MICROBIAL-ASSISTED NANOBIOREMEDIATION OF METHYL RED

A DISSERTATION

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BIOTECHNOLOGY

Submitted by

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CANDIDATE'S DECLARATION

I **Ananya Chugh, Roll No. 2K22/MSCBIO/08** hereby certify that the work which is being presented in the thesis entitled **"Microbial-Assisted Nanobioremediation Of Methyl Red"** is in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Prof. Jai Gopal Sharma.

The matter presented in the thesis has not been submitted by me for the award of any degree of this or any other Institute.

My review paper is accepted in SCI/SCI expanded/SSCI/Scopus indexed journal with the following details:

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CERTIFICATE

Certified that **Ananya Chugh (2k22/MSCBIO/08)** has carried out their search work presented in this thesis entitled **"Microbial-Assisted Nanobioremediation Of Methyl Red"** for the award of the degree of Master of Science and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. This thesis embodies results of original work, and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other Institution.

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“Microbial-Assisted Nanobioremediation of Methyl Red”

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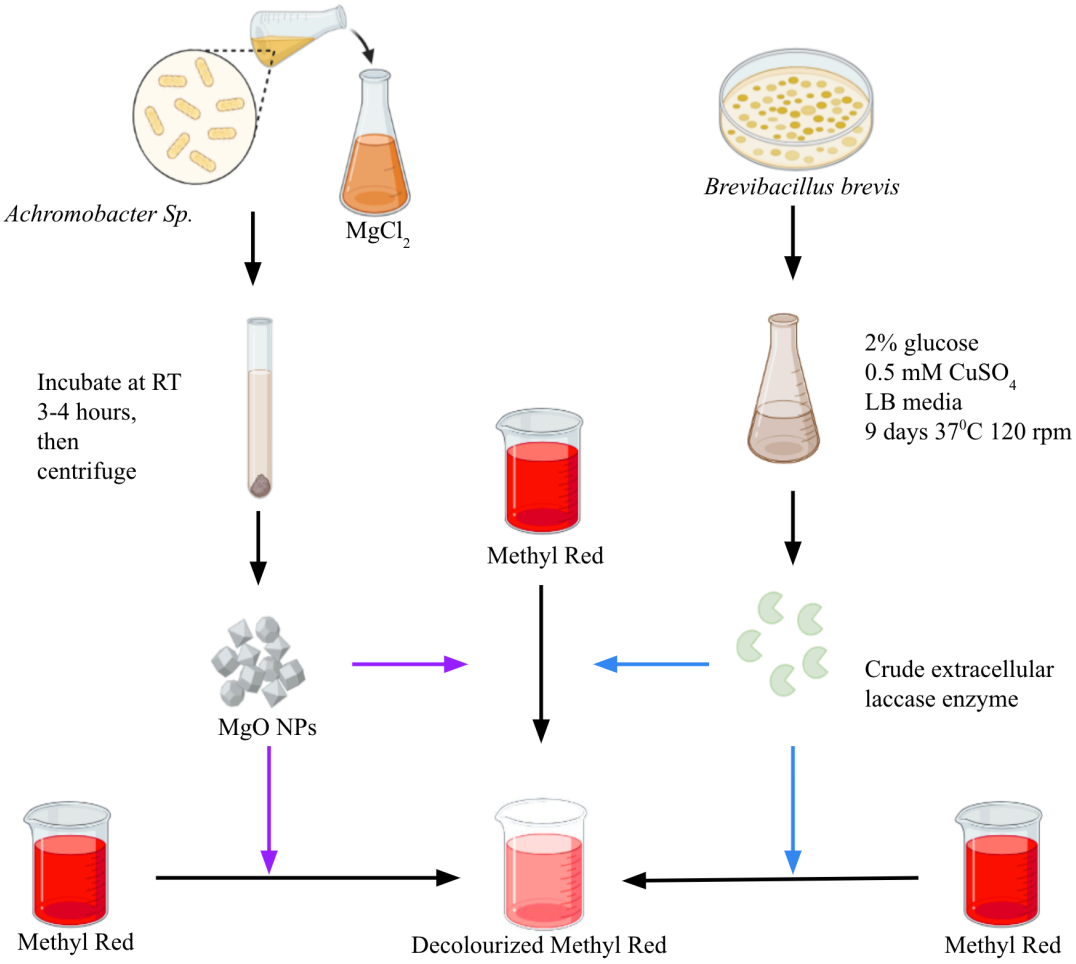
ABSTRACT

The textile industry is known to consume much more water than any other industry, such that approximately 200L of water is consumed for each kilogram of fabric. In India, the textile industry alone consumes 425,000,000 gallons of water daily. The two main processes that consume the most water are the dyeing of the fabric and washing the fabric. The problem arises, as the dye does not bind properly to the fabric and thus gets discarded as effluent into the water bodies. Apart from this the wastewater also contains tannins, lignins, high pH, high BOD and COD and heavy metals thus causing severe impact on the environment. Keeping in mind the size of the industry, it is crucial to find efficient ways to treat the effluent before releasing it into water channels. On a large scale, many physical and chemical techniques are being used. However, researchers are still in search of less hazardous, efficient and inexpensive techniques for the remediation of dye effluents. The work in this dissertation was done to understand a relatively less-tested combination of techniques. The aim was to use a microbial-assisted nanobioremediation technique to remediate a monoazo dye i.e. Methyl Red.

We synthesised MgO nanoparticles (MgO NPs) using *Achromobacter sp.* To our knowledge, it is the first time MgO NPs are being synthesised from *Achromobacter sp.* We have extracted laccase enzyme from *Brevibacillus bevris*. We used both MgO and bacterial laccase to remediate MR. And as per our knowledge, this experimental design is also being done for the first time. We saw only crude laccase degraded MR with Decolourization efficiency(DE) being 20.5%. The DE of only MgO to degrade MR was 23.3%. While the DE when both MgO and crude extracellular laccase enzyme were used together was 37.7%.

We can observe that there is a nearly 80 % increase in the DE from only laccase to using both laccase and MgO together. As speculated, combining MgO and laccase enzyme increased the degradation of MR.

GRAPHICAL ABSTRACT



LIST OF PUBLICATIONS

1. The manuscript entitled "Unveiling Future Advancements in Azo Dye Degradation and Enhanced Bioelectricity Production using Microbial Fuel Cells" has been accepted in Research Journal of Biotechnology
2. A paper entitled “Docking Study of Environmental Dyes: Insights into Affinity and Bioremediation Potential of Laccase Enzyme” was presented at the 14th International Conference On Science And Innovative Engineering – 2024 (ICSIE – 2024) held on April 27th – 28th, 2024.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/ Abbreviations	**Meaning/Full form**
MgO	Magnesium Oxide
NPs	Nanoparticles
MR	Methyl Red
FT-IR	Fourier transform-infrared spectroscopy
XRD	X-ray diffraction
CI	Colour Index
LB	Luria Broth
DE	Decolourization Efficiency
Δ	Delta
θ	Theta
φ	Phi
ψ	Psi
α	Alpha

CHAPTER 1

INTRODUCTION

The first ever synthetic dye mauve was an accidental discovery by a teenage chemist William Henry Perkin in his home lab in the 1850s. Almost immediately after this, a whole new synthetic dye industry began(Travis, 1988). A wide variety of colour ranges and most importantly cheaper dyes were now available in the market. As a result, fashion and textile production grew exponentially, but so did the environmental burden of untreated effluents being released into water bodies and polluting them (Akter et al., 2023). Keeping in mind the size of the industries using dyes today, the entire world generates approximately 60 x 10^7 - 90 x 10^9 kgs of dying process effluent annually and out of this 1/10th of this penetrates the environment via industrial wastewater (Chauhan et al., 2017).

Bioremediation i.e. the use of microorganisms to remediate pollutants from soil and water has been more promising and comparatively inexpensive than other physical and chemical techniques of remediation (Juwarkar et al., 2010). However, using only one technique may not be feasible and as efficient. In response to these challenges, innovative solutions are emerging at the nexus of nanotechnology and bioremediation. One such promising approach is microbial-assisted nanobioremediation, the combination of nanoparticle's catalytic properties with microbe-mediated enzyme activity.

Nanotechnology has managed to make its way into all industries including food, agriculture, medicine, cosmetics, etc. This is because nanomaterials have offered a whole new set of properties to already existing bulk materials like a higher ratio of surface area to volume. Metal Oxide nanoparticles are one class of nanomaterials that have recently gained interest to remediate dyes owing to their catalytic activity (Chaudhary et al., 2023). And studies have shown that biologically synthesised nanoparticles as opposed to chemically synthesised nanoparticles are less toxic, consume less energy and are

eco-friendly (Das et al., 2017). Noman et al. (Noman et al., 2020) used Cu NPS synthesized from Escherichia sp. as photocatalysts for degradation and reported 83.90%, and 76.84% for congo red and reactive black-5, respectively at 100 mg L^{-1} dye concentration (Noman et al., 2020). Green synthesized NPs have also gained a lot of interest because it is also cost-effective, eco-friendly and have efficient dye degradation potential. As reported by Laouini et al. (Laouini et al., 2021) 84.50 % decolorization efficiency of Congo red was obtained after a 50-minute reaction with Ag NPs synthesised using leaf extracts of a plant commonly called Date palm (Laouini et al., 2021).

Bioremediation of dye using the Laccase enzyme has been prominently done over the past decade. Laccase is an oxidoreductase which, by catalyzing the oxidation of aromatic molecules, both phenolic and non-phenolic, has been effective in removing various synthetic dyes including azo dyes (Kanagaraj et al., 2015).

In this study, we have used Methyl Red (MR) as our model dye. We synthesised MgO nanoparticles (MgO NPs) from soil isolate *Achromobacter sp*. To our knowledge, it is the first time MgO NPs are being synthesised from *Achromobacter sp.* Along with this we have extracted laccase enzyme from *Brevibacillus bevris* which is also a soil isolate. We used both MgO and bacterial laccase to remediate MR. And as per our knowledge, this experimental design is also being done for the first time. We speculate that combining MgO and laccase enzyme should increase the degradation of MR.

