M.Sc Biotechnology

IN-SILICO TARGETING OF WASTEWATER POLLUTANTS WITH LACCASE ENZYME USING MOLECULAR DOCKING

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CANDIDATE'S DECLARATION

I Swati Shandilay, Roll No. 2K21/MSCBIO/52 student of M.Sc. Biotechnology, hereby declare that the project Dissertation titled "**In-silico targeting of Wastewater pollutant with Laccase enzyme using Molecular Docking**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi **SWATI SHANDILAY**
Date: 30/May/2023

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**In-silico targeting of Wastewater pollutant with Laccase enzyme using Molecular Docking**" which is submitted by Swati Shandilay, Roll No. 2K21/MSCBIO/52, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the students 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
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ABSTRACT

Water resource preservation, environmental preservation, and public health protection all depend on effective wastewater treatment. It aids in preventing water body contamination, upholds ecological harmony, and assures a sustainable water supply for a variety of uses. It is important to remove toxins, pollutants, and other undesirable elements from wastewater as part of the wastewater treatment process. Its objective is to safely transform wastewater such that it can either be released into the environment or recycled for a variety of uses. In recent years, molecular docking has been successfully applied to the research of the biodegradation mechanism for environmental remediation. Although molecular docking has mostly been used in the fields of biology and medicine, it has shown to be a practical and economical way to accurately comprehend how proteins or enzymes interact with their ligands. This article seeks to provide an overview of how molecular docking has been used to investigate how organic contaminants and enzymes interact. The basic understanding of molecular docking, including its theory, available software tools, and key databases, is summarised in the paper's opening paragraphs. For successful docking research, it is essential to comprehend these factors. Following that, the review concentrates on five different categories of pollutants: phenols, BTEX (benzene, toluene, ethylbenzene, and xylenes), nitrile, polycyclic aromatic hydrocarbons (PAHs), and high polymers like lignin and cellulose. Through docking studies, the molecular interactions of these contaminants with enzymes are examined. The report also provides a detailed explanation of several removal procedures employing docking technology. The docking investigations shed light on how contaminants interact with enzymes and travel through the degradation process. Researchers can create better environmental remediation solutions by comprehending these mechanisms. Although molecular docking has some interesting uses in the study of biodegradation, the publication notes that more research is still required to apply the findings to actual environmental settings. It is crucial to verify the findings of docking studies using experimental data and to take into account the numerous environmental variables that could have an impact on the biodegradation process. In conclusion, this research discusses the use of molecular docking to investigate the interaction between organic contaminants and biodegradation enzymes. The fundamentals of molecular docking are covered, as well as the molecular features

of various contaminants and removal strategies. Although molecular docking has potential, further study is required to close the gap between theoretical discoveries and practical implementations.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

EPA	Environmental Protection agency
BTEX	Benzene, Toulene, Ethylbenzene, Xylene
PCB	Polychlorinated Biphenyl
PAH	Polycyclic Aromatic Hydrocarbon
OH	Hydroxyl
HRP	Horeseradish Peroxidase
Fe	Iron
VOC	Volatile Organic Comounds
PDB	Protein Data Bank
EM	Electron Microscopy
GO	Gene Ontology
SMD	Small Molecule Database
NCBI	National Center for Biotechnology Information
NLM	National Library of Medicine
ADME	Absorption, Distribution, Metabolism, Excretion
Qsar	Quantitative structure-activity relationship
SIB	Swiss Institute of Bioinformatics
FFT	Fast Fourier Transform
UCSF	University of California, San Francisco
PLIP	Protein-Ligand Interaction profiler
HB	Hydrogen Bond
VdW	VanderWaal
TRP	Tryptophan
HSD	Histidine
TYR	Tyrosine

CHAPTER 1

INTRODUCTION

Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and hydrocarbon derivatives are only a few examples of the organic pollutants that have grown to be a major problem for environmental pollution. Due to their persistence, toxicity, and ability to have a negative impact on ecosystems and human health, these pollutants are regarded as being dangerous. The Environmental Protection Agency (EPA) has designated phenols, PAHs, nitriles, and BTEX (benzene, toluene, ethylbenzene, and the three xylene isomers) as priority control pollutants in environmental samples [1] [2].

Given the volatility and persistence of these contaminants, addressing the environmental risks they cause is difficult. However, biodegradation holds promise among the chemical, physical, and biological approaches for pollution treatment because of a number of benefits [3]–[6].

1. Complete breakdown: Through the action of microorganisms, biodegradation has the capacity to totally break down organic contaminants into simpler, non-toxic chemicals. By using the pollutants as a source of energy and carbon, this process transforms the contaminants into safe byproducts [7].

2. Lower cost: In general, biodegradation has lower operational and maintenance expenses than other techniques like chemical or physical treatments. It doesn't require expensive chemical reagents or energy-intensive processes because it relies on the action of naturally occurring microbes [8].

3. Minimal secondary pollution: Compared to other remediation techniques, biodegradation often results in very little secondary pollution. Physical and chemical processes could leave behind byproducts or residues that need to be properly disposed

of, raising further environmental issues. Contrarily, biodegradation primarily results in the production of innocuous byproducts like carbon dioxide and water [9].

The efficiency of biodegradation can, however, vary depending on the specific contaminants, environmental factors, and the presence of compatible microbial populations, so it's crucial to keep this in mind. The biodegradation process can be influenced by variables like pH, temperature, oxygen availability, and the presence of co-contaminants.

Understanding the properties of the pollutant, site-specific conditions, and the appropriate selection of microbial cultures or tactics to boost microbial activity are essential for implementing biodegradation as a successful remediation strategy. For the biodegradation process to be efficient and effective in reducing organic pollutants, monitoring and optimisation are also necessary.

In conclusion, although biodegradation shows promise as a potentially efficient and environmentally friendly method for addressing organic pollutant contamination, careful consideration and evaluation of site-specific factors are required to determine its suitability and success in a specific remediation scenario.

CHAPTER 2

LITERATURE REVIEW

The investigation of the biodegradation pathway for environmental remediation has in fact successfully used molecular docking in recent years. Although molecular docking has traditionally been used primarily in the domains of biology and medicine, it can be used in a variety of other fields due to its adaptability.

The binding interactions between a tiny molecule (ligand) and a target protein or enzyme can be predicted computationally using molecular docking. It can reveal details about the molecular recognition and binding modalities of the ligand within the protein's active site by mimicking the docking procedure.

Molecular docking can be used to explore the interactions between pollutants or other environmental contaminants and the enzymes or proteins that are involved in their biodegradation. Researchers can learn how to speed up the biodegradation process and create more effective bioremediation techniques by comprehending the interactions and binding processes between the pollutants and the degrading enzymes.
One benefit of molecular docking is how convenient and inexpensive it is in comparison to experimental techniques. An extensive spectrum of researchers can do docking simulations using software tools and processing resources. Furthermore, with improvements in computational methods and algorithms, molecular docking accuracy has considerably increased, enabling more accurate predictions of ligand-protein interactions.

Overall, molecular docking has shown to be a useful method for studying biodegradation mechanisms for environmental remediation, offering a low-cost way to accurately comprehend interactions between proteins or enzymes and ligands.

Enzymatic catalysis is frequently used to biodegrade organic pollutants. Specialised proteins called enzymes function as biological catalysts, accelerating chemical reactions without being eaten. They are essential for dissolving intricate chemical compounds into more manageable components.

Enzymes bind to organic contaminants at their active sites during biodegradation. The portion of the enzyme's surface known as the active site is designed precisely to accommodate the pollutant molecule, enabling precise and selective binding. The building blocks of proteins, called amino acids, are normally arranged in a particular way to create the active site.

The enzyme's active site attracts the organic contaminant, which then undergoes a number of chemical processes that break it down into smaller molecules. These reactions may involve enzymatic processes like hydrolysis, oxidation, reduction, or other mechanisms. The organic pollutant eventually decomposes into less toxic or dangerous for the environment simpler components like carbon dioxide and water.

Exploring biodegradation pathways at the molecular level has revealed details on the specific enzymes involved, their active sites, and the chemical reactions they promote. The creation of enzymes with improved organic pollutant degrading capabilities and the development of more effective bioremediation procedures can both benefit from an understanding of these processes.

2.1 Phenols

A hydroxyl (-OH) group is joined to an aromatic ring in phenols, a category of organic molecules. Based on the number of phenol units, they can be divided into two primary categories: polyphenols, which have several phenol units, and simple phenols, which have a single phenol unit.

Phenols are synthesised naturally by plants and microorganisms in addition to through industrial methods [10], [11] . Phenols can build up in soil, groundwater, and surface water after being introduced into the environment, providing potential dangers to ecosystems [12].

Microorganisms can be crucial in the context of water treatment for phenol pollution. *Pseudomonas spp.* and *Acinetobacter spp.* are two bacterial species that have been used for their capacity to break down phenols and remove them from polluted water sources [13].

