 ml of chloroform/methanol solution (in a ratio of 2:1) to each sample.

- Thoroughly mix the suspension.

- Centrifuge the samples for 7 minutes at 6000 rpm.

- After centrifugation, the supernatant will contain the extracted fluorescein product.

3.8.4. Measurement of Fluorescence Intensity:

- Transfer the supernatant from each sample to separate cuvettes.

- Use a fluorometer to measure the fluorescence intensity of the supernatant.

- Set the excitation wavelength to 490 nm and the emission wavelength to 519 nm.

- Record the fluorescence intensity for each sample, including the positive and negative controls

- Prepare positive and negative controls:

- Positive control: Take a separate container with 2 ml of B. brevis culture.

- Negative control: Take a separate container with only FDA solution and no culture.

3.8.2. Incubation:

- Add 20 ml of phosphate buffer (pH = 7.4) to each culture sample (B. brevis and B. brevis_CeNPs).

- Add 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 shaking at 150 rpm.

- Incubate the samples for 40 minutes to allow the enzyme activity present in the cultures to hydrolytically cleave FDA.

3.8.3. Extraction:

- Add 20 ml of chloroform/methanol solution (in a ratio of 2:1) to each sample.

- Thoroughly mix the suspension.

- Centrifuge the samples for 7 minutes at 6000 rpm.

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Chapter 04

Results

4.1 Effect of Cerium (IV) Oxide Nanoparticle on Bacterial Growth

A comparative analysis was conducted to investigate the impact of Cerium(IV) oxide nanoparticles (CeO2) on the growth of Brevibacillus brevis. The study aimed to understand how different concentrations of cerium nanoparticles affect bacterial growth.

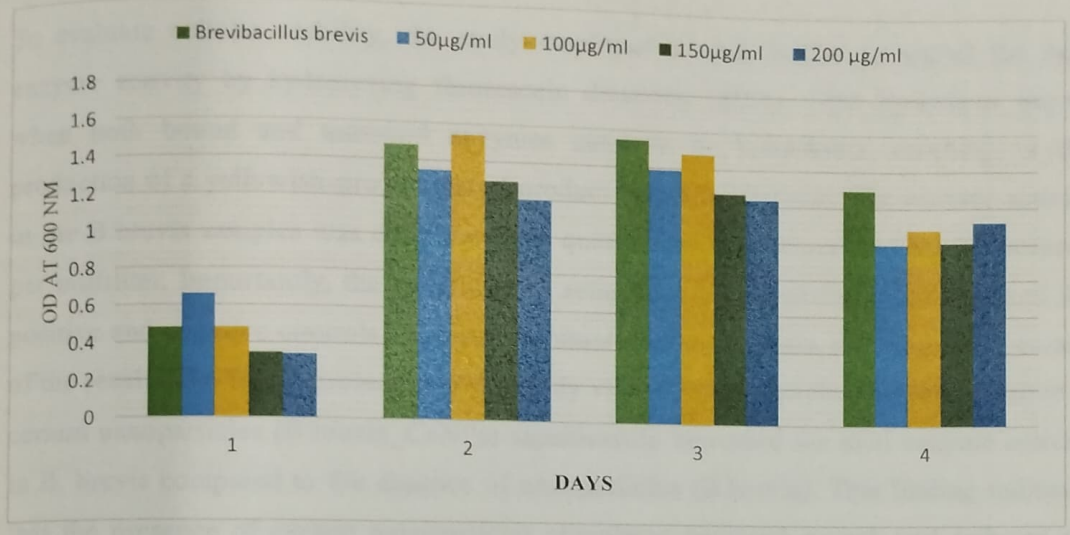
Under normal conditions, Brevibacillus brevis exhibited the typical growth curve with distinct phases: lag, log, stationary, and death. The growth rate was rapid, reaching high optical density values (OD600nm = 1.48) within 2 days of cultivation, and the stationary phase was achieved after approximately 48 hours.