CHAPTER 2

LITERATURE REVIEW

2.1 Textile Dyes

Synthetic dyes serve a crucial role in colouration processes within various industries like food, textiles, cosmetics, leather, etc. There are 5 major classifications of dyes based on the chromophore groups present namely nitro/nitroso dyes, trimethyl methane dyes, azo dyes, indigo dyes and anthraquinone dyes as shown in Figure 2.1. The characteristic colour of dyes originates from the chromophoric, auxochromic or conjugated aromatic structures. It is chromogenic groups that enable white light to be transformed into coloured light by selectively absorbing energy (Benkhaya et al., 2020; Jamee & Siddique, 2019).

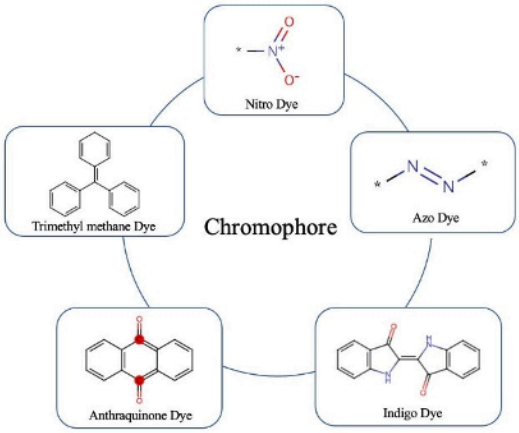

Figure 2.1: Classification of dye based on chromophore

Since seventy per cent of all dyes used in the industry are azo dyes, azo dye is the most significant. These dyes have a characteristic -N=N-, called the azo linkage. Azo dyes are

categorized according to how many azo links the dyes contain: (i) monoazo, (ii) disazo, (iii) trisazo and (iv) polyazo. Also according to their chemical structure, a corresponding Colour Index (CI) number is given to each dye (Gregory, 1990; Gürses et al., 2016).
Subsequent modifications have been introduced to dyes which have further increased the yields and colour properties, leading to a diverse range of dyes. However, the discharge of textile dyes poses a global concern due to the high concentration of pigments and additives they contain. Approximately ten to fifteen per cent of the colour used is discharged into neighbouring waterways untreated (Saba et al., 2021).

2.2 Hazards of Textile dyes

The use of synthetic dyes is directly or indirectly harmful to all life forms. The dyeing process out of all the processes in the textile industry, is the most hazardous. This is because a significant amount of chemicals and dyes do not adhere to the fibre and instead are released as a part of dye effluent (Subburaj et al., 2024). The main problem arises when this effluent enters the waterbodies in an untreated form. Firstly this effluent affects the aesthetic quality of water bodies including malodor, and unclean appearance and even impedes the penetration of sunlight; affecting photosynthesis. There are elevated levels of chemical and biological oxygen demand (COD and BOD) (Lellis et al., 2019). Because of their recalcitrant behaviour, they persist for a prolonged time and can go up the food chain due to bioaccumulation. These synthetic dyes also have the presence of auxiliary compounds and heavy metals like sulphur, acetic acid, chromium, cobalt, nickel, etc. All of these make the effluent even more toxic. Further, if these effluents are allowed to irrigate the fields, the soil productivity is lost. And if these effluents flow into rivers or get leached into underground water reserves they can directly impact the drinking water (Kant, 2011). These dyes are mutagenic, allergenic, teratogenic and non-biodegradability (Nandhini et al., 2019). Thus making it necessary to remediate the dye effluents before being released into the water bodies.

2.3 Bioremediation

By definition, bioremediation is the process of cleaning up pollutants that may be found in soil, water, or the air by using bacteria, fungi, plants, or their enzymes (P. Singh et al., 2020). For many years natural bioremediation for wastewater treatment has been observed. However, the intentional use of microorganisms to clean up toxic waste is comparatively a newer approach (Omokhagbor Adams et al., 2020). There are two major bioremediation techniques based on the site of application:

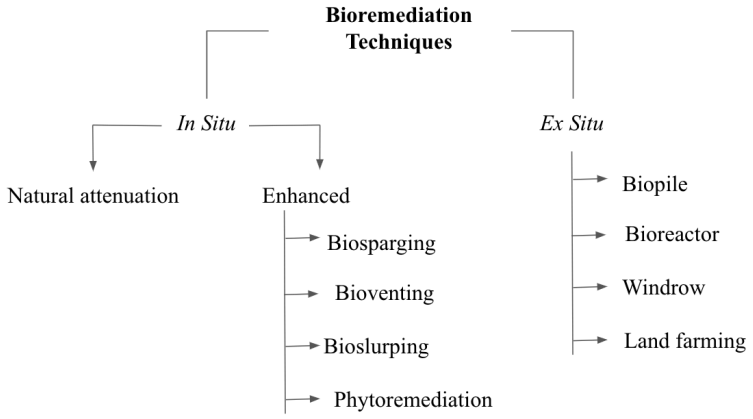

Figure 2.2: Schematic diagram of 2 major bioremediation techniques based on site

In-situ techniques include treating the polluted substances at the site of pollution. In theory, these techniques are less expensive than *ex-situ* techniques. Some of these techniques are intrinsic bioremediation i.e. natural attenuation occurs, while in other cases the remediation can be enhanced like phytoremediation, bioventing, biosparging and biosplurging. On the other hand, *ex-situ* techniques involve excavating pollutants from the site to another site for their treatment (Azubuike et al., 2016).

2.3.1 Bioremediation of textile dyes

The primary characteristics of textile wastewater are the dye effluent, high salt concentration, increased temperature, high BOD and COD and variable pH. There are

physical treatments that include physical separation methods like sedimentation and filtration. Also, there are chemical methods that include adding coagulants and flocculants. However, both these processes are very harmful to the environment and not very cost-effective (Lim et al., 2010). So, there has been an increase in interest in the third category of treatment i.e. bioremediation. Using bacteria, fungi and especially now microalgae to treat textile wastewater (Premaratne et al., 2021). To remove these textile dyes from the water, the microorganism uses one or more of the following mechanisms:

a. **Biosorption** - it is a passive mechanism, that involves using dead cells and is a metabolically independent process. Allows the contaminants to get absorbed onto the surface. This is especially helpful for toxic contaminants.

b. **Bioaccumulation** - it is an active mechanism, that involves the transport of the contaminants into the living cell and accumulates there (Filote et al., 2021).

c. **Biodegradation** - it involves a one-step or chain reaction to convert organic pollutants into simpler less toxic molecules.

2.4 Laccase Enzyme

Laccase is a member of the superfamily of multicopper oxidases (MCOs). Due to the presence of a cuperdoxin-like domain in all MCOs, they can reduce oxygen to water (Janusz et al., 2020). Laccase is a glycoprotein which may be monomeric, dimeric, or tetrameric. It is considered a versatile biocatalyst and can degrade many compounds, including the effluents found in industrial wastes (Arregui et al., 2019). It is produced mostly in fungi, higher plants and even some bacteria like *S.lavendulae, S.cyaneus,* etc (Shraddha et al., 2011).

2.4.1 Laccase Enzyme to remediate Textile dyes

A wide range of aromatic compounds are oxidized in conjunction with the reduction of O_2 to H_2O by laccase (Shraddha et al., 2011). The efficacy of laccase in decolorizing dyes varies depending on the type and location of substituents on the aromatic ring of the dye (Martins et al., 2001). Martins et al., 2001 showed that dye analogues with sulfonic

groups at para positions to azo bonds were found to undergo greater decolourization than analogues with sulfonic groups at meta positions (Martins et al., 2001). Apart from this, it has been shown that dyes' redox potential is directly related to their decolourization, although further research is needed to understand this relationship (Zille et al., 2004). Laccase is successful in oxidizing most dyes, but there is a wide range of degrees and rates of decolourization.

2.4.2 Mechanism of laccase enzyme degrading Azo dye

Azo dye has the characteristic -N=N- linkage that gives it a recalcitrant nature. Laccase a multicopper oxidase's mechanism is based on a non-specific free radical formation to degrade dyes and form simpler compounds instead of toxic aromatic amines. The basic mechanism includes reduction of Cu (II) to Cu (I) and in return oxidizing the azo dye and cleaving the azo linkage, thus degrading the dye and producing simplified products (Chacko & Kalidass, 2011).

2.5 Nanomaterials

By definition and literal translation, nanomaterials are tiny materials that are in the 1- 100 nm size range (I. Khan et al., 2019). Nanomaterials have offered a whole new set of properties to already existing bulk materials. One such example is the increased ratio of surface area to volume and because of this, the surface contains many atoms, which leads to the catalytic property in the nanoparticles. There are two methods for creating NPs - the top down method means breaking bulk material to give NPs and the bottom up method entails starting at the atom level and going up to making NPs. Recently, methods such as physical vapour deposition, sol-gel, optical lithography, and biological synthesis have become increasingly significant in the production of nanomaterials. Nanomaterials are classified based on - dimensionality (at least one dimension in nanosize), chemical composition and porosity (Harish et al., 2022).