The chemical phenol itself is a significant raw material with several industrial uses. The manufacture of bakelite, oils, cokes, textiles, dyes, insecticides, and pharmaceuticals all heavily rely on it. As a result, it is among the main contaminants in industrial effluent.

It's vital to remember that phenol and its vapours have the potential to be toxic and damaging to people's health. They may irritate and harm the eyes, skin, and respiratory system. The strong chemical characteristics of phenol produce denaturation of proteins, which is what causes the corrosive effects on mucosal membranes and skin. As a result, when handling phenol and working with chemicals that include phenol, the required safety precautions and safeguards should be implemented [14].

According to the results of the molecular docking, phenol and laccase interact through hydrogen bonds and hydrophobic interactions [14] . This phenol-laccase binding on microbe surfaces may prevent the microorganism from effectively interacting with hydrophobic organic pollutants present in the aqueous phase [15]. To put it another way, the presence of phenol might make it more difficult for the microbe to break down hydrophobic organic contaminants.

However, it is feasible to lessen phenol's toxicity to cells and speed up phenol elimination by using certain surfactants. Surfactants, such as water and a hydrophobic material, are substances that can lessen the surface tension between two phases [16] . When *Candida tropicalis* is used as the degrading bacteria, the inclusion of surfactants can improve the interaction between the microbe and phenol, increasing the effectiveness of the degradation [17]–[19] [15].

Surfactants can improve the dispersion of hydrophobic organic pollutants in the aqueous phase and increase their accessibility to the microbe by lowering surface tension. This enables *Candida tropicalis* to efficiently breakdown phenol and other organic contaminants that are hydrophobic [15], [17].

It's important to note that the compatibility of a certain surfactant with the microorganism and pollutants present in the system should be taken into consideration. The toxicity of phenol and *Candida tropicalis*' ability to degrade it may be affected differently by various surfactants. Therefore, to find the best surfactant for a particular situation, significant thought and trial are required.

An enzyme called horseradish peroxidase (HRP) has been employed in a number of processes, including the purification of phenol-containing wastewater. Phenols are organic substances that are frequently present in industrial wastewater and can be hazardous to the environment [20]–[22].

Phenols can be oxidised by HRP, which produces less hazardous chemicals as a result. This is accomplished by catalysing a process with hydrogen peroxide that converts phenols into less dangerous, simpler compounds. Wastewater polluted with phenol can be effectively treated using this enzymatic oxidation method.

2.2 Nitriles

The interactions between nitriles and many degrading enzymes, particularly amidase, nitrile hydratase, and nitrilase, were explicitly shown using visual models [23].

A hydroxide ion may be adsorbed into acrylonitrile due to nucleophilicity and activation, according to a comparison of the relative locations of crystal water and the chemical compound. In this context, it is said that the metal atom in the enzyme plays a crucial part as a Lewis acid in the electrical receptor [24].

There are specific instructions for the Co-type nitrile hydratase-mediated biodegradation of acrylonitrile. Three phases make up the process:

1. Water molecules give the cobalt ion at the active site an OH group, forming the Co_2^+-OH complex.
2. A carbon-nitrogen double bond and a C-OH bond are formed when an oxygen atom from these complex attacks a carbon atom of the carbon-nitrogen triple bond in acrylonitrile.

3. A serine residue takes away the hydrogen atom from the C-OH bond. Acrylonitrile is transformed into acrylamide through this process [25].

According to the statement, Fe-type nitrile hydratase degrades differently from Co-type nitrile hydratase. Molecular docking and analysis revealed that the oxygen atom of the carbonyl group from the glutamine residue can activate a water molecule and take part in a chemical reaction in the case of Fe-type nitrile hydratase [26].

2.3 BTEX

The acronym BTEX stands for the isomers of benzene, toluene, ethylbenzene, and xylene, which are all aromatic hydrocarbons. These substances are frequently present in oil and are created as byproducts of various refining techniques, such as the catalytic reforming of naphtha [27].

The petrochemical industry relies heavily on the catalytic reforming process to transform low-octane hydrocarbons into higher-octane compounds that can be utilised to make petrol. The typical feedstock for catalytic reforming is naphtha, a light component of crude oil. The naphtha is heated and passed over a catalyst during this process, causing chemical reactions that result in the synthesis of aromatic hydrocarbons.

BTEX, also known as benzene, toluene, ethylbenzene, and xylene, are useful chemical intermediates with several industrial uses. They are sometimes referred to as volatile organic compounds (VOCs), and because of their toxicity and propensity for contaminating groundwater, they are linked to issues with the environment and human health [28]. As a result, petrochemical businesses have put policies in place to cut back on BTEX compound emissions and lessen their negative effects on the environment.
Findings suggest that an in-silico approach based on molecular docking and molecular similarity search can be used to identify and screen proteins with toluene. Additionally, toluene's considerable binding to six proteins, including DNA polymerase, haemoglobin, serum albumin, and cytochrome P450 2E1, was successfully demonstrated by the docking data [29] . Catechol-2,3-dioxygenase (EC 1.13.11.2), a typically multimeric enzyme, splits the benzene rings of a variety of environmental pollutants, such as

naphthalene, xylene, toluene, and derivatives of the biphenyl ring, and its catalytic action is dependent on Fe [30].

It's an intriguing idea to use docking technology in the realm of materials to investigate adsorption capacity. Studying the interactions between molecules and materials can be done using docking, which is frequently employed in molecular biology and drug discovery.

It is a good idea to study the zeolite's ability to bind benzene using supercagep-based molecular docking. The porous nature of zeolites allows for the selective adsorption of particular molecules based on their size, shape, and polarity [31]. By using molecular docking, one may forecast and examine interactions between benzene molecules and the zeolite surface, revealing information about the adsorption procedure and capacity.

In order to explore other pollutants' adsorption, benzene could potentially be replaced with them. The effectiveness of materials for environmental cleanup can be evaluated using this application. Researchers can assess the potential for adsorption of various pollutants and create more potent adsorbents for eliminating toxins from water or the air by virtually docking them with various materials.

2.4 PAHs (Polycyclic Aromatic Hydrocarbons)

In fact, PAHs (polycyclic aromatic hydrocarbons) are made of hydrogen and carbon atoms and are made up of two or more fused benzene rings. They are organic substances that are created when carbon-containing substances like oil, coal, petrol, and tobacco are burned partially.

PAHs include anthracene, phenanthrene, pyrene, and benzo[a]pyrene. These substances are recognised by their distinctive ring structures and are frequently present in a variety of environmental sources, such as industrial processes, exhaust emissions, and some forms of fossil fuels.

PAHs are classified as neutral and non-polar molecules since they are primarily composed of hydrocarbons. They don't have any polar functional groups or substantial charges, which makes them somewhat insoluble in water but soluble in organic solvents.

Their fondness for oil and coal, where they can amass, is also a result of their non-polar nature.

It's crucial to remember that PAHs have raised significant environmental and health concerns because of their potential for toxicity and carcinogenicity. Exposure to PAHs, especially by ingestion, absorption, or skin contact, has been linked to negative health impacts in people and can be dangerous for both ecological and social systems. In order to reduce potential risks, efforts are conducted to monitor and control PAH levels in a variety of sectors and environmental contexts [32], [33].

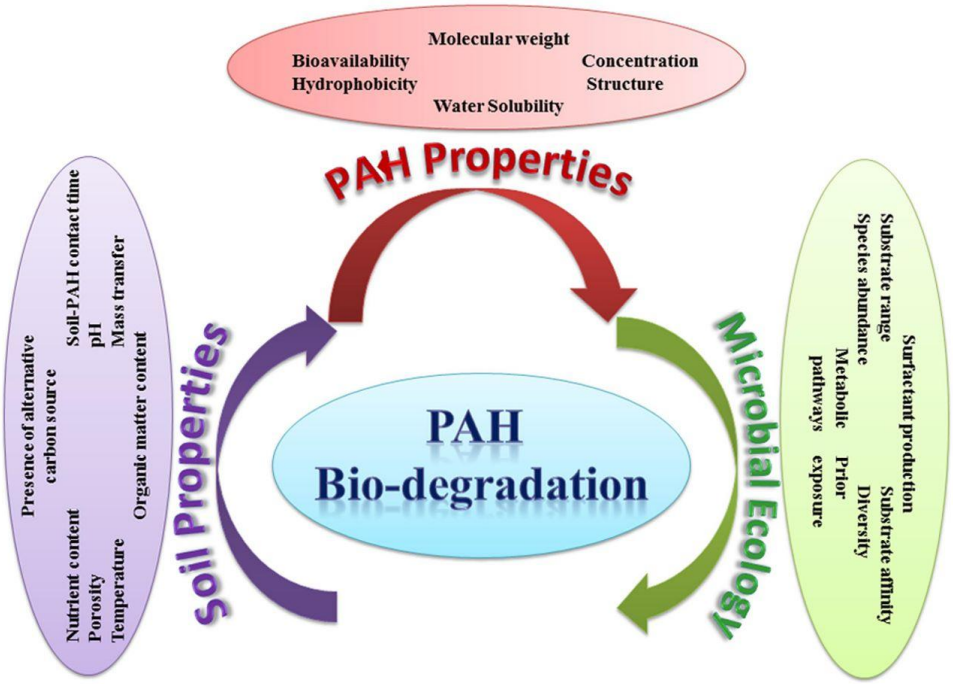


Figure 1: Abiotic abd Biotic factor influencing the breakdown of PAHs in soil

Polycyclic aromatic hydrocarbons, or PAHs, are created when organic materials like wood, tobacco, and fossil fuels are pyrolyzed or burned insufficiently. Because of their poor reactivity and resilience to deterioration, PAHs are recognised as persistent pollutants in the environment.