However, when Brevibacillus brevis was exposed to varying concentrations of CeO2 nanoparticles (50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml), a noticeable reduction in the log phase growth was observed. This suggests that the presence of cerium nanoparticles has a microbiostatic effect on Brevibacillus brevis, and the effect is concentration-dependent.

In the absence of CeO2 nanoparticles, the growth curve of Brevibacillus brevis showed rapid and robust growth, reaching high OD values within a short period. On the other hand, cultures exposed to 100 µg/ml of CeO2 nanoparticles exhibited steeper slopes during the exponential phase compared to the control samples without CeO2. These cultures achieved the highest OD values (1.54) within 2 days.

Interestingly, when Brevibacillus brevis was grown in the presence of 150 µg/ml of CeO2, the growth curve displayed a shallower slope and lower OD600nm values compared to samples with 50 µg/ml and 100 µg/ml of CeO2. This suggests that within this concentration range, neither the maximum specific growth rate nor the maximum final OD values can be reliable indicators of the effects of nanoparticles on microbial growth.

Overall, the study highlights the concentration-dependent microbiostatic effect of CeO2 nanoparticles on Brevibacillus brevis. The growth curves demonstrate how different concentrations of CeO2 can alter the growth behavior of the bacteria, emphasizing the need to consider nanoparticle concentration when studying their impact on microbial growth.



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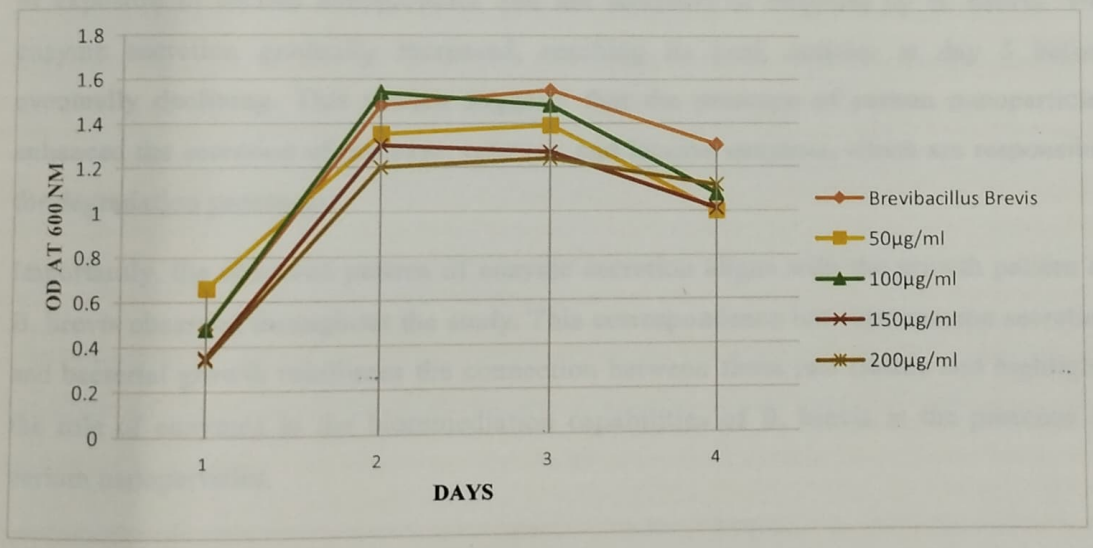

(b)

Figure 4: Growth curve of B. brevis under the influence of CeO_2 nanoparticles compared to the normal growth curve of B. brevis.

4.2 FDA analysis

To evaluate cellular viability, the study employed a method that measured the total enzyme activity by hydrolyzing fluorescein diacetate (FDA). FDA hydrolysis occurs when both bound and unbound enzymes catalyze its breakdown, resulting in the production of a yellowish-green-colored product called fluorescein. The enzyme activity in the B.brevis samples was determined by quantifying the amount of FDA hydrolyzed per milliliter. Importantly, the experimental setup maintained sterile conditions, and the positive and negative controls exhibited minimal FDA hydrolysis, ensuring the accuracy of the results.The figure presented in the study visually demonstrates that the presence of cerium nanoparticles (B.brevis_CeNPs) significantly increased the total enzyme activity in B. brevis compared to the absence of nanoparticles (B.brevis). This finding indicates that the presence of cerium nanoparticles stimulated bacterial growth and induced the secretion of enzymes crucial for bioremediation processes.