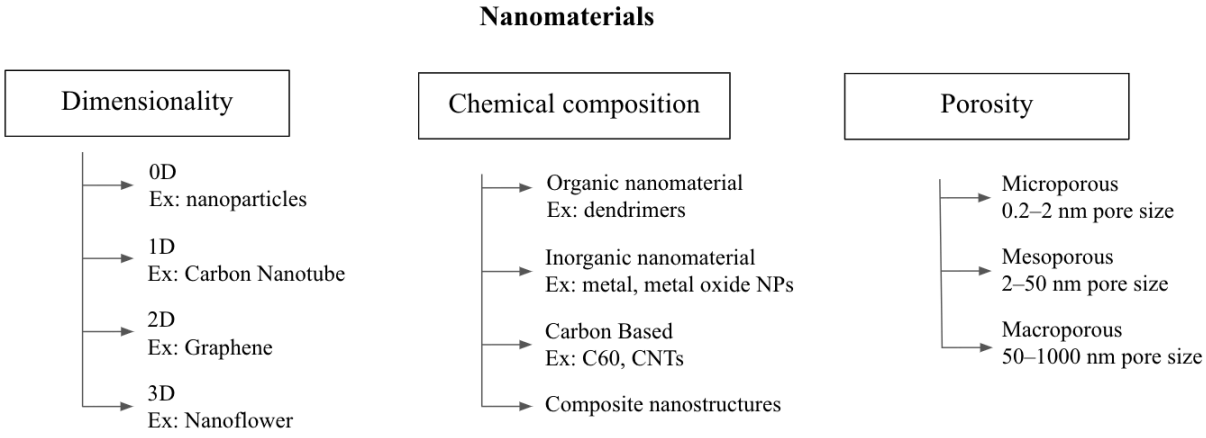

Figure 2.3: Classification of Nanomaterials

2.5.1 Synthesis of Nanoparticles

The most widely used methods to synthesise nanoparticles are physical and chemical methods, which are normally low-cost and produce high volume. However, these methods often need toxic chemicals for the procedure and end up contaminating the NPs formed and also could be harmful to the environment. However, metallic nanoparticles have become very important because of their broad-spectrum applications. And thus non-toxic, eco-friendly and inexpensive techniques are needed to synthesise these nanoparticles (Pantidos, 2014). One such technique is biological synthesis, which includes using bacteria, fungi and plants to synthesise these metallic nanoparticles. Biologically synthesised nanoparticles are better stable and have oxidation resistance. In general, nanoparticle synthesis involves the following steps - (i) reduction, (ii) stabilization, (iii) nucleation, (iv) aggregation,
(v) capping (F. Khan et al., 2022).

a. Bacterial Synthesis

Metal nanoparticles are synthesized by microbes in the presence of reducing enzymes. It could be intracellular or extracellular synthesis Bacterial synthesis offers various advantages bacteria are fast growing, easy to maintain and most importantly the growth

conditions can be manipulated. This manipulation allows for a change in the shape and size of the nanoparticle which has a direct impact on the function and therefore its applications (Ghosh et al., 2021).

b. Phyto-mediated synthesis

Using plants is another environmentally friendly approach. The plant extracts have active phytochemicals like terpenoids, alkaloids, phenols; and enzymes that reduce the metal precursor to form nanoparticles (F. Khan et al., 2022).

c. Fungal synthesis

This also has two mechanisms intracellular and extracellular methods. The fungal culture filtrate/fungal mycelia culture contains metabolites like mevastatin and cyclosporine, and enzymes like ACCases and peroxidases reduce the metal ions to form nanoparticles (Pantidos, 2014).

2.5.2 Nanoparticles for remediation of Textile dyes

When visible light is present and the photon's energy is higher than the threshold, the excited electron leaves the Valance band (VB) and enters the Conduction band (CB), opening a gap/hole in the VB. The electron in the CB reacts with O_2 and reduces it to form superoxide 0_2^- which is a potent reducing agent, While the hole in VB reacts with the H_2O adsorbed and forms hydroxyl free radical which behaves as a strong oxidizing agent. The dye gets adsorbed onto the surface of the nanoparticle, and both these oxidizing and reducing agents react with the dye and therefore degrade it into simpler molecules (Nandhini et al., 2019; Yadav et al., 2022).

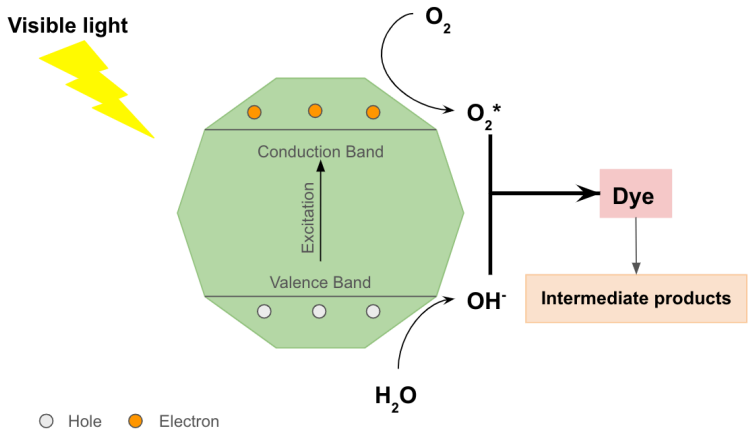

Figure 2.4: Mechanism of Nanoparticle Remediating Dye. (The green decagon represents a metallic nanoparticle)

2.6 Nanobioremdiation

The fact is to remediate contaminated sites one technology alone may be expensive and not as effective because of the recalcitrant characteristics of the pollutants. This is where combining more than one technology comes in to achieve synergism and ultimately an increased efficiency to remediate pollutants. One such combination is Microbial nano-bioremediation or simple nanobioremediation. It involves the abiotic factor of the nanoparticles that can adsorb, absorb and even degrade some pollutants owing to the photocatalyst activity. And the second is the biotic factor i.e. using complete microbes or microbial enzymes to reduce/oxidise pollutants. Apart from enzymes other biotic processes like biostimulation and biotransformation can also remediate the pollutants (Cecchin et al., 2017; Y. Singh & Saxena, 2022).

2.7 In silico analysis

Molecular docking is a very fast, inexpensive and easy technique to understand the binding affinity between protein/ receptor and small molecule/ligand. Based on the flexibility of ligand and/or target protein docking has 3 types (Agarwal & Mehrotra, 2016).

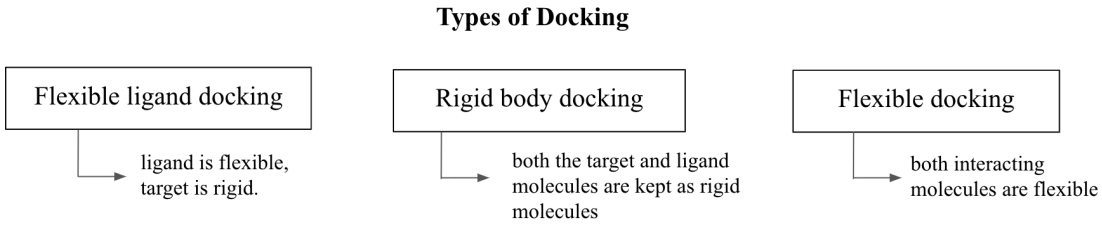

Figure 2.5: Types of docking

One such open source tool is Autodock Vina which is used in this study. It is extensively employed in drug discovery to screen extensive libraries of compounds for potential drug candidates (Trott & Olson, 2010).

In the drug development sector, molecular docking has saved a lot of money and time because it allows the scanning of large libraries of drugs in one go. Similarly, molecular docking is proving to be useful in the environmental pollutant degradation domain; by predicting binding affinities of pollutants with the potential enzymes, decoding degradation pathways and eventually helping in designing more efficient and targeted remediation strategies (Basharat et al., 2017; Liu et al., 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1 Culturing *Achromobacter Sp.*

i) The bacterium *Achromobacter Sp*. was obtained from The Environmental and Industrial Biotechnology lab, at Delhi Technological University.
ii) The nutrient broth was prepared and autoclaved.
ii) Achromobacter *Sp*. were grown and maintained in NB for 24 hours at $37^{O}C$.

3.2 Bacterial synthesis of MgO NPs

The protocol from Mohanasrinivasan et al., 2018 was followed with certain modifications (Mohanasrinivasan et al., 2018).
i) Prepared 0.2M $MgCl_2$ and 0.4M NaOH.
ii) Overnight grown culture was diluted with sterile nutrient broth in a 1:3 ratio.
iii) Added 40 ml of 0.2M $MgCl_2$ to the diluted media
iv) Dropwise added 0.4 M NaOH
v) For control, no $MgCl_2$ was added to the diluted media
vi) Placed in a water bath for 15-20 minutes.
vii) Let the whitish-brown precipitate formed settle down.
viii) Removed from water bath and allowed to sit undisturbed for 3 hours
ix) Centrigufed the media for 15' at 2800 g. Discard supernatant and washed the pellet twice with distilled water.
x) Calcinated at $300^{O}C$ for 4 hours to make it into oxide form.

3.3 Antimicrobial activity of MgO NPs

To test antimicrobial activity simple disc diffusion assay was performed.

i) Prepared Nutrient Agar Plates.

ii) On two plates streaked *E. coli* (gram-negative) and the other two streaked *Brevibacillus brevis* (gram-positive).

iii) A disc of 5 % solution of MgO NPs and 400ug/ml solution of Rifaximin was prepared.

iv) Place the disc on each plate and leave undisturbed at 37°C for 24 hours.

v) The zones of inhibition were observed by measuring the clearance zone around the disc by a scale.

3.4 Characterization of MgO NPs

3.4.1 UV-Vis Spectroscopy

Metallic Nanoparticles interact with specific wavelengths of light. NPs have varied optical properties owing to the shape, diameter, concentration, and RI of NPs surface. So UV-Vis Spectrophotometry can help to characterize the nanoparticle (Philip, 2008).

i) To obtain an absorption spectrum we dissolved the MgO NPs in dilute acid and measured absorbance through 300-800 nm wavelength using Eppendorf BioSpectrometer.

3.4.2 Fourier transform - infrared spectroscopy (FT-IR)

FTIR is used to determine the functional groups and thus the composition of biomolecules. The IR causes different types of vibration - stretching and bending in the bonds of atoms. Thus these vibrational signals are detected and converted into an FTIR spectrum (%Transmittance vs wavenumber cm^{-1}). Thus in the FTIR spectrum, there are two main regions - 1200 cm^{-1} to 400 cm^{-1} region is the fingerprint region. This region is specific to the biomolecule and is very essential for the characterization and identification of the molecule. The region from 4000 cm^{-1} to 1200 cm^{-1} contains distinct peaks for functional groups. For instance, a broad centred at about 3400 cm^{-1} is a characteristic signal of the stretching of alcohols (-OH). The broadness of a peak reflects the polarity/ H

bonding of the atoms. FTIR can be used to characterize nanoparticles owing to the optical properties of their surface (*12.7*, 2024; Ramírez-Hernández et al., 2019).

i) In this study, a PerkinElmer Fourier transform infrared (FT-IR) spectrometer with a wavelength from 400 to 4500 cm^{-1} was used for the characterization of the NPs formed.

3.4.3 X-ray diffraction

XRD is another non-damaging characterization tool. The wavelength of X-ray is comparable to the inter-atomic distances, allowing this technique to accurately predict the atomic distances and consequently the crystallographic structure of the molecule. The diffraction spectrum consists of a graph of intensities vs the detected angle 2 θ. Further using Bragg's law, one can find the distance between the atomic planes.