Although PAHs can be subjected to physical and chemical processes such as adsorption, breakdown, and attenuation, biodegradation has been identified as one of the most efficient ways to convert PAHs into water (H_2O) and carbon dioxide (CO_2).

Biodegradation refers to the breakdown of organic compounds by living organisms, particularly microorganisms [34].

Microorganisms like bacteria and fungus have the enzymes necessary to metabolise and break down PAHs. These enzymes use a series of biochemical processes to convert the complex PAH molecules into simpler ones. The final byproducts of biodegradation are the environmentally safe gases carbon dioxide and water.

The particular PAH chemical, environmental circumstances (such as temperature, pH, oxygen availability), existing microbial populations, and the presence of co-contaminants are some of the factors that affect the biodegradability of PAHs. Due to variations in their chemical characteristics and structures, certain PAHs biodegrade more quickly than others.

Through a variety of methods, PAH biodegradation can be accelerated in environmental remediation schemes. Introduce specialised microorganisms or microbial groups that can break down PAHs into contaminated areas as part of bioremediation. Enhancing environmental factors, such as offering oxygen and nutrients, can be used in conjunction with this strategy to encourage microbial activity and growth.

It's vital to remember that the individual conditions and complexity of the PAH-contaminated site can affect how efficient biodegradation is as a remediation strategy. To get satisfactory results, it could be essential to combine various remediation techniques, such as biodegradation and physical or chemical treatments.

Overall, biodegradation has tremendous potential to convert PAHs into harmless byproducts while being effective and environmentally acceptable, making it a key area of attention in the field of environmental rehabilitation.

2.4.1 Environmental and Chemico-physical Parameters Affecting Degradation

Polycyclic aromatic hydrocarbons (PAHs) can be degraded by enzymes in response to a variety of chemico-physical parameters as well as other factors [35]. The effectiveness, reaction kinetics, and selectivity of PAH breakdown by enzymes are significantly

influenced by these variables. The following are some of the crucial factors and variables that can influence enzymatic PAH degradation:

1. Substrate characteristics: The molecular size, hydrophobicity, solubility, and volatility of PAHs, as well as their chemical and physical makeup, can have a big impact on how quickly they are degraded by enzymes. Based on these characteristics, enzymes might favour certain PAHs over others.

2. Enzyme specificity: The degree of specificity that various enzymes have for PAHs varies. Due to variations in their active site structures and binding affinities, some enzymes may be more effective at destroying particular PAHs than others.

3. Enzyme concentration: The quantity of enzymes in the reaction mixture can have an impact on how quickly and effectively PAHs are degraded. If other parameters are not limiting, higher enzyme concentrations may improve the rate of breakdown.

4. Temperature and pH: Both the activity and stability of enzymes are influenced by temperature and pH levels. To ensure the highest level of enzyme activity for PAH breakdown, ideal temperature and pH ranges should be maintained.

5. Co-factors and co-substrates: Co-factors and co-substrates are frequently needed by enzymatic reactions to speed up the degradation process. The effectiveness and selectivity of PAH degradation may be impacted by the concentration and availability of these compounds.

6. Inhibitors are present: Some substances or environmental elements can prevent the breakdown of PAHs by enzymatic means. Inhibitors may directly interfere with the enzymatic reaction or compete with PAHs for enzyme binding sites, decreasing the efficiency or selectivity of breakdown.

7. Agents that trigger the synthesis of certain enzymes involved in PAH breakdown can be found in some compounds or substances. These inducers can increase enzyme activity and boost PAH breakdown processes' effectiveness.

8. Environmental factors can influence the enzymatic PAH breakdown process, including oxygen availability, moisture content, and the presence of other microbes or organic matter. The overall effectiveness and selectivity of the degradation process may be affected by the competitive or inhibitory effects created by these circumstances.

Understanding and improving these chemico-physical characteristics, along with other variables, can help with the development of methods to improve the efficiency, kinetics, and selectivity of enzymatic PAH degradation.

While anaerobic conditions refer to the lack of oxygen, aerobic conditions refer to the presence of oxygen in the surrounding environment or reaction system. The aerobic condition significantly affects the effectiveness, reaction kinetics, and selectivity of the degradation process when it comes to the enzymatic breakdown of polycyclic aromatic hydrocarbons (PAHs).

Microorganisms, such as bacteria or fungi, that have the enzymes necessary to degrade these sophisticated chemical molecules frequently perform PAH breakdown.

CHAPTER - 3

MATERIALS AND METHODS

3.1. Material Used

This is a brief description of softwares and database that are used for the in-silico molecular docking

3.1.1. PDB (Protein Data Bank

In the realm of structural biology, the PDB (Protein Data Bank) is a highly acknowledged and often utilised resource. It is a database that gives users access to biological macromolecules like proteins, nucleic acids, and complex assemblies that have had their three-dimensional structures determined by experimental means. The Worldwide Protein Data Bank (wwPDB) organisation, a partnership between numerous institutions worldwide, is in charge of managing the PDB.

Important aspects of the PDB:

1. The PDB serves as a central repository for structural data on biological macromolecules that have been determined through experimental means. It includes comprehensive atomic coordinates and other crucial details on the structures of proteins, nucleic acids, and complexes. Every entry in the PDB, which represents a distinct structure, is given a special identification number called the PDB ID.

2. **Protein Structures and Macromolecular Complexes:** The Protein Data Bank (PDB) is home to a sizable collection of protein structures, including those of enzymes, receptors, antibodies, and other useful proteins. It also includes complexes created by interactions between proteins, nucleic acids, and ligands, as well as the structures of nucleic acids like DNA and RNA. The database contains

information on a wide variety of species, including bacteria, viruses, plants, and animals.

3. **Experimental Techniques:** The PDB contains details of the experimental techniques used to establish the structures. This covers procedures including cryo-electron microscopy (cryo-EM), NMR spectroscopy, X-ray crystallography, and hybrid approaches. Researchers can evaluate the calibre and dependability of the structures thanks to the availability of the specifics of the experimental setup and data collection techniques.

4. **Structural Annotations and Metadata:** The PDB contains a plethora of structural annotations and metadata for each entry. This comprises details regarding the sequence of the protein or nucleic acid, biological purpose, ligand-binding locations, post-translational modifications, and other pertinent characteristics. Information about biological assembly, crystal packing specifics, and experimental settings are possible additional annotations.

5. Protein structure visualisation and analysis are made possible by the PDB's tools and resources. The representational styles that users can view structures in include ribbon, space-filling, and cartoon models. In-depth examination and analysis of structural characteristics, including as ligand interactions, active sites, and conformational changes, are also possible with the aid of interactive viewers and molecular visualisation software.

6. **Cross-referencing and Data Integration:** To give more information and improve data analysis, the PDB integrates with other databases and resources. Researchers can access further data about proteins, functional annotations, and ligands connected to the structures thanks to cross-references to other databases including UniProt, Gene Ontology (GO), and Small Molecule Database (SMD).

7. **Data Deposition & Community Contribution:** Researchers from all around the world may deposit data at the PDB. It motivates researchers to communicate their empirically discovered structures, adding to the body of knowledge and improving

academic study. By depositing the data in the PDB, it is made possible for other researchers to expand on the findings and conduct additional analysis.

3.1.2. PubChem

The National Centre for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM) of the United States, maintains the extensive database PubChem. It is a useful tool for scientists working in the domains of chemistry, biology, and bioinformatics. The biological functions, chemical compositions, and characteristics of tiny organic compounds are all covered in PubChem.

The three main parts of the database are substances, compounds, and bioassays. Unique chemical substances are included in the substances section along with information about them, such as their chemical structures, synonyms, and connections to associated resources. In-depth details on specific chemical compounds are provided in the compounds section, including references to scholarly articles, information on their physical and chemical properties, and experimental and projected characteristics. Information on the biological activities of chemicals is provided in the bioassays section. This information includes assay descriptions, results, and bioactivity ratings.