Moreover, the study unveiled an interesting relationship between the duration of exposure to cerium nanoparticles and the secretion of enzymes by B. brevis. The enzyme secretion gradually increased, reaching its peak activity at day 5 before eventually declining. This pattern suggests that the presence of cerium nanoparticles enhanced the secretion of protease, esterase, and laccase enzymes, which are responsible for degradation processes.

Importantly, the observed pattern of enzyme secretion aligns with the growth pattern of B. brevis observed throughout the study. This correspondence between enzyme secretion and bacterial growth reinforces the connection between these two factors and highlights the role of enzymes in the bioremediation capabilities of B. brevis in the presence of cerium nanoparticles.

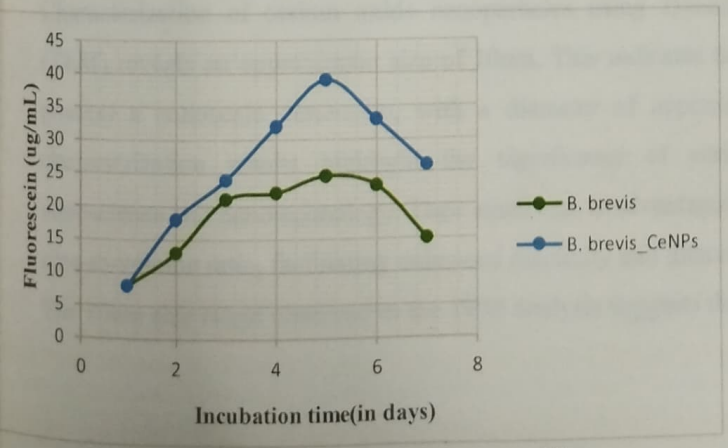

Figure 5: Total enzyme activity of B. brevis in the presence or absence of CeNPs.

4.3 Mechanism of how cerium nanoparticle support brevibacillus brevis growth

4.3.1. Enzyme activity enhancement: At the optimal concentration of cerium nanoparticles, they can act as catalysts and enhance the activity of certain enzymes within B. brevis. CeNPs can provide a suitable microenvironment or surface for enzyme binding, facilitating their catalytic function. This can lead to increased metabolic activity and improved growth of B. brevis. However, at higher concentrations, the excess CeNPs may disrupt the proper functioning of enzymes, affecting their activity and leading to a decline in bacterial growth.

4.3.2. Reactive oxygen species (ROS) modulation: Cerium nanoparticles possess antioxidant properties and can scavenge ROS, which are byproducts of normal cellular metabolism. By reducing oxidative stress, CeNPs can protect B. brevis from ROS-induced damage and maintain cellular integrity, promoting bacterial growth. However, beyond the critical concentration, excessive CeNPs may interfere with ROS regulation, leading to an imbalance in redox homeostasis and hampering bacterial growth.

4.3.3. Membrane stability: Cerium nanoparticles can interact with the bacterial cell membrane and affect its stability. At the optimal concentration, CeNPs can reinforce the membrane structure, enhancing its integrity and fluidity. This can facilitate nutrient uptake and waste elimination, promoting bacterial growth. However, higher concentrations of CeNPs can disrupt the membrane structure and compromise its functionality, adversely affecting B. brevis growth.