$2l \sin \theta = n \lambda,$ (3.1)

l is the distance between atomic planes

θ is the diffraction angle

λ is the wavelength

The width of the peaks obtained indicates the crystal size. A sharp, high-intensity peak indicates a well-ordered crystalline material (Sharma et al., 2012). It has been studied that for very small nanoparticles the size itself affects the XRD pattern (Vorontsov & Tsybulya, 2018).

i) For this study the X-ray diffractometer (XRD) pattern was observed using a Bruker, D8 Discover High Resolution X-Ray Diffractometer.

3.4.4 Zeta Potential

Zeta potential measures the electric charge on the surface of particles in a colloidal suspension. In other words, it quantifies the potential difference existing between the fluid layer surrounding the particle and the dispersion medium. It also provides information about the particle's tendency to aggregate (Clogston & Patri, 2011). Nanoparticles with zeta potential > +30 mV or < -30 mV are highly cationic and highly anionic respectively and therefore will repel enough to not aggregate and stay stable. When the value is

between -10 mV and +10 mV it is considered neutral and suggests the particles can aggregate easily. Finally, if the values are between ±10 mV and ±30 mV, it indicates moderate stability and there may be aggregation under certain conditions (Gupta & Trivedi, 2018; Rasmussen et al., 2020).

i) The stability of MgO nanoparticles was assessed by a zeta potential (ZP) analyser (Malvern Zetasizer).

3.5 To extract extracellular laccase enzyme from *Brevibacillus brevis*

The protocol from Bozoglu et al., 2013; Peter et al., 2014; Rajeswari & Bhuvaneswari, 2016 were followed with modifications (Bozoglu et al., 2013; Peter et al., 2014; Rajeswari & Bhuvaneswari, 2016).

i) The bacterium *Brevibacillus brevis* was obtained from The Environmental and Industrial Biotechnology lab, at Delhi Technological University.

ii) The bacteria was grown and maintained in Nutrient Broth for 24 Hours at $37^{O}C$ at 120 rpm.

iii) To extract extracellular laccase enzyme from *Brevibacillus brevis*, the bacteria were grown under varied concentrations of $CuSO_4$ to obtain the maximum possible enzyme activity.5 different setups were created-

a. T1 : 2% glucose, 0.1 mM $CuSO_4$, LB media
b. T2 : 2% glucose, 0.5 mM $CuSO_4$, LB media
c. T3 : 2% glucose, 1 mM $CuSO_4$, LB media
d. T4 : 2% glucose, LB media
e. T5 : LB media

The pH of each of these setups was maintained at 7-8.

iv) Each of these setups was incubated for 9 days and each day the enzyme activity was calculated by testing it with Guaiacol as the substrate.

3.6 Enzyme activity of extracellular laccase enzyme

The protocol from Jhadav et al., 2009 and Sheikhi et al., 2012 were followed with modifications (Jhadav et al., 2009; Sheikhi et al., 2012).

i) 20 mM guaiacol solution was prepared in 0.1 M phosphate buffer.

ii) Reaction setup had 1 ml of crude laccase enzyme extract and 1.5ml 20mM guaiacol

iii) Monitored the absorbance change at 470 nm for 40 minutes of incubation.

iv) The enzyme activity was calculated using (Agrawal & Verma, 2019):

$$\mathbf{EA = (\Delta A \times Vt \times Df \times 10^6)/ (t \times \varepsilon \times d \times Vs)} \quad (3.2)$$

Where (a) EA = Enzyme activity ($\mu mol\ min^{-1}\ L^{-1}$)

(b) ΔA = final absorbance − initial absorbance

(c) Vt = total reaction volume (millilitre)

(d) Df =dilution factor

(e) t = time (minutes)

(f) ε = molar extinction co-efficient ($M^{-1}\ cm^{-1}$)

(g) d= optical path (cm)

(h) Vs= sample volume (millilitre)

v) Using the Spectrophotometric technique, the protein concentration was determined by measuring the crude enzyme extract's absorbance at 280 nm (Boyer, 2012).

3.7 Nanobioremdiation of Methyl Red

i) Methyl red solution of concentration 100mg/ml was prepared. The experiment setup was as follows:

a. Test 1- 20ml MR + 10mg MgO NPs
b. Control 1 - 20ml MR
c. Test 2 - 50ml MR + 1ml laccase enzyme
d. Control 2 - 50ml MR
e. Test 3 - 10ml MR + 5mg MgO + 250 ul laccase enzyme
f. Control 3 - 10ml MR

ii) Place the falcons on a shaker incubator at 120 rpm and 35°C for 4 days.

iii) A control was set up - a dye sample with no enzyme for treatment.

iv) The decolourization efficiency (DE) was determined by employing an Eppendorf BioSpectrometer to track changes in absorbance at 425 nm.

v) The decolourization efficiency percentage was calculated using

$$\% \text{ DE} = C_0 - C_1 \div C_0 \times 100 \quad (3.3)$$

where (a) C_0 initially recorded absorbance and (ii) C_1 finally recorded absorbance (Moussavi & Mahmoudi, 2009).

3.8 *In-silico Analysis*

i) The laccase protein sequence of *Bacillus amyloliquefaciens* (phylogenetically close to *Brevibacillus brevis*) was retrieved from the NCBI database with GenBank ID KJD53679.1.

ii) The 3D structure of the sequence was modelled using Swiss-Model online server with PDB ID 6DZD 1RW0 as a template.

iii) The model was evaluated using the PROCHECK feature at UCLA-DOE LAB - SAVES v6.0 server to obtain the Ramachandran Plot.

iv) PubChem database was used to retrieve the 3D structure SDF file of MR (CID 10303).

v) OpenBable was used to convert the SDF file to PDB format using.

vi) AutoDock Vina tool 1.5.6 was used for the molecular docking.

vii) The laccase protein was prepared by adding the hydrogen and Kollman charges and water molecules were deleted.

viii) Torsions were set to the dye structure.

ix) The grid of 40 × 40 × 40 cubic along the x, y, and z-axis was fixed around the laccase active site.

x) The docking was set between MR and Laccase.

xi) After the completion of the docking search, the best conformation of protein-ligand (laccase-MR) with the most negative binding affinity was chosen.

xii) Discovery Studio was used to visualise and examine the list of amino acids of the active site involved in interactions within the complex.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Bacterial synthesis of MgO NPs

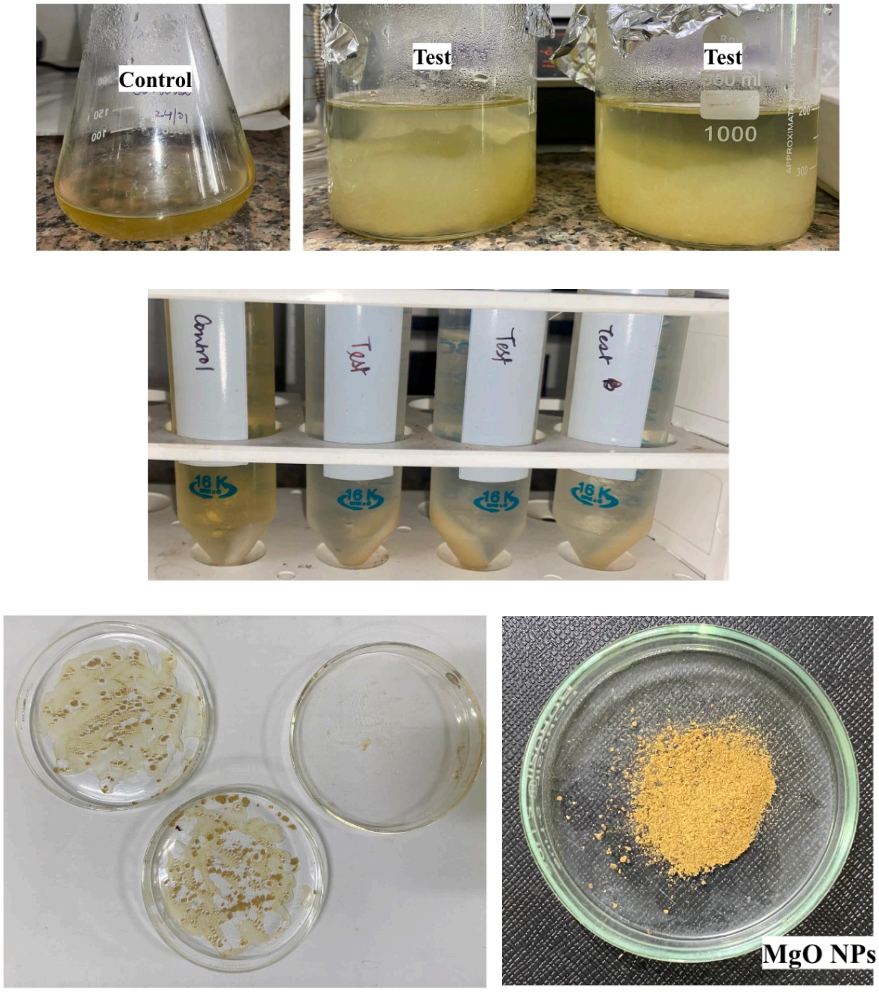

Figure 4.1: Steps involved in Bacterial synthesis of MgO NPs

After preparing the control and test cultures as mentioned in Chapter 3. We observe a dense white-yellowish precipitate. Upon centrifugation, the pellets were collected, which was followed by calcination leading to the formation of powdered MgO NPs.

4.2 Antimicrobial activity of MgO NPs

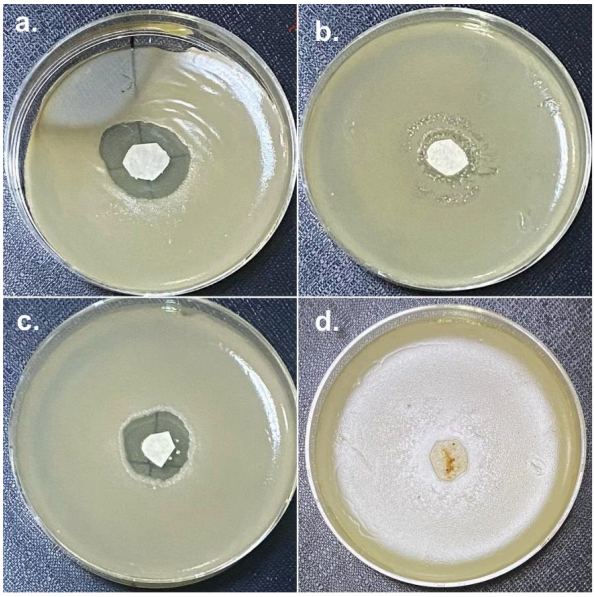

Figure 4.2 : Disc Diffusion Assay a) Rifaximin + *Brevibacillus brevis* b) MgO + *Brevibacillus brevis* c) Rifaximin + *E. coli* d) MgO + *E. coli*

Table 4.1: Diameters (cm) of the zone of inhibition

	Brevibacillus brevis	*E. coli*
Rifaximin	1.8 cm	1.5 cm
MgO	1.6 cm	partial zone of inhibition with a diameter of 1.1 cm

We tested the MgO NPs' antibacterial capabilities using the Kirby-Bauer method. As observed in Figure 6 a clear zone of inhibition is observed for *B. brevis* (Gram-positive). However, for *E. coli* (Gram-negative) there was a partial zone of inhibition.