To make it easier to retrieve and explore data, PubChem provides a variety of search and analytical tools. Chemical names, identifiers, molecular formulas, and chemical structures can all be used by users to search the database. Additionally, PubChem offers tools for compound grouping, substructure searching, and chemical structure similarity searches.

Data from PubChem can be used by researchers for a variety of purposes. It makes it possible to find potential therapeutic targets, explore the chemical world in search of new drugs, and forecast compounds' biological activities and toxicological profiles. Structure-activity relationship (SAR) models can also be developed more easily, and chemical and biological data can be analysed for research and development reasons.

Additionally, PubChem is linked to other well-known databases, including PubMed, giving users access to more details on the biological and medicinal implications of the

chemicals and substances of interest. It is a useful tool for researchers working in the fields of chemical biology, small molecule research, and drug discovery.

3.1.3. BIOVIA Discovery Studio Visualizer

Modern drug development and research depend heavily on bioinformatics, which makes it possible to analyse and interpret enormous amounts of biological data. Dassault Systèmes created the comprehensive toolkit known as BIOVIA Discovery to enable bioinformatics processes. It offers researchers a variety of data management, analysis, visualisation, and predictive modelling functionalities.

A comprehensive software package called BIOVIA Discovery provides bioinformatics researchers with tools for data management, analysis, visualisation, and predictive modelling. Researchers can prioritise drug candidates, hasten the drug development process, and obtain insightful knowledge into biological systems thanks to its broad range of tools and features. BIOVIA Discovery facilitates the discovery of novel therapeutics and advances in bioinformatics research by integrating a variety of data formats, supporting advanced analysis methods, and fostering cooperation.

I will examine the main characteristics and uses of BIOVIA Discovery in the area of bioinformatics research in this paper.

Data Management and Integration: BIOVIA Discovery has strong data management features that make it possible for researchers to effectively organise, store, and retrieve biological data. It makes it easier to integrate different types of data, such as genomes, proteomics, chemical structures, and experimental results.

a. **Data Integration:** BIOVIA Discovery enables the integration of data from a variety of sources, including internal data repositories, external databases, and internally produced experimental data. Through this connection, data accessibility is improved and cross-domain analysis and interpretation are made easier.

b. **Data Visualisation:** The programme gives researchers strong visualisation tools to explore and examine intricate biological datasets. It offers interactive visualisations, allowing academics to learn more about the connections, trends, and patterns in the data.

c. **Data Mining:** BIOVIA Discovery uses cutting-edge data mining methods like association rule mining, clustering, and classification. These tools allow for the meaningful extraction of data from massive datasets, the discovery of hidden patterns, and the generation of hypotheses for further research.

Analysis Tools and Algorithms for Bioinformatics Research: BIOVIA Discovery provides a broad range of analysis tools and algorithms. These resources help scientists comprehend biological mechanisms, find biomarkers, and forecast the effects of genetic variants.

a. **Sequence Analysis:** The programme offers capabilities for sequence alignment, motif detection, and the discovery of conserved sections. These traits make it easier to identify functional components and evolutionary connections when comparing and annotating DNA and protein sequences.

b. **Structure Analysis:** With the aid of BIOVIA Discovery, protein structures may be analysed and visualised, making it easier to comprehend how proteins fold, stay stable, and interact with one another. It provides tools for molecular dynamics simulations, protein-ligand docking, and protein structure prediction, aiding efforts in structure-based drug discovery and protein engineering.

c. **Network Analysis:** The software has tools for examining gene regulatory networks, protein-protein interactions, and biological pathways using network analysis. Researchers can use these technologies to find important participants, comprehend network dynamics, and find brand-new therapeutic targets.

The ability to prioritise and assess new drug candidates is made possible by the predictive modelling and virtual screening features that BIOVIA Discovery integrates.

a. **QSAR and ADME Models:** The programme facilitates the creation and use of absorption, distribution, metabolism, and excretion (ADME) and quantitative structure-activity relationship (QSAR) models. These models help with lead optimisation and compound selection by forecasting the biological activity, toxicity, and pharmacokinetic characteristics of drugs.

b. **Virtual Screening:** The virtual screening methods in BIOVIA Discovery make it easier to find prospective drug candidates from vast compound libraries. In order to rank compounds according to their expected binding affinities and drug-like characteristics, it makes use of docking algorithms, pharmacophore modelling, and machine learning techniques.

Cooperation and Workflow Management: By offering capabilities for data sharing, project management, and version control, BIOVIA Discovery fosters cooperation and streamlines bioinformatics workflows.

a. Collaboration is made possible by the software, which makes it easier to share data, coordinate projects, and communicate with your team. In a centralised setting, it enables numerous researchers to collaborate, discuss discoveries, and trade ideas.

b. **Workflow Management:** Researchers can create and use bioinformatics pipelines using BIOVIA Discovery's workflow management features. Complex analytic procedures are made simpler by its support for automation, reproducibility, and the integration of different analysis tools.

3.1.4. SwissDock

Understanding the interactions between tiny molecules (ligands) and target proteins is a key task in the discipline of bioinformatics. It aids in the investigation of drug binding mechanisms, the prediction of protein-ligand complexes, and the development of novel medicinal substances. The Swiss Institute of Bioinformatics (SIB) created SwissDock, a potent molecular docking programme that has won widespread acclaim for its precision, effectiveness, and user-friendly design. We shall examine the main characteristics and uses of SwissDock in the context of bioinformatics research in this post.

The optimal orientation and binding affinity of a ligand molecule to a target protein are predicted using the computational method known as molecular docking. It entails sampling and scoring various ligand conformations and orientations within the binding site of the protein. The objective is to determine the most energetically advantageous

docking position, which can shed light on the ligand-protein interactions and direct drug discovery efforts.

Features and Capabilities of SwissDock: SwissDock is a web-based molecular docking platform that combines a number of tools and algorithms to carry out precise and effective simulations of protein-ligand docking. It has many characteristics and abilities that make it a popular option for bioinformatics researchers, including:

a. **User-Friendly Interface:** Researchers may readily input their protein and ligand structures, select docking parameters, and view the docking results thanks to SwissDock's user-friendly interface. Both seasoned professionals and industry newbies can use the platform.

b. **Ligand and Target Preparation:** Using SwissDock, users can complete activities including completing missing atoms, enhancing hydrogen bonds, and assigning charges to their ligand and target protein structures. With the help of these preprocessing techniques, the input structures are made appropriate for precise docking simulations.

c. **Docking techniques:** SwissDock uses a variety of docking techniques, such as a fast Fourier transform (FFT)-based docking method and a molecular dynamics-based approach. In order to determine the optimal docking posture, these algorithms sample several ligand conformations and orientations within the binding region of the protein.

d. **Scoring and Ranking:** SwissDock uses a variety of scoring procedures to gauge the ligand and protein's ability to bind to one another. These scoring formulae take into account variables including hydrogen bonds, electrostatic interactions, van der Waals forces, and shape complementarity. Based on the expected binding energies or scores, the software ranks the docking poses.

e. **Visualisation and Analysis:** To assist researchers in analysing the docking data, SwissDock offers visualisation tools. It allows for the interactive investigation of

docked complexes, allowing users to look at the interactions between ligands and proteins, hydrogen bonding patterns, and residues on binding sites that are important for complex formation.

3.1.5. Chimera

In the realm of bioinformatics, UCSF Chimera is a flexible piece of software that provides a wide range of features for molecular visualisation, analysis, and modelling. With its user-friendly interface and sophisticated functionality, researchers may learn a lot about intricate biomolecular structures and functions. UCSF Chimera supports tasks like structure-based drug design, protein engineering, and molecular dynamics simulations, advancing bioinformatics research and assisting in the creation of novel therapeutic approaches.

The ability to visualise and analyse intricate molecular structures is essential for understanding biological processes and developing new therapeutic approaches in the field of bioinformatics. The University of California, San Francisco (UCSF) created UCSF Chimera, a potent programme that has become well-known for its extensive set of molecular visualisation, analysis, and modelling features. In the framework of bioinformatics research, we shall examine the salient characteristics and uses of UCSF Chimera in this paper.

a. Proteins, nucleic acids, and other molecular structures, as well as tiny compounds, can all be visualised and analysed using the wide range of capabilities offered by UCSF Chimera. Researchers can study and interact with complicated biomolecular systems using its user-friendly interface, gaining important knowledge about their structures and functions.

b. A highly interactive and adaptable 3D visualisation environment is provided by UCSF Chimera, allowing users to rotate, zoom, and explore molecular structures. The exploration and study of intricate macromolecular assemblies are made easier by the support for a wide range of depiction approaches, such as surface representations, ribbon diagrams, and molecular surfaces.

c. Molecular dynamics simulations, which replicate how biomolecules behave and move over time, can be visualised and examined by researchers using Chimera. In

order to better comprehend protein motions and conformational changes, it offers tools for trajectory analysis, calculation of crucial dynamics parameters, and visualisation of dynamic features.

d. UCSF Chimera incorporates a number of tools for sequence and structure analysis, allowing researchers to carry out tasks like sequence alignment, structure superposition, and structural motif identification. These characteristics are helpful for contrasting protein structures, locating conserved areas, and researching functional domains.

e. **Molecular Modelling and Simulation:** Based on experimental results or computational predictions, researchers can create and improve 3D models of biomolecules using UCSF Chimera's powerful molecular modelling and simulation capabilities.