4.4 Characterization of nanoparticles

The properties of the cerium oxide nanoparticles used in the study assessed through the application of two techniques: dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Characterization of cerium oxide nanoparticles using Transmission Electron Microscopy (TEM) reveals an approximate size of 10nm. This indicates that the cerium oxide particles possess a nanoscale dimension, with a diameter of approximately 10 nanometers. The characterization results highlight the significance of cerium oxide nanoparticles in nanoscience and nanotechnology. Their small size is advantageous, as it offers a large surface area-to-volume ratio, facilitating enhanced reactivity and interaction with biological systems. The 10nm size range observed in the TEM analysis suggests that these nanoparticles possess

desirable properties for various applications, such as catalysis, biomedicine, and environmental remediation.

Through the utilization of DLS analysis, it was established that the average size of the cerium (IV) oxide nanoparticles measured 1778. The DLS analysis provided valuable information about the size of the cerium (IV) oxide nanoparticles. The average size of 1778 indicates the typical diameter or dimension of the nanoparticles in the sample.

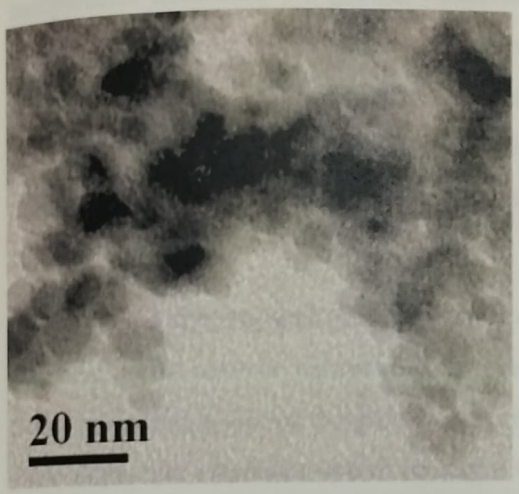

Figure 6: Transmission Electron Microscope (TEM) image of CeO_2 nanoparticle showing the size of the nanoparticle to be 10 nm (approx).

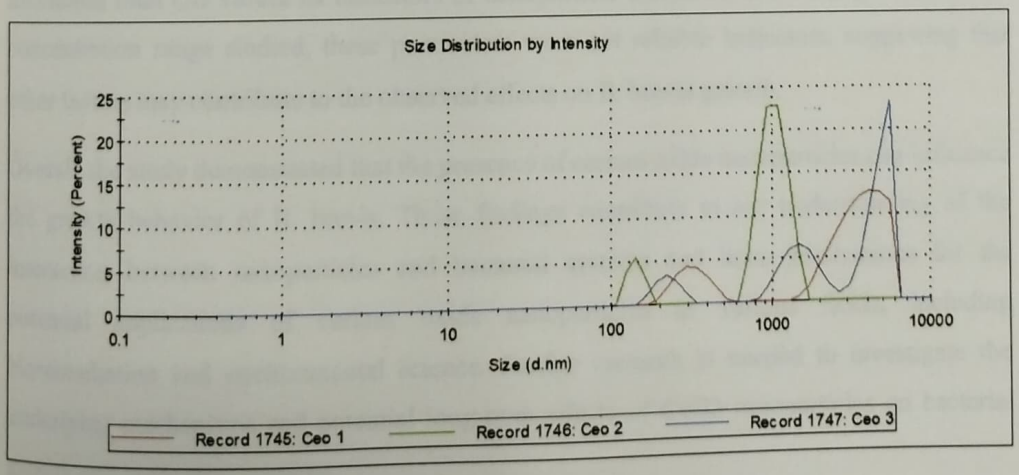

Figure 7: Size distribution intensity graph of CeO_2 nanoparticle as revealed by DLS.

CHAPTER 5

CONCLUSION AND FUTURE SCOPE

In conclusion, the study investigated the impact of cerium oxide nanoparticles (CeO2) on the growth of Brevibacillus brevis. The findings revealed that the presence of CeO2 nanoparticles influenced the growth behavior of B. brevis in a concentration-dependent manner. Under normal conditions, B. brevis exhibited a typical growth curve with distinct phases of lag, log, stationary, and death. However, when exposed to different concentrations of CeO2 nanoparticles, a reduction in the log phase was observed, indicating a microbiostatic effect. Higher concentrations of CeO2 nanoparticles led to a more significant reduction in the log phase.