4.3 Characterization of MgO NPs

4.3.1 UV-Vis Spectrophotometry

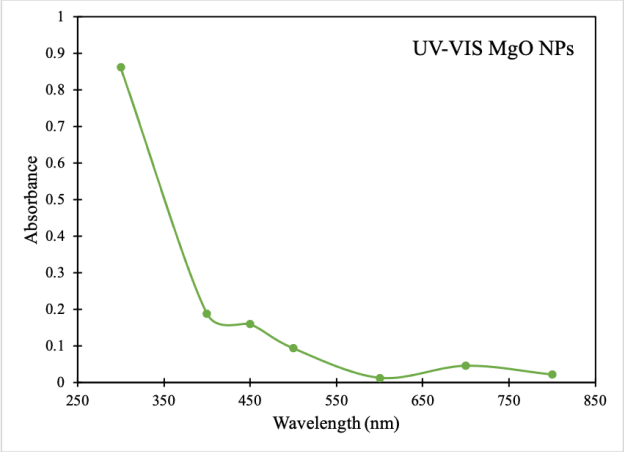

Figure 4.3: Absorbance spectrum of MgO NPs

The absorption spectrum as seen in Figure 7 was obtained. The peak between 260-330nm indicated the presence of MgO NPs (Abdel-Aziz et al., 2020).

4.3.2 Fourier transform infrared spectroscopy (FTIR)

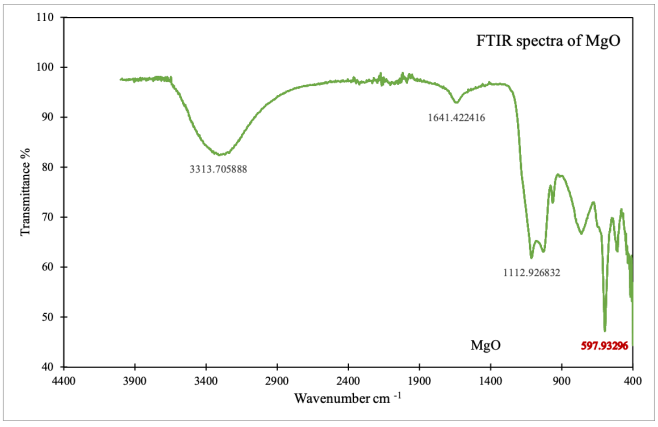

Figure 4.4: FTIR spectrum of MgO NPs

According to the literature, the peaks that appear between the wavenumber 400 and 700 cm^{-1} in the fingerprint region indicate the presence of MgO at the nanoscale and we rightly observed the peak at 597.93 cm^{-1} (S & Kavitha, 2023). Apart from the broad small peak at 3313 cm^{-1} indicated the presence of the -OH functional group. The peak at 1641 cm^{-1} is a result of the amide group (O=C–NH). And the Amide group (C-N stretching) was identified with the peak at 1112 cm^{-1}(Hirphaye et al., 2023; Ratnam et al., 2020; S & Kavitha, 2023).

4.3.3 X-Ray Diffraction (XRD)

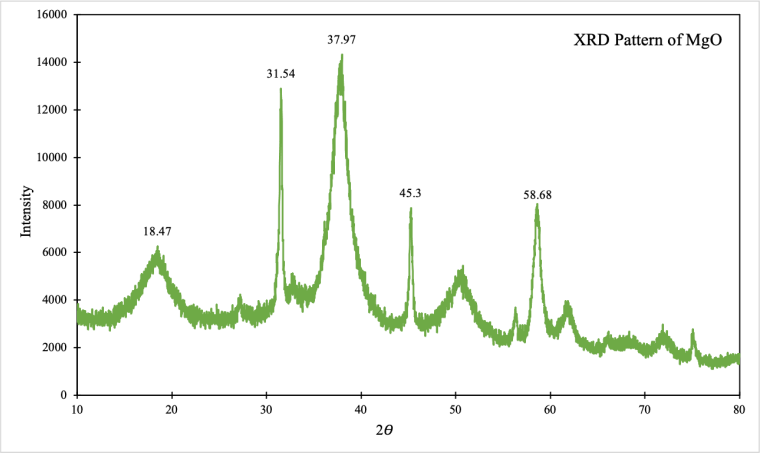

Figure 4.5: XRD pattern of MgO NPs

The presence of sharp peaks suggests that nanoparticles are highly crystalline. The XRD study revealed a sequence of diffraction peaks occurring at 2θ angles of 18.47°, 31.54°, 37.97°, 45.3°, and 56.68°. The XRD pattern obtained for the MgO NPs produced was confirmed with the standard values of MgO (as reported by JCPDS-International Centre for Diffraction Data).

4.3.4 Zeta potential

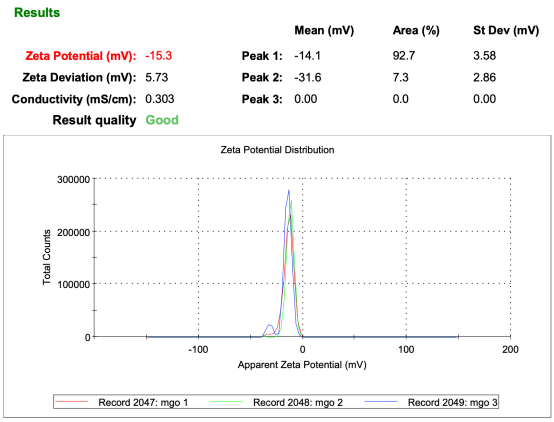

Figure 4.6: Zeta Potential of MgO NPs

From the zeta potential -15.3mV we can conclude that the nanoparticles formed are only moderately stable and the net charge on the surface is negative. While there will be some electrostatic repulsion that could prevent immediate aggregation, it won't be stable for the long term and tends to aggregate under some specific conditions (Abdel-Aziz et al., 2020; Pugazhendhi et al., 2019).

4.4 Extraction of extracellular laccase from *Brevibacillus brevis*

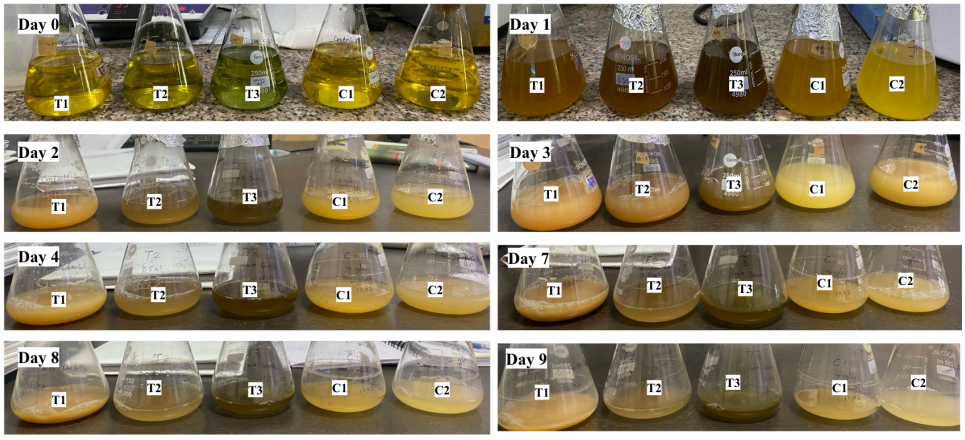

Figure 4.7: Production of extracellular Laccase enzyme from *Brevibacillus brevis*

As mentioned in Chapter 3, five different setups were prepared to ensure maximum production of laccase enzyme over 9 days. The T2 setup with 0.5mM $CuSO_4$ was best in terms of bacterial growth. Each of the flasks was maintained at $37^{O}C$ and 120 rpm. Each day the supernatant was collected and the enzyme activity of the crude extracellular enzyme was calculated.

4.5 Enzyme activity of extracellular laccase enzyme

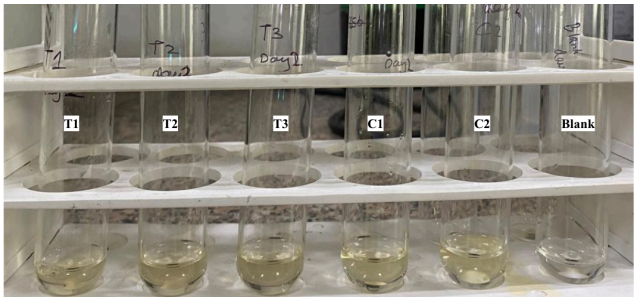

Figure 4.8: Enzyme activity of Laccase enzyme using Guaiacol as the substrate.

The absorbance change (Δ A) due to the reaction with guaiacol was measured each day for 9 days. The ΔA was used to calculate the enzyme activity. It was observed that the 7th day showed a maximum production with an enzyme activity of 0.241 μmol min^{-1} L^{-1}. The protein concentration for the same was 26.067 ug/ml calculated by the Spectrophotometric Assay. Therefore the suprnatant collected on the 7th day was further used as the crude extracellular laccase enzyme for the degradation of methyl red.