A. **Homology models**, which predict the three-dimensional structure of a protein based on its sequence similarity to experimentally known structures, can be created using Chimera. It generates precise models using cutting-edge algorithms and offers insightful information about how proteins' structure and function relate to one another.

B. **Molecular docking**, a computer method that forecasts the binding mechanism and affinities of small compounds to target proteins, is incorporated into UCSF Chimera. Utilising Chimera, researchers can analyse binding interactions, dock ligands into protein binding sites, and evaluate the stability of the resultant complexes.

C. **Calculations of electrostatic potentials on molecular surfaces:** Chimera enables users to compute and view electrostatic potentials on molecular surfaces, facilitating comprehension of protein-ligand interactions and protein-protein recognition. The research of protein binding sites, the identification of crucial residues involved in ligand binding, and the development of novel medicinal drugs can all benefit greatly from this property.

D. **Integration & Extensibility:** Researchers can import and export data from a variety of sources using UCSF Chimera's support for a wide range of file formats. It effectively interfaces with other programmes and databases, enabling the transfer of data and streamlining intricate bioinformatics research workflows.

E. Chimera's plugin architecture enables researchers to increase its capability by creating original plugins or by making use of those already created by the UCSF Chimera community. Chimera can be customised to meet particular research objectives and integrated into current bioinformatics processes because to its extensibility.

F. **Database Connectivity:** Users can immediately access and view experimental structures using UCSF Chimera's ability to connect to external databases like the Protein Data Bank (PDB). This connection makes it easier to get current structural data and do comparative analysis.

3.1.6. PLIP

In many areas of bioinformatics, such as drug discovery, protein engineering, and structural biology, an understanding of how proteins and small molecules interact is crucial. A potent bioinformatics tool for analysing and visualising protein-ligand interactions is called PLIP (Protein-Ligand Interaction Profiler). It gives scientists useful information on the binding mechanisms, important residues, and non-covalent interactions present in protein-ligand complexes. We shall examine the main characteristics and uses of PLIP in the context of bioinformatics research in this paper.

1. **Protein-Ligand Interaction Analysis:** Using a wide range of computational techniques, PLIP enables researchers to conduct thorough investigations of protein-ligand interactions. It analyses three-dimensional protein-ligand complex structures and produces thorough analyses and visualisations that draw attention to key interactions and how they affect ligand binding.

2. The automatic identification of binding sites inside protein structures by PLIP is a crucial step in the research of ligand recognition and protein function. It allows

researchers to concentrate on certain areas of interest since it finds cavities, pockets, and active locations.

3. **Interaction Profiling:** PLIP analyses the protein-ligand non-covalent interactions, such as hydrogen bonds, hydrophobic contacts, salt bridges, and interactions involving the - stacking group. These interactions are quantified, highlighting their strengths and frequency while also revealing important details about the stability of the protein-ligand complex.

4. **Residue Analysis:** PLIP characterises the contributions made by the major residues involved in ligand binding. It aids in understanding the molecular basis of ligand recognition and binding specificity by providing details on the kind and strength of interactions for each residue.

PLIP provides visualisation tools to aid in the analysis of protein-ligand interactions and the development of a deeper comprehension of the intricate structure-function links.

a. **Interactive 3D visualisations of protein-ligand complexes** are produced using PLIP, enabling researchers to investigate the structure and interactions of the complex. It highlights the important residues involved in ligand binding and offers an intuitive picture of the ligand-binding site.

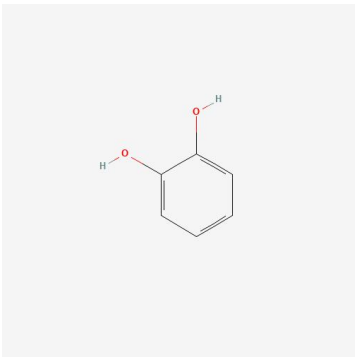
b. **Interaction Diagrams:** Using PLIP, interaction diagrams are created that show how a protein interacts with a ligand non-covalently. These diagrams make it easy to identify key interactions that influence ligand binding and give a clear summary of the binding modes.

c. **Binding Affinity Estimation:** To calculate the binding affinity between a protein and its ligand, PLIP connects with external tools and databases. Researchers can pick potential therapeutic candidates by using molecular docking or scoring methods to acquire insights into the strength of the protein-ligand interaction.

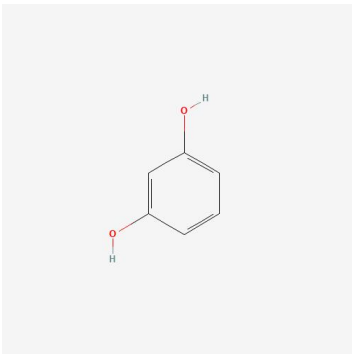
3.2. Workflow

2.2.1. Receptor (enzyme) Ligand (Organic Pollutant) Interaction:

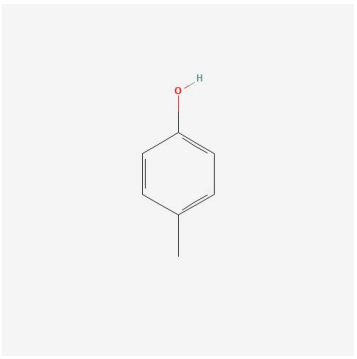
The receptor (enzyme) studies were collected from Protein Data Bank (PDB) (https://www.rcsb.org/), and the in-silico ligand (organic pollutant) structure data was taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Using Biovia Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download), the docking of the target enzyme laccase, 2H5U, and 10 ligands (organic pollutants) including Catechol, Resorcinol, Cresol, Hydroquinone, Naphthol, Bisphenol, and Guaiacol was prepared. Removing hetero atoms from things like water, non-amino acid groups, and other ligand compounds is necessary to prepare the ligand and receptor.



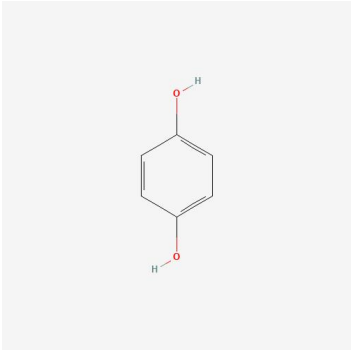
3. Catechol



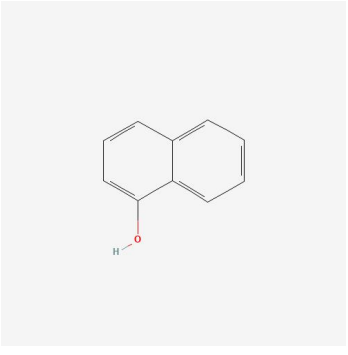
2. Resorcinol



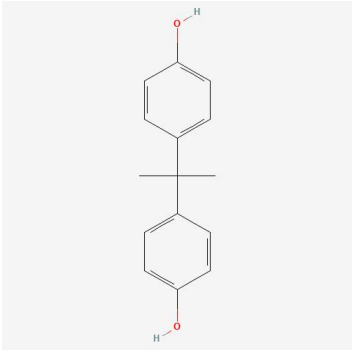
1. Cresol



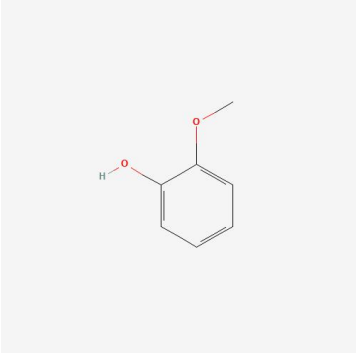
4. Hydroquinone



5. Naphthol



6. Bisphenol



7. Guaiacol

Figure 2: The 2D structure of organic pollutants used for docking analysis. 1. Catechol, 2. Resorcinol, 3. Cresol, 4. Hydroquinone, 5. Naphthol, 6. Bisphenol and 7. Guaiacol

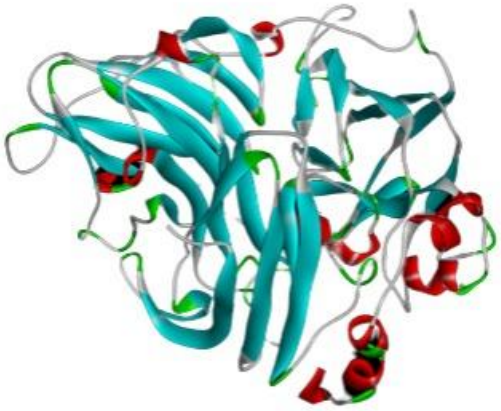


Figure 3: 3D structure of Laccase enzyme

3.2.2. Molecular Docking

The designing of ligands and receptors typically marks the beginning of in-silico molecular docking. The target enzyme was docked against a few selected organic contaminants using the SwissDock internet server (http://www.swissdock.ch/docking#).