The study also demonstrated that the growth curves of B. brevis with CeO2 nanoparticles showed different patterns compared to the control samples without CeO2. The presence of CeO2 nanoparticles altered the growth dynamics, resulting in variations in the exponential phase slopes and maximum optical density (OD) values. These effects were concentration-dependent, with different concentrations of CeO2 nanoparticles leading to varying growth patterns.

Furthermore, the study highlighted the importance of considering specific growth rate and maximum final OD values as indicators of nanoparticle effects on microbial growth. In the concentration range studied, these parameters were not reliable indicators, suggesting that other factors may contribute to the observed effects on B. brevis growth.

Overall, the study demonstrated that the presence of cerium oxide nanoparticles can influence the growth behavior of B. brevis. These findings contribute to our understanding of the interaction between nanoparticles and bacterial systems and have implications for the potential applications of cerium oxide nanoparticles in various fields, including bioremediation and environmental science. Further research is needed to investigate the underlying mechanisms and potential long-term effects of CeO2 nanoparticles on bacterial growth and ecological systems.

The impact of cerium oxide nanoparticles on Brevibacillus brevis growth is an interesting area of study that can have several future research implications. Here are some potential directions and areas of focus for the future scope of this study:

Toxicity Assessment: Investigate the toxic effects of cerium oxide nanoparticles on Brevibacillus brevis at different concentrations and exposure durations. Assess cell viability, growth kinetics, and metabolic activity using various assays to understand the potential adverse effects on bacterial growth.

Mechanistic Studies: Elucidate the underlying mechanisms of how cerium oxide nanoparticles interact with Brevibacillus brevis. Explore the cellular uptake, internalization pathways, and intracellular localization of nanoparticles. Study the impact on cellular processes such as oxidative stress, DNA damage, and protein synthesis.

Genomic Analysis: Perform whole-genome sequencing or transcriptomic analysis to identify the genetic and molecular responses of Brevibacillus brevis to cerium oxide nanoparticles exposure. Identify specific genes or pathways that are activated or repressed in response to nanoparticle exposure, shedding light on the bacterial defense mechanisms or stress response pathways involved.

Nanoparticle-Microbe Interactions: Investigate the physical and chemical interactions between cerium oxide nanoparticles and Brevibacillus brevis at the nano-bio interface. Study the surface properties, agglomeration behavior, and surface charge of nanoparticles and their influence on bacterial attachment, biofilm formation, and overall growth dynamics.

Environmental Implications: Assess the potential environmental implications of cerium oxide nanoparticles on Brevibacillus brevis in real-world scenarios. Investigate the effects of nanoparticles under varying environmental conditions, such as temperature, pH, and the presence of other contaminants or organic matter.

Biotechnological Applications: Explore the potential biotechnological applications of Brevibacillus brevis in the context of cerium oxide nanoparticles. Assess the ability of Brevibacillus brevis to remediate or degrade nanoparticles, or investigate their potential in nanoparticle synthesis or surface modification.

These are some potential areas of future research that can contribute to our understanding of the impact of cerium oxide nanoparticles on Brevibacillus brevis growth. It is important to note that such studies should consider proper controls, replicate experiments, and adhere to safety and ethical guidelines for working with nanoparticles.

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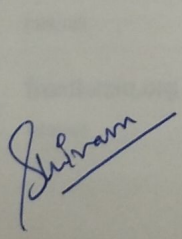