4.6 Nanobioremdiation of Methyl Red

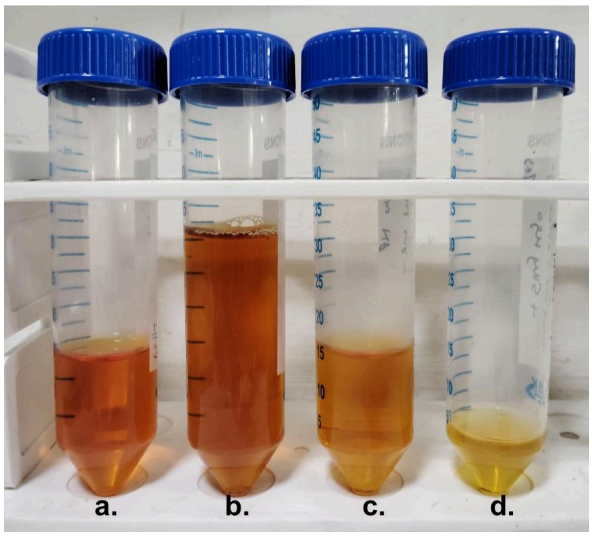

Figure 4.9: Decolourization of Methyl Red. a. Control b. treated with crude laccase only c. treated with MgO NPs only d. treated with both crude laccase and MgO NPs for 72 hours.

The decolourization experiment was set up as mentioned in Chapter 3. The variation in absorbance was recorded daily at a wavelength of 425nm. Further, the decolourization efficiency (DE) was calculated. In this study, we saw only crude laccase degraded MR with DE being 20.5%. The DE of only MgO to degrade MR was 23.3%. While the DE when both MgO and crude extracellular laccase enzyme were used together was 37.7%. We can observe that there is a nearly 80 % increase in the DE from only laccase to using both laccase and MgO together.

4.7 *In silico* Analysis

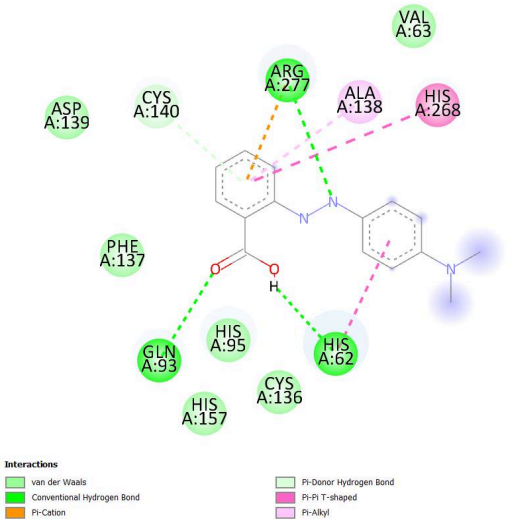

Figure 4.10: Visualization of active site amino aid of laccase involved in the interaction with Methyl Red.

The laccase protein was modelled using the homology modelling technique available on Swiss-Model online server. Thus the Ramachandran plot analysis was done using PROCHECK and showed that 91.3% of amino acids in the protein model were found to be in the favoured region. From the molecular docking analysis using Autodock vina tool 1.5.6, the predicted average binding energy between the extracellular laccase and Methyl red was found to be − 6.5 kcal/mol. Further, the Laccase-MR complex was visualised using Discovery Studio (Fig. 14). The amino acids involved in the binding and degradation of the dyes are GLN 93, HIS 62, HIS 268, ALA 138, ARG 277, VAL 63, HIS 95, HIS 157, CYS 136, CYS 140, ASP 139, and PHE 137. Laccase and MR definitely interact, but they do not interact strongly, which may explain why only 20.5% of the MR was degraded.

CHAPTER 5

CONCLUSION AND FUTURE PROSPECTS

Textile dye effluents are widely recognized as highly detrimental to both the environment and human well-being. Various physical and chemical techniques like flocculation, aggregation, membrane separation, etc have been applied over the years. But, one technique is not enough and is not always feasible. Apart from this, these techniques are often harmful to the environment. Thus the search for a more eco-friendly, sustainable and inexpensive technique had begun. One such technique is bioremediation; which uses microorganisms to remediate pollutants. However, again one technique is often not very efficient.

Since the 1980s when modern nanotechnology came into existence, it has taken its place in almost every industry. And so was the introduction of nanomaterials to remediate pollutants. Further, researchers have tried various combinations of using nanomaterials with physical, chemical and biological means to remediate pollutants.

In this study, the goal was to understand the use of the nanoparticle and biological technique to remediate Methyl red. Methyl red is a mono-azo dye, which belongs to an important class of textile dye i.e. azo dyes. We synthesised MgO nanoparticles using bacterial synthesis protocol using Achromobacter sp., which according to our knowledge is novel. The NPs synthesized were characterized by FTIR, UV-VIS spectrophotometry, Zeta potential and XRD. Further, we extracted crude extracellular laccase from *B. brevis*. Four treatment setups for MR were created which included control, only crude laccase, only MgO, and the third setup with MgO and crude laccase together. At the end of 72 hours, we saw 20.5%, 23.3% and 37.5% degradation of MR respectively in each setup. As we speculated, we saw an 80% increase in the decolourization efficiency of MR when MgO NPs and Laccase were used together as opposed to laccase alone.

The decolourization efficiency of laccase degradation of methyl red is not very high. This finding is also supported by the predicted binding affinity of -6.5 kcal/mol. This may be due to the fact that there is much documentation indicating that the structure of the dye and its accessibility to the active site of the enzyme directly impact the effectiveness of degradation (Martins et al., 2001; Zille et al., 2004). But to understand more specifically MR interacting with laccase further studies are required. Most dyes are oxidized by laccase, but the extent of decolourization and the rate of degradation can vary significantly. Apart from this at the experimental level, the prospects could include- i) varying the size of the nanoparticle to observe change in its properties and thus its DE. ii) purifying the extracellular laccase using dialysis and affinity chromatography columns. iii) understand if there is any cross-interaction between laccase and MgO NPs which could affect the enzyme activity. iv) one can try to immobilize the enzyme on the surface of the nanoparticles which could increase reusability and may as well increase DE. v) using test samples from the actual polluted water sources.

On a large scale, the prospects include testing the efficacy and scalability of microbial-assisted nano bioremediation for dye removal. Therefore paving the way for sustainable industrial practices and significant environmental benefits.

REFERENCES

12.7: Interpreting Infrared Spectra. (2024, March 24). Chemistry LibreTexts. https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Organic_Chemistry_II_(Morsch_et_al.)/12%3A_Structure_Determination_-_Mass_Spectrometry_and_Infrared_Spectroscopy/12.07%3A_Interpreting_Infrared_Spectra

Abdel-Aziz, M. M., Emam, T. M., & Elsherbiny, E. A. (2020). Bioactivity of magnesium oxide nanoparticles synthesized from cell filtrate of endobacterium *Burkholderia rinojensis* against *Fusarium oxysporum*. *Materials Science and Engineering: C*, *109*, 110617. https://doi.org/10.1016/j.msec.2019.110617

Agarwal, S., & Mehrotra, R. (2016). Mini Review_ An overview of Molecular Docking. *JSM Chemistry*, *2*, 1024.

Agrawal, K., & Verma, P. (2019). Laccase: Addressing the ambivalence associated with the calculation of enzyme activity. *3 Biotech*, *9*(10), 365. https://doi.org/10.1007/s13205-019-1895-1

Akter, T., Protity, A. T., Shaha, M., Al Mamun, M., & Hashem, A. (2023). The Impact of Textile Dyes on the Environment. In A. Ahmad, M. Jawaid, M. N. Mohamad Ibrahim, A. A. Yaqoob, & M. B. Alshammari (Eds.), *Nanohybrid Materials for Treatment of Textiles Dyes* (pp. 401–431). Springer Nature. https://doi.org/10.1007/978-981-99-3901-5_17

Arregui, L., Ayala, M., Gómez-Gil, X., Gutiérrez-Soto, G., Hernández-Luna, C. E., Herrera de los Santos, M., Levin, L., Rojo-Domínguez, A., Romero-Martínez, D., Saparrat, M. C. N., Trujillo-Roldán, M. A., & Valdez-Cruz, N. A. (2019). Laccases: Structure, function, and potential application in water bioremediation. *Microbial Cell Factories*, *18*(1), 200. https://doi.org/10.1186/s12934-019-1248-0

Azubuike, C. C., Chikere, C. B., & Okpokwasili, G. C. (2016). Bioremediation techniques–classification based on site of application: Principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, *32*(11), 180. https://doi.org/10.1007/s11274-016-2137-x

Basharat, Z., Bibi, M., & Yasmin, A. (2017). Implications of Molecular Docking Assay for Bioremediation. In *Handbook of Research on Inventive Bioremediation Techniques*. https://doi.org/10.4018/978-1-5225-2325-3.ch002

Benkhaya, S., M'rabet, S., & El Harfi, A. (2020). Classifications, properties, recent synthesis and applications of azo dyes. *Heliyon*, *6*(1), e03271. https://doi.org/10.1016/j.heliyon.2020.e03271

Boyer, R. F. (2012). *Biochemistry Laboratory: Modern Theory and Techniques*. Prentice Hall.

Bozoglu, C., Adiguzel, A., Nadaroglu, H., & Efe, D. (2013). Purification and Characterization of Laccase from newly isolated Thermophilic Brevibacillus sp. (Z1) and its applications in removal of Textile Dyes. *Research Journal of Biotechnology*, *8*.

Cecchin, I., Reddy, K. R., Thomé, A., Tessaro, E. F., & Schnaid, F. (2017). Nanobioremediation: Integration of nanoparticles and bioremediation for sustainable remediation of chlorinated organic contaminants in soils. *International Biodeterioration & Biodegradation*, *119*, 419–428. https://doi.org/10.1016/j.ibiod.2016.09.027

Chacko, J., & Kalidass, S. (2011). Enzymatic Degradation of Azo Dyes–A Review. *INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES Volume*, *1*.