3.2.3. Docking Interaction Analysis

Values of affinity binding (G) were recorded and examined. The interactions between organic contaminants and the chosen target laccase enzyme were examined by UCSF Chimera. (https://www.cgl.ucsf.edu/chimera/). Through the study of amino acids and related data, such as density maps and sequence alignments, UCSF Chimera provides dynamic molecular structure visualisation and analysis. Organic pollutants with high binding energies were chosen after the binding energies of organic pollutants and the control were compared.

3.2.4. PLIP Analysis

The downloaded outputs from SwissDock were analyzed via PLIP to identify by which amino acid the organic pollutants binds to the enzyme. Organic contaminants with high binding energies were chosen after the binding energies of the ligands and standards were compared.

The specific amino acids in the enzyme that interact with the organic contaminants were discovered by the PLIP analysis. Hydrogen bonds, VanderWaals interactions, hydrophobic contacts, and electrostatic interactions are only a few of the interactions covered by PLIP. The amino acids implicated in the organic pollutants' binding to the enzyme were identified by examining the output.

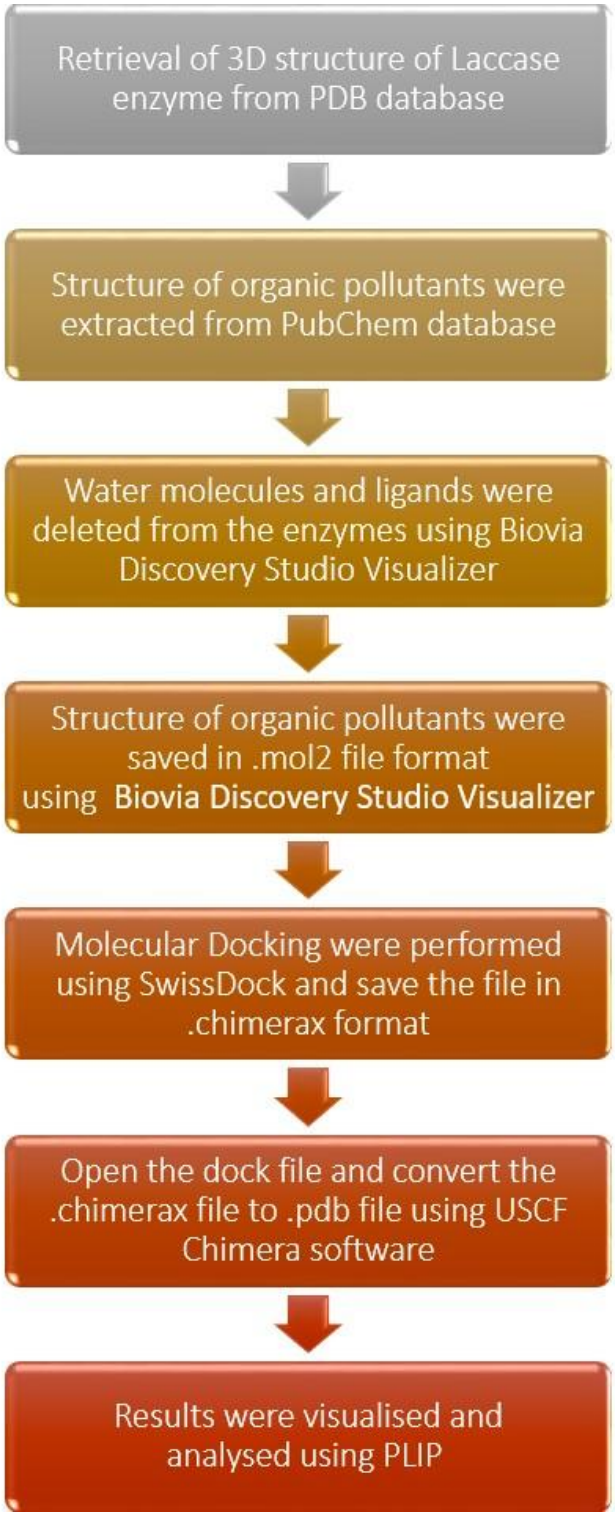


Figure 4: Flowchart of protocol followed

CHAPTER - 4

RESULT AND DISCUSSION

The docking results show that Catechol, Resorcinol, Cresol, Hydroquinone, Naphthol, Bisphenol, and Guaiacol form hydrogen bonds (HB) and VanderWaal (VdW) interactions with the laccase enzyme residues of TRP107A, HSD109Z, and TYR116A, and that these interactions have free energy binding values of -6.32, -6.59, -6.30, -6.36, -6.24, -7.34 respectively.

The laccase enzyme residues and organic contaminants have been discovered to interact through hydrogen bonding and VanderWaals interactions, which suggests a high degree of compatibility and affinity. The organic contaminants may be able to successfully bind to the laccase enzyme's active site, according to the favourable free energy of binding.

These findings have significant ramifications since they indicate that the above-mentioned organic pollutants can be catalysed and degraded by the laccase enzyme. Strong VanderWaals interactions between the contaminants and the enzyme suggest a long-lasting binding in the active site, which is essential for effective degradation.

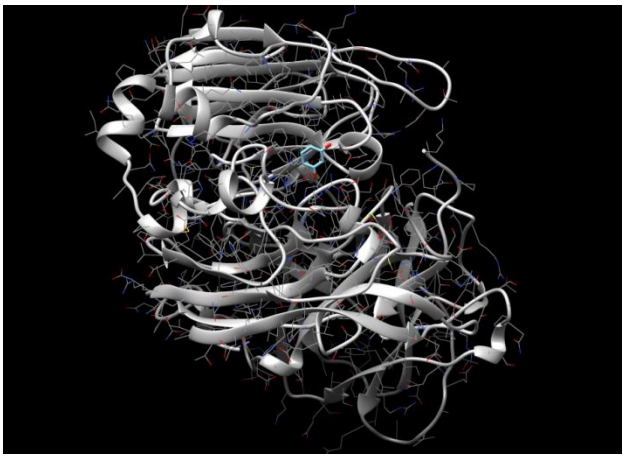
The laccase enzyme's catalytic activity and its capacity to bind with and breakdown these organic contaminants are both highly dependent on the discovered residues TRP107A, HSD109Z, and TYR116A. Additional research into the precise mechanisms and enzymatic procedures involved in the breakdown of these pollutants may provide light on the potential uses of laccase in pollution and environmental cleanup.

These results offer information on laccase's ability to break down organic pollutants and advance our knowledge of the molecular connections between organic pollutants and the enzyme. They lay the groundwork for future research aimed at improving the enzymatic breakdown of organic contaminants and investigating laccase's potential as a biotechnological tool for wastewater treatment and environmental remediation.

Table 1: Binding energy (in kcal/mol) of selected organic pollutants

S.No.	Organic Pollutants	PubChem ID	ΔG
1.	Catechol	289	-6.32
2.	Resorcinol	5054	-6.59
3.	Cresol	2879	-6.30
4.	Hydroquinone	785	-6.36
5.	Naphthol	7005	-6.24
6.	Bisphenol	6623	-7.34
7.	Guaiacol	460	-6.25

Resorcinol



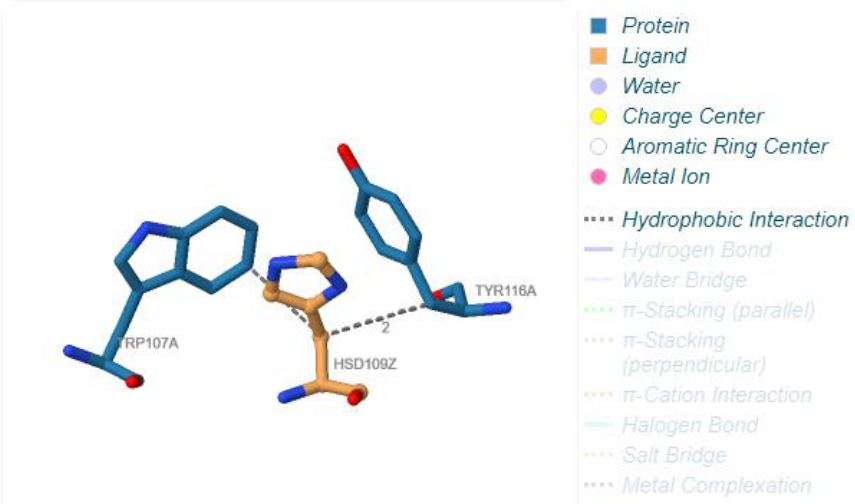
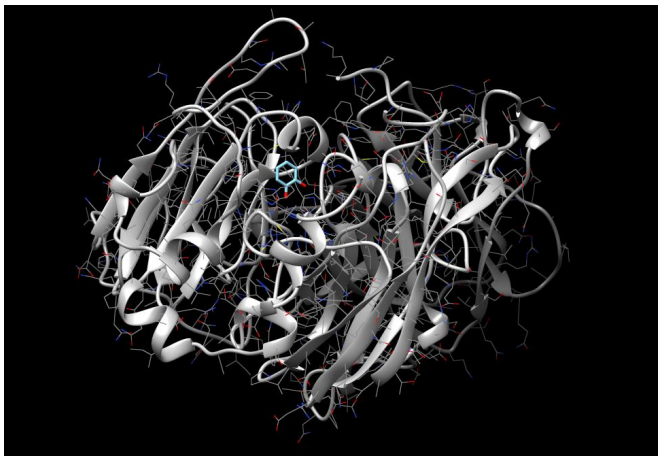