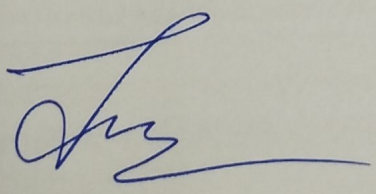

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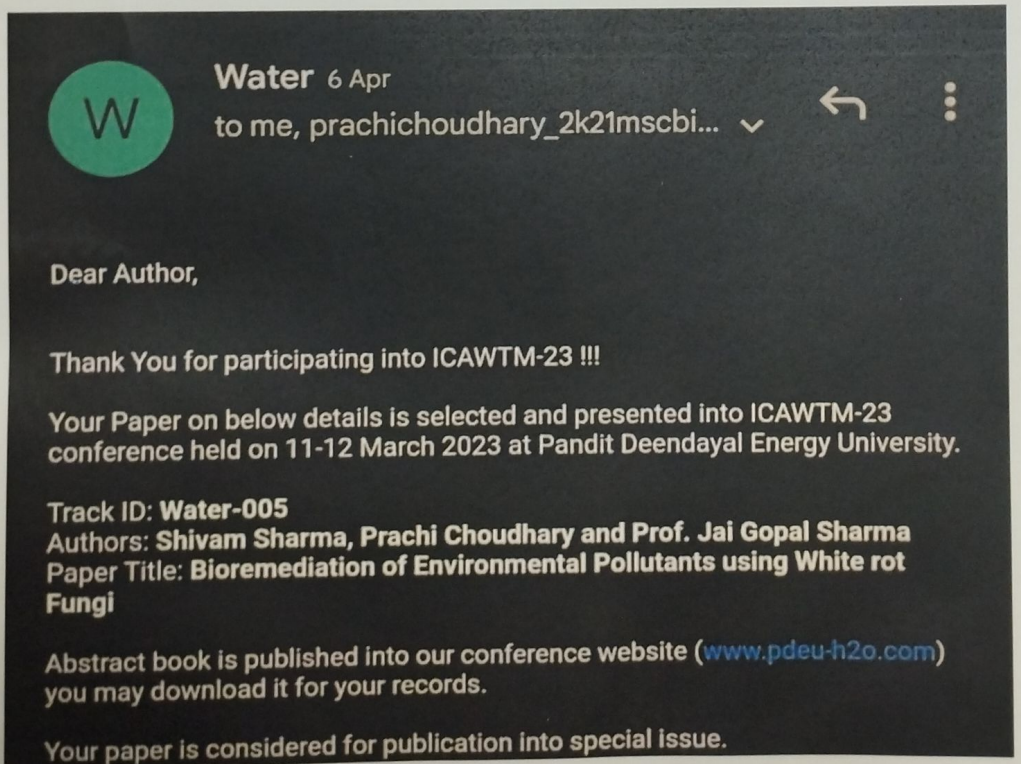

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Bioremediation of Environmental Pollutants using White rot Fungi

Shivam Sharma, Prachi Choudhary, Prof. Jai Gopal Sharma *

Delhi Technological University

Abstract: Environmental pollutants can have deleterious effects on human health and the environment. In order to mitigate these effects, various methods have been employed including chemical and physical degradation of the pollutants. However, these methods are often costly, ineffective, and/or toxic to humans and animals. Thus, researchers are exploring alternative strategies for remediation such as the use of fungi. WRF utilizing lignin as an energy source comprises fungi that break down xenobiotics and recalcitrant pollutants using peroxidases enzyme. White rot fungi degrade lignin and a bunch of other stuff with a lot of mechanisms. For lignin and environmental pollutants to be metabolized, both oxidative and reductive reactions are needed. The fungi make peroxidases that do both direct and indirect oxidation. Therefore, white rot fungi offer an excellent tool for bioremediation because they require very little energy input and work without the need for toxic chemicals or exposure to humans. One of the nutrients that promote the synthesis of enzymes, which influences pollution breakdown, is nitrogen. Different wood-rotting fungal strains should be examined for their capacity to break down xenobiotic substances using non-sterile soil samples. White rot fungi may be able to maximize pollutant degradation, which can support the creation of practical bioremediation techniques that are both effective and economical. Laboratory experiments have demonstrated the degradation of many hazardous chemicals and wastes. In addition to bacterial contamination, scaling up the process remains a technical challenge.

Keywords: *White Rot Fungi; Recalcitrant Pollutants; Peroxidases; Xenobiotics; Bioremediation; Lignin*