Chaudhary, P., Ahamad, L., Chaudhary, A., Kumar, G., Chen, W.-J., & Chen, S. (2023). Nanoparticle-mediated bioremediation as a powerful weapon in the removal of environmental pollutants. *Journal of Environmental Chemical Engineering*, *11*(2),

109591. https://doi.org/10.1016/j.jece.2023.109591
Chauhan, P. S., Goradia, B., & Saxena, A. (2017). Bacterial laccase: Recent update on production, properties and industrial applications. *3 Biotech*, *7*(5), 323. https://doi.org/10.1007/s13205-017-0955-7
Clogston, J. D., & Patri, A. K. (2011). Zeta potential measurement. *Methods in Molecular Biology (Clifton, N.J.)*, *697*, 63–70. https://doi.org/10.1007/978-1-60327-198-1_6
Das, R. K., Pachapur, V. L., Lonappan, L., Naghdi, M., Pulicharla, R., Maiti, S., Cledon, M., Dalila, L. M. A., Sarma, S. J., & Brar, S. K. (2017). Biological synthesis of metallic nanoparticles: Plants, animals and microbial aspects. *Nanotechnology for Environmental Engineering*, *2*(1), 18. https://doi.org/10.1007/s41204-017-0029-4
Filote, C., Roșca, M., Hlihor, R. M., Cozma, P., Simion, I. M., Apostol, M., & Gavrilescu, M. (2021). Sustainable Application of Biosorption and Bioaccumulation of Persistent Pollutants in Wastewater Treatment: Current Practice. *Processes*, *9*(10), Article 10. https://doi.org/10.3390/pr9101696
Ghosh, S., Ahmad, R., Zeyaullah, M., & Khare, S. K. (2021). Microbial Nano-Factories: Synthesis and Biomedical Applications. *Frontiers in Chemistry*, *9*. https://doi.org/10.3389/fchem.2021.626834
Gregory, P. (1990). Classification of Dyes by Chemical Structure. In D. R. Waring & G. Hallas (Eds.), *The Chemistry and Application of Dyes* (pp. 17–47). Springer US. https://doi.org/10.1007/978-1-4684-7715-3_2
Gupta, V., & Trivedi, P. (2018). Chapter 15—In vitro and in vivo characterization of pharmaceutical topical nanocarriers containing anticancer drugs for skin cancer treatment. In A. M. Grumezescu (Ed.), *Lipid Nanocarriers for Drug Targeting* (pp. 563–627). William Andrew Publishing. https://doi.org/10.1016/B978-0-12-813687-4.00015-3
Gürses, A., Açıkyıldız, M., Güneş, K., & Gürses, M. S. (2016). Classification of Dye and Pigments. In A. Gürses, M. Açıkyıldız, K. Güneş, & M. S. Gürses (Eds.), *Dyes and Pigments* (pp. 31–45). Springer International Publishing. https://doi.org/10.1007/978-3-319-33892-7_3
Harish, V., Tewari, D., Gaur, M., Yadav, A. B., Swaroop, S., Bechelany, M., & Barhoum, A. (2022). Review on Nanoparticles and Nanostructured Materials: Bioimaging, Biosensing, Drug Delivery, Tissue Engineering, Antimicrobial, and Agro-Food Applications. *Nanomaterials*, *12*(3), 457. https://doi.org/10.3390/nano12030457
Hirphaye, B. Y., Bonka, N. B., Tura, A. M., & Fanta, G. M. (2023). Biosynthesis of magnesium oxide nanoparticles using Hagenia abyssinica female flower aqueous extract for characterization and antibacterial activity. *Applied Water Science*, *13*(9), 175. https://doi.org/10.1007/s13201-023-01987-2
Jamee, R., & Siddique, R. (2019). Biodegradation of synthetic dyes of textile effluent by microorganisms: An environmentally and economically sustainable approach. *European Journal of Microbiology and Immunology*, *9*(4), 114–118. https://doi.org/10.1556/1886.2019.00018
Janusz, G., Pawlik, A., Świderska-Burek, U., Polak, J., Sulej, J., Jarosz-Wilkołazka, A., & Paszczyński, A. (2020). Laccase Properties, Physiological Functions, and Evolution. *International Journal of Molecular Sciences*, *21*(3), 966. https://doi.org/10.3390/ijms21030966
Jhadav, A., Vamsi, K. K., Khairnar, Y., Boraste, A., Gupta, N., Trivedi, Dr. S., Patil, P., Gupta, G., Gupta, M., Mujapara, A. K., Joshi, B., & Mishra, D. (2009). Optimization of production and partial purification of laccase by Phanerochaete chrysosporium using submerged fermenation. *International Journal of Microbiology Research*, *1*. https://doi.org/10.9735/0975-5276.1.2.9-12

Juwarkar, A. A., Singh, S. K., & Mudhoo, A. (2010). A comprehensive overview of elements in bioremediation. *Reviews in Environmental Science and Bio/Technology*, *9*(3), 215–288. https://doi.org/10.1007/s11157-010-9215-6

Kanagaraj, J., Senthilvelan, T., & Panda, R. C. (2015). Degradation of azo dyes by laccase: Biological method to reduce pollution load in dye wastewater. *Clean Technologies and Environmental Policy*, *17*(6), 1443–1456. https://doi.org/10.1007/s10098-014-0869-6

Kant, R. (2011). *Textile dyeing industry an environmental hazard*. *2012*. https://doi.org/10.4236/ns.2012.41004

Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P., & Ahmad, F. (2022). Green Nanotechnology: Plant-Mediated Nanoparticle Synthesis and Application. *Nanomaterials*, *12*(4), 673. https://doi.org/10.3390/nano12040673

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, *12*(7), 908–931. https://doi.org/10.1016/j.arabjc.2017.05.011

Laouini, S. E., Bouafia, A., Soldatov, A. V., Algarni, H., Tedjani, M. L., Ali, G. A. M., & Barhoum, A. (2021). Green Synthesized of Ag/Ag2O Nanoparticles Using Aqueous Leaves Extracts of Phoenix dactylifera L. and Their Azo Dye Photodegradation. *Membranes*, *11*(7), Article 7. https://doi.org/10.3390/membranes11070468

Lellis, B., Fávaro-Polonio, C. Z., Pamphile, J. A., & Polonio, J. C. (2019). Effects of textile dyes on health and the environment and bioremediation potential of living organisms. *Biotechnology Research and Innovation*, *3*(2), 275–290. https://doi.org/10.1016/j.biori.2019.09.001

Lim, S.-L., Chu, W.-L., & Phang, S.-M. (2010). Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresource Technology*, *101*(19), 7314–7322. https://doi.org/10.1016/j.biortech.2010.04.092

Liu, Z., Liu, Y., Zeng, G., Shao, B., Chen, M., Li, Z., Jiang, Y., Liu, Y., Zhang, Y., & Zhong, H. (2018). Application of molecular docking for the degradation of organic pollutants in the environmental remediation: A review. *Chemosphere*, *203*, 139–150. https://doi.org/10.1016/j.chemosphere.2018.03.179

Martins, M. A., Ferreira, I. C., Santos, I. M., Queiroz, M. J., & Lima, N. (2001). Biodegradation of bioaccessible textile azo dyes by Phanerochaete chrysosporium. *Journal of Biotechnology*, *89*(2–3), 91–98. https://doi.org/10.1016/s0168-1656(01)00318-2

Mohanasrinivasan, V., Subathra Devi, C., Mehra, A., Prakash, S., Agarwal, A., Selvarajan, E., & Jemimah Naine, S. (2018). Biosynthesis of MgO Nanoparticles Using Lactobacillus Sp. And its Activity Against Human Leukemia Cell Lines HL-60. *BioNanoScience*, *8*(1), 249–253. https://doi.org/10.1007/s12668-017-0480-5

Moussavi, G., & Mahmoudi, M. (2009). Removal of azo and anthraquinone reactive dyes from industrial wastewaters using MgO nanoparticles. *Journal of Hazardous Materials*, *168*(2), 806–812. https://doi.org/10.1016/j.jhazmat.2009.02.097

Nandhini, N. T., Rajeshkumar, S., & Mythili, S. (2019). The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. *Biocatalysis and Agricultural Biotechnology*, *19*, 101138. https://doi.org/10.1016/j.bcab.2019.101138

Noman, M., Shahid, M., Ahmed, T., Niazi, M. B. K., Hussain, S., Song, F., & Manzoor, I. (2020). Use of biogenic copper nanoparticles synthesized from a native *Escherichia* sp. As photocatalysts for azo dye degradation and treatment of textile effluents. *Environmental Pollution*, *257*, 113514. https://doi.org/10.1016/j.envpol.2019.113514

Omokhagbor Adams, G., Tawari Fufeyin, P., Eruke Okoro, S., & Ehinomen, I. (2020). Bioremediation, Biostimulation and Bioaugmention: A Review. *International Journal of Environmental Bioremediation & Biodegradation*, *3*(1), 28–39. https://doi.org/10.12691/ijebb-3-1-5

Pantidos, N. (2014). Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants. *Journal of Nanomedicine & Nanotechnology*, *05*(05). https://doi.org/10.4172/2157-7439.1000233

Peter, J. K., Vandana, P., Masih, H., & Kumar, Y. (2014). Production Optimization and Partial Purification of Laccases from Bacterial Consortium. *International Journal of Engineering Research & Technology*, *3*(6). https://doi.org/10.17577/IJERTV3IS060531

Philip, D. (2008). Synthesis and spectroscopic characterization of gold nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *71*(1), 80–85. https://doi.org/10.1016/j.saa.2007.11.012

Premaratne, M., Nishshanka, G., Liyanaarachchi, V., Nimarshana, P., & Ariyadasa, T. U. (2021). Bioremediation of textile dye wastewater using microalgae: Current trends and future perspectives. *Journal of Chemical Technology & Biotechnology*, *96*(12), 3249–3258. https://doi.org/10.1002/jctb.6845

Pugazhendhi, A., Prabhu, R., Muruganantham, K., Shanmuganathan, R., & Natarajan, S. (2019). Anticancer, antimicrobial and photocatalytic activities of green synthesized magnesium oxide nanoparticles (MgONPs) using aqueous extract of *Sargassum wightii*. *Journal of Photochemistry and Photobiology B: Biology*, *190*, 86–97. https://doi.org/10.1016/j.jphotobiol.2018.11.014

Rajeswari, M., & Bhuvaneswari, V. (2016). Production of extracellular laccase from the newly isolated Bacillus sp. PK4. *African Journal of Biotechnology*, *15*(34), Article 34. https://doi.org/10.5897/AJB2016.15509

Ramírez-Hernández, A., Aguilar-Flores, C., & Aparicio-Saguilán, A. (2019). Fingerprint analysis of FTIR spectra of polymers containing vinyl acetate. *DYNA*, *86*(209), 198–205.