Figure 5: 1. Docking of Resorcinol with Laccase enzyme, 2. PLIP analysis

Catechol



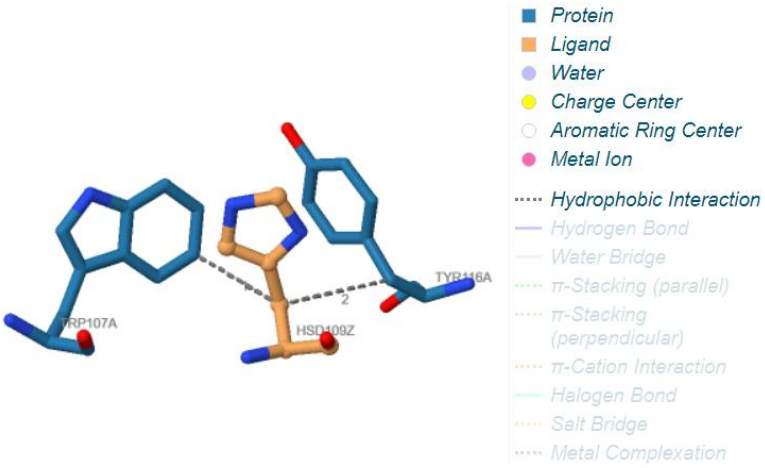
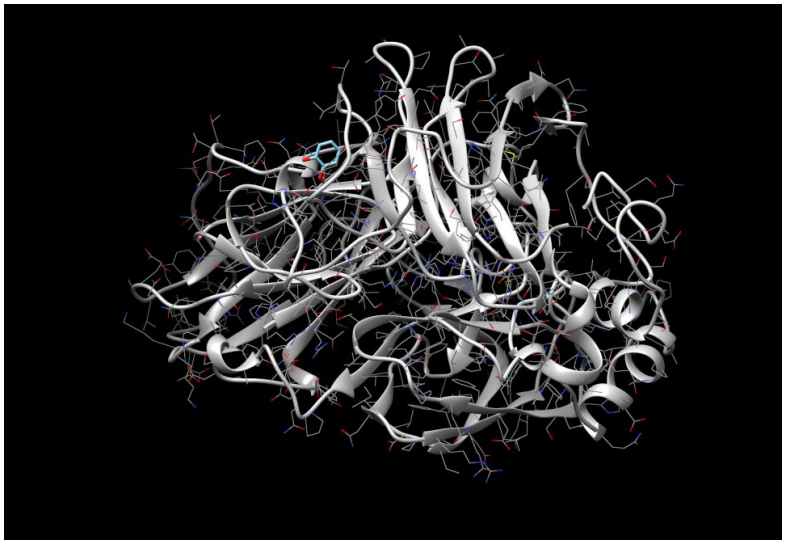