Rasmussen, M. K., Pedersen, J. N., & Marie, R. (2020). Size and surface charge characterization of nanoparticles with a salt gradient. *Nature Communications*, *11*(1), 2337. https://doi.org/10.1038/s41467-020-15889-3

Ratnam, M. V., Karthikeyan, C., Rao, K. N., & Meena, V. (2020). Magnesium oxide nanoparticles for effective photocatalytic degradation of methyl red dye in aqueous solutions: Optimization studies using response surface methodology. *Materials Today: Proceedings*, *26*, 2308–2313. https://doi.org/10.1016/j.matpr.2020.02.498

S, A., & Kavitha, H. P. (2023). Magnesium Oxide Nanoparticles: Effective Antilarvicidal and Antibacterial Agents. *ACS Omega*, *8*(6), 5225–5233. https://doi.org/10.1021/acsomega.2c01450

Saba, B., Kjellerup, B. V., & Christy, A. D. (2021). Eco-friendly bio-electro-degradation of textile dyes wastewater. *Bioresource Technology Reports*, *15*, 100734. https://doi.org/10.1016/j.biteb.2021.100734

Sharma, R., Bisen, D. P., Shukla, U., & Sharma, B. G. (2012). X-ray diffraction: A powerful method of characterizing nanomaterials. *Recent Research in Science and Technology*. https://updatepublishing.com/journal/index.php/rrst/article/view/933

Sheikhi, F., Roayaei Ardakani, M., Enayatizamir, N., & Rodriguez-Couto, S. (2012). The Determination of Assay for Laccase of Bacillus subtilis WPI with Two Classes of Chemical Compounds as Substrates. *Indian Journal of Microbiology*, *52*(4), 701–707. https://doi.org/10.1007/s12088-012-0298-3

Shraddha, Shekher, R., Sehgal, S., Kamthania, M., & Kumar, A. (2011). Laccase: Microbial Sources, Production, Purification, and Potential Biotechnological Applications. *Enzyme Research*, *2011*, 217861. https://doi.org/10.4061/2011/217861

Singh, P., Singh, V. K., Singh, R., Borthakur, A., Madhav, S., Ahamad, A., Kumar, A., Pal, D. B., Tiwary, D., & Mishra, P. K. (2020). Chapter 1 - Bioremediation: A sustainable approach for management of environmental contaminants. In P. Singh, A. Kumar, & A. Borthakur (Eds.), *Abatement of Environmental Pollutants* (pp. 1–23). Elsevier.

https://doi.org/10.1016/B978-0-12-818095-2.00001-1
Singh, Y., & Saxena, M. K. (2022). Insights into the recent advances in nano-bioremediation of pesticides from the contaminated soil. *Frontiers in Microbiology*, *13*, 982611. https://doi.org/10.3389/fmicb.2022.982611
Subburaj, S., Paramasivam, M., Lafta, M. H., Gangadhar, L., Subburaj, S., Paramasivam, M., Lafta, M. H., & Gangadhar, L. (2024). A Review on Elimination of Colour and Dye Products from Industrial Effluent. In *Water Purification—Present and Future*. IntechOpen. https://doi.org/10.5772/intechopen.112475
Travis, A. S. (1988, August 1). *William Henry Perkin: A Teenage Chemist Discovers How to Make the First Synthetic Dye from Coal Tar. | Textile Chemist & Colorist | EBSCOhost*. https://openurl.ebsco.com/contentitem/gcd:31810351?sid=ebsco:plink:crawler&id=ebsco:gcd:31810351
Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334
Vorontsov, A. V., & Tsybulya, S. V. (2018). Influence of Nanoparticles Size on XRD Patterns for Small Monodisperse Nanoparticles of Cu0 and TiO2 Anatase. *Industrial & Engineering Chemistry Research*, *57*(7), 2526–2536. https://doi.org/10.1021/acs.iecr.7b04480
Yadav, S., Punia, S., Sharma, H., & Gupta, A. (2022). Nano-remediation for the decolourisation of textile effluents: A review. *Nanofabrication*, *7*. https://doi.org/10.37819/nanofab.007.226
Zille, A., Ramalho, P., Tzanov, T., Millward, R., Aires, V., Cardoso, M. H., Ramalho, M. T., Gübitz, G. M., & Cavaco-Paulo, A. (2004). Predicting dye biodegradation from redox potentials. *Biotechnology Progress*, *20*(5), 1588–1592. https://doi.org/10.1021/bp049963i

LIST OF PUBLICATIONS WITH ACCEPTANCE LETTERS

DTU Delhi Technological University

Dr. Jai Gopal Sharma <sharmajaigopal@dce.ac.in>

Regarding Status of review papers

World Researchers Associations <info@worldresearchersassociations.com> Tue, May 7, 2024 at 12:52 AM
To: "Dr. Jai Gopal Sharma" <sharmajaigopal@dce.ac.in>

Dear Author,

It is a pleasure to accept your manuscript entitled "Unveiling Future Advancements in Azo Dye Degradation and Enhanced Bioelectricity Production using Microbial Fuel Cells" in its current form for publication in the Research Journal of Biotechnology.

Thank you for your fine contribution. On behalf of the editors, we appreciate your research work and its quality and we look forward to your continued contributions to the Journal.

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This is to certify that Dr./Mr./Ms. Ananya Chugh from Delhi Technological University has presented a paper titled Docking Study of Environmental Dyes: Insights into Affinity and Bioremediation Potential of Laccase Enzyme
in the "14th International Conference on Science & Innovative Engineering" held on 27th & 28th April 2024 at Prince Dr. K. Vasudevan College Of Engineering & Technology
Dr. Antony V.Samrot, M.E., Ph.D., Director (Research, Innovation and Postgraduate Studies) Manipal University College, Malaysia
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EDUCATION

Delhi Technological University, India 2022-present
M.Sc. Biotechnology | CGPA- 9.72/10

Sri Venkateswara College, University of Delhi, India 2019-2022
B.Sc. Hons Biochemistry | Average CGPA-9.04/10.0

Queens Valley School, India 2016-2019
Class XII (AISCE) | 93.2% | Subjects- PCMB

Class X (AISSCE) |CGPA 10.0 /10.0

INTERNSHIPS AND PROJECTS

1. **Research intern**

Kusuma School of Biological Sciences, IIT Delhi.

JUNE 2023 - AUGUST 2023

- EPS profiling of Acinetobacter baumannii upon exposure to sub-MIC colistin.

2. **Research intern**

Sri Venkateswara College, University of Delhi

JUNE 2021 - AUGUST 2021

- In-Silico Approaches to Understanding Transcriptomics and Proteomics of Mycobacterium tuberculosis.

3. **Research intern**

Sri Venkateswara College, University of Delhi

JUNE 2020 - AUGUST 2020

- Systematic Review and Meta-analysis: Association between Occupational Exposure to Air Pollutants and Pulmonary Health in Construction Workers.

ACADEMIC TRAINING

Data Analysis for Biologists (R programming)

IIT Guwahati, NEPTL coursel JANUARY-MARCH 2023

Bioinformatics and Computational Biology

Sri Venkateswara College, University of Delhi | SEPTEMBER 2020 – JANUARY /2021

Online Summer Training - Research Methodology 2.0

Shivaji College, University of Delhi | July 2021

PUBLICATION & CERTIFICATION

- **Chugh** A. Rai R, Sharma J.G. "Unveiling Future Advancements in Azo Dye Degradation and Enhanced Bioelectricity Production using Microbial Fuel Cells" - **manuscript accepted** in Research Journal of Biotechnology
- **Chugh** A, Khurana N, Verma K, Sehgal I, Rolta R, Vats P, Phartyal R, Salaria D, Kaushik N, Choi EH, Verma M, Kaushik NK. Changing Dynamics of SARS-CoV-2: A Global Challenge. Applied Sciences. 2022; 12(11):5546. https://doi.org/10.3390/app12115546 [**Published review paper**]
- "In Silico Evidence for Extensive Ser/Thr Phosphorylation of Mycobacterium tuberculosis Two Component Signalling Systems" (Commentary) doi: 10.18520/cs/v123/i9/1164-1169 [**Published Current Science Journal**]
- A, Chugh, Sehgal I, Khurana N, Verma K, Rolta R, Vats P, Salaria D, Fadare Oa, Awofisayo O, Verma A, Phartyal R, and Verma M. 2023. “Comparative Docking Studies of Drugs and Phytocompounds for Emerging Variants of SARS-CoV-2.” 3 Biotech 13(1). doi: 10.1007/s13205-022-03450-6. [**Published research paper**]

ACHIEVEMENTS, CO-CURRICULAR & EXTRA-CURRICULAR ACTIVITIES

- Research & Content Head- National Service Schemel Sri Venkateswara College | University of Delhi | 2021-2022

  Chief Editor - Darpan Magazine | Annual Magazine - National Service Scheme | 2022
- Amalgam-In-Charge - Catalysis, The Biochemical Society | Department of Biochemistry | University of Delhi | 2021-2022
- Awarded for outstanding performance in Chemistry in AISSCE Class XII examinations.
- Awarded for Excellence in Academics (All Subjects) in Class X CBSE examinations.

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CANDIDATE'S DECLARATION

I **Ananya Chugh, Roll No. 2K22/MSCBIO/08** hereby certify that the work which is being presented in the thesis entitled **"Microbial-Assisted Nanobioremediation Of Methyl Red"** is in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Prof. Jai Gopal Sharma.

The matter presented in the thesis has not been submitted by me for the award of any degree of this or any other Institute.

My review paper is accepted in SCI/SCI expanded/SSCI/Scopus indexed journal with the following details:

Title of the paper: Unveiling Future Advancements in Azo Dye Degradation and Enhanced Bioelectricity Production using Microbial Fuel Cells.

Name of Authors: Ananya Chugh, Riya Rai, Jai Gopal Sharma

Journal name: Research Journal of Biotechnology

Journal Indexing: Scopus

Status of paper: Accepted

Date of paper acceptance: May 7, 2024

Place: Delhi

Date: 06/06/24

Ananya Chugh

2k22/MSCBIO/08

DEPARTMENT OF BIOTECHNOLOGY

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CERTIFICATE

Certified that **Ananya Chugh (2k22/MSCBIO/08)** has carried out their search work presented in this thesis entitled **"Microbial-Assisted Nanobioremediation Of Methyl Red"** for the award of the degree of Master of Science and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. This thesis embodies results of original work, and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other Institution.

Prof. Yasha Hasija
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