Figure 5: 1. Docking of Catechol with Laccase enzyme, 2. PLIP analysis

Guaiacol



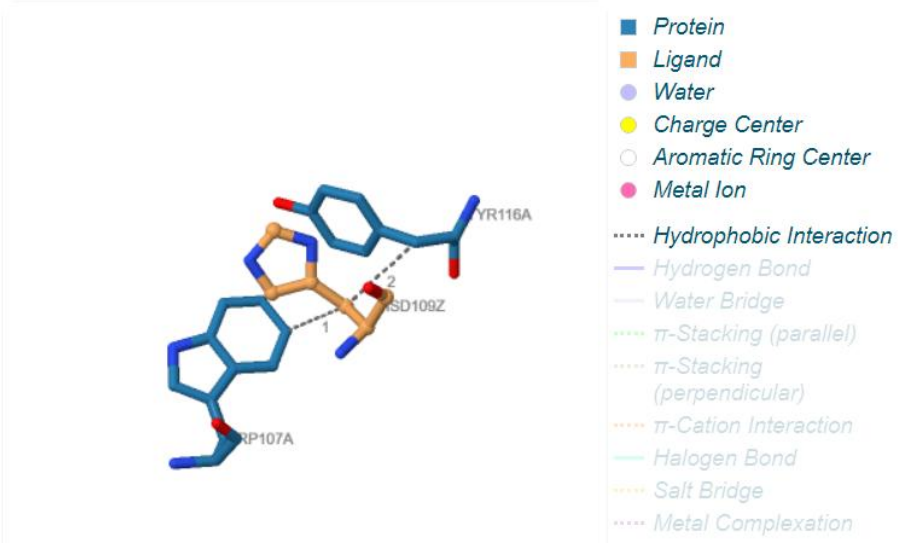


Figure 6: 1. Docking of Guaiacol with Laccase enzyme, 2. PLIP analysis

Hydroquinone

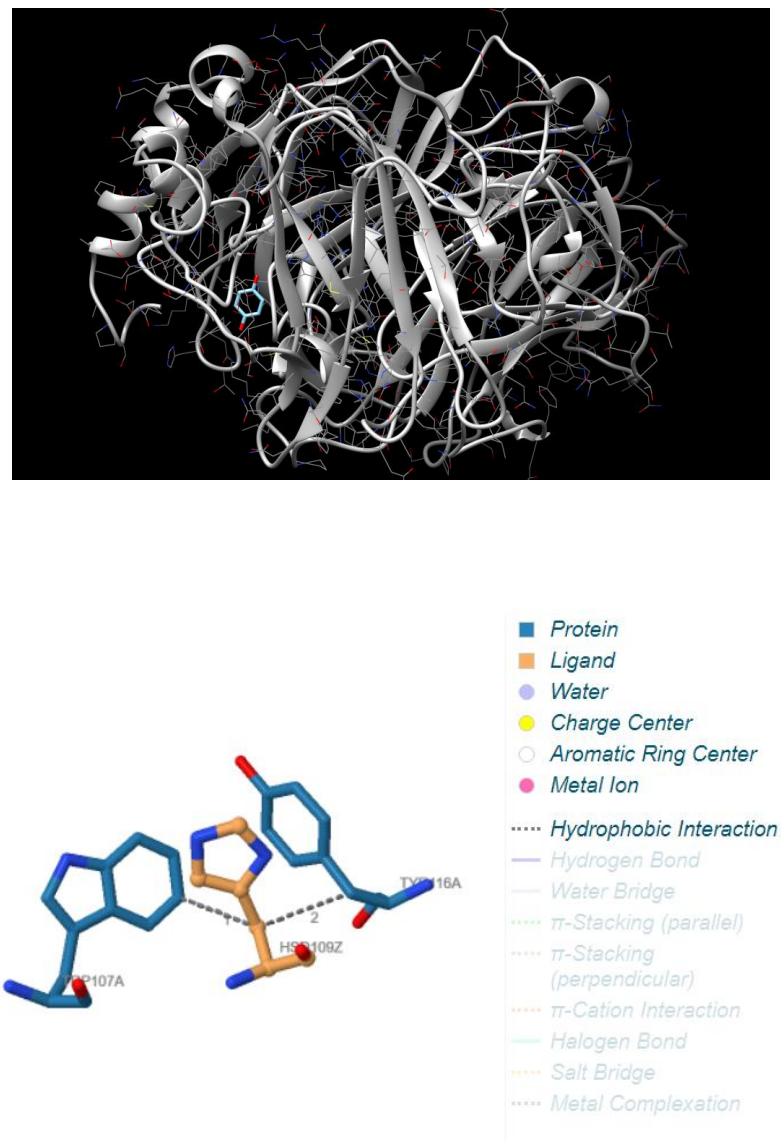


Figure 7: 1. Docking of Hydroquinone with Laccase enzyme, 2. PLIP analysis

Bisphenol

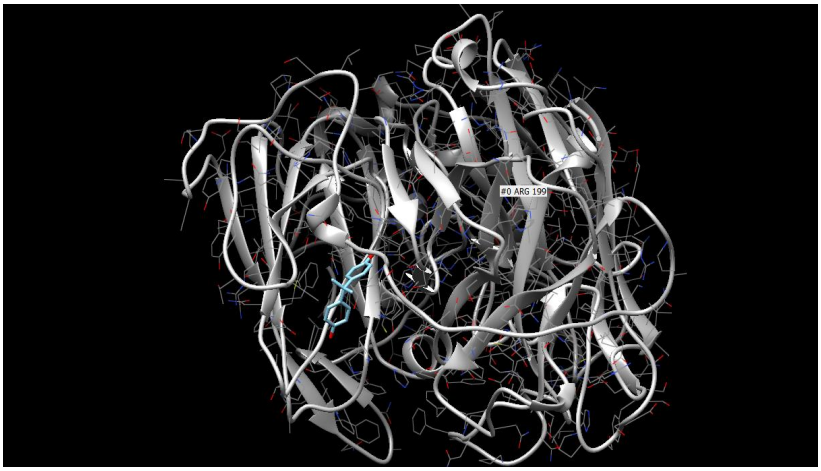


Figure 8: 1. Docking of Bisphenol with Laccase enzyme

Cresol

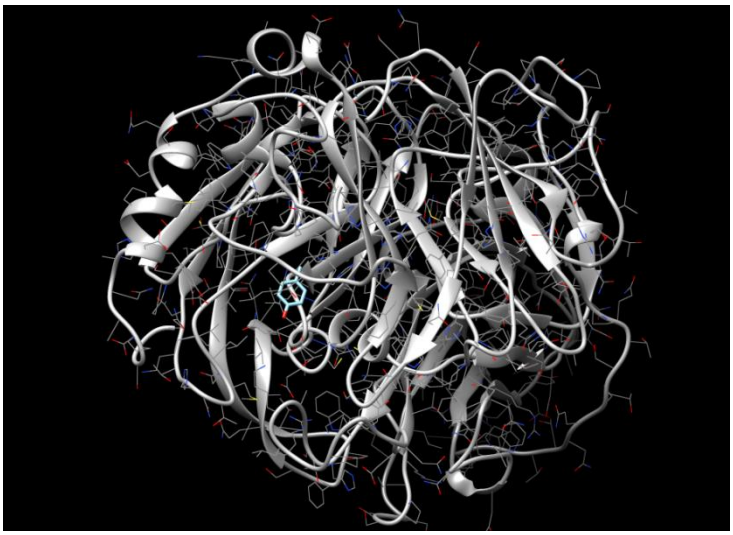


Figure 9: 1. Docking of Cresol with Laccase enzyme

CHAPTER - 5

CONCLUSION AND FUTURE PERSPECTIVE

In recent years, researchers have concentrated on the effectiveness of contaminant removal by regulating and changing reaction conditions, with less attention paid to underlying causes like the metamorphosis of enzymes. Enzymes, however, are crucial to biodegradation. Studies on the interactions and alterations between enzyme and substrate are useful for directing related investigations. Hydrogen bonds, hydrophobic interactions, electrostatic interactions, and more are examples of interactions. The major takeaway from this review is that molecular docking is a promising technique. Due to its simplicity and inexpensive cost, molecular docking was widely used in various research disciplines. In particular, molecular docking has the ability to predict and explain the biological reaction's mechanism. Not all benefits from science and technological advancements will benefit humanity, and a number of new contaminants need to be eliminated. The findings contribute to our understanding of the molecular interactions between organic pollutants and the enzyme laccase and the ability of laccase to degrade organic pollutants. They establish the foundation for future studies that aim to enhance the enzymatic breakdown of organic pollutants and look into laccase's potential as a biotechnological tool for wastewater treatment and environmental remediation. In conclusion, using this technology to study reaction mechanisms will be encouraging for cleaning up the environment. However, due to various conditions and attention, there are numerous difficulties in fusing theory and reality. At the atomic level, the mechanisms of electron transfer and enzymatic reactions are difficult to understand.

REFERENCES

[1] L. Keith and W. Telliard, “ES&T Special Report: Priority pollutants: I-a perspective view,” *Environ Sci Technol*, vol. 13, no. 4, pp. 416–423, Apr. 1979, doi: 10.1021/es60152a601.

[2] G. Zeng, M. Chen, and Z. Zeng, “Risks of neonicotinoid pesticides.,” *Science*, vol. 340, no. 6139, p. 1403, Jun. 2013, doi: 10.1126/science.340.6139.1403-a.

[3] C. H. Walker, *Organic Pollutants*. CRC Press, 2008. doi: 10.1201/9781420062595.

[4] G. Zeng, M. Chen, and Z. Zeng, “Shale gas: Surface water also at risk.,” *Nature*, vol. 499, no. 7457, p. 154, Jul. 2013, doi: 10.1038/499154c.

[5] X. Hu *et al.*, “Adsorption of chromium (VI) by ethylenediamine-modified cross-linked magnetic chitosan resin: isotherms, kinetics and thermodynamics.,” *J Hazard Mater*, vol. 185, no. 1, pp. 306–14, Jan. 2011, doi: 10.1016/j.jhazmat.2010.09.034.

[6] Y. Feng *et al.*, “Adsorption of Cd (II) and Zn (II) from aqueous solutions using magnetic hydroxyapatite nanoparticles as adsorbents,” *Chemical Engineering Journal*, vol. 162, no. 2, pp. 487–494, Aug. 2010, doi: 10.1016/j.cej.2010.05.049.

[7] R. Margesin and F. Schinner, “Biodegradation and bioremediation of hydrocarbons in extreme environments.,” *Appl Microbiol Biotechnol*, vol. 56, no. 5–6, pp. 650–63, Sep. 2001, doi: 10.1007/s002530100701.

[8] T. Fan *et al.*, “Biosorption of cadmium(II), zinc(II) and lead(II) by Penicillium simplicissimum: Isotherms, kinetics and thermodynamics.,” *J Hazard Mater*, vol. 160, no. 2–3, pp. 655–61, Dec. 2008, doi: 10.1016/j.jhazmat.2008.03.038.

[9] M. Chen, P. Xu, G. Zeng, C. Yang, D. Huang, and J. Zhang, “Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs.,” *Biotechnol Adv*, vol. 33, no. 6 Pt 1, pp. 745–55, Nov. 2015, doi: 10.1016/j.biotechadv.2015.05.003.

[10] S. Hättenschwiler and P. Vitousek, “The role of polyphenols in terrestrial ecosystem nutrient cycling.,” *Trends Ecol Evol*, vol. 15, no. 6, pp. 238–243, Jun. 2000, doi: 10.1016/s0169-5347(00)01861-9.

[11] A. Khoddami, M. A. Wilkes, and T. H. Roberts, “Techniques for analysis of plant phenolic compounds.,” *Molecules*, vol. 18, no. 2, pp. 2328–75, Feb. 2013, doi: 10.3390/molecules18022328.

[12] L. Gianfreda, F. Sannino, M. A. Rao, and J. M. Bollag, “Oxidative transformation of phenols in aqueous mixtures.,” *Water Res*, vol. 37, no. 13, pp. 3205–15, Jul. 2003, doi: 10.1016/S0043-1354(03)00154-4.

[13] V. K. Dhatwalia and M. Nanda, “Biodegradation of Phenol,” 2016, pp. 198–214. doi: 10.4018/978-1-4666-9734-8.ch010.

[14] Y. Zhang *et al.*, “Effect of Triton X-100 on the removal of aqueous phenol by laccase analyzed with a combined approach of experiments and molecular docking.,” *Colloids Surf B Biointerfaces*, vol. 97, pp. 7–12, Sep. 2012, doi: 10.1016/j.colsurfb.2012.04.001.

[15] G. Zeng *et al.*, “Effect of monorhamnolipid on the degradation of n-hexadecane by Candida tropicalis and the association with cell surface properties.,” *Appl Microbiol Biotechnol*, vol. 90, no. 3, pp. 1155–61, May 2011, doi: 10.1007/s00253-011-3125-6.

[16] Z. Liu *et al.*, “Investigation on the reaction of phenolic pollutions to mono-rhamnolipid micelles using MEUF.,” *Environ Sci Pollut Res Int*, vol. 24, no. 2, pp. 1230–1240, Jan. 2017, doi: 10.1007/s11356-016-7851-2.

[17] Z.-F. Liu, G.-M. Zeng, J. Wang, H. Zhong, Y. Ding, and X.-Z. Yuan, “Effects of monorhamnolipid and Tween 80 on the degradation of phenol by Candida tropicalis,” *Process Biochemistry*, vol. 45, no. 5, pp. 805–809, May 2010, doi: 10.1016/j.procbio.2010.01.014.

[18] Z.-F. Liu *et al.*, “Effect of saponins on cell surface properties of Penicillium simplicissimum: performance on adsorption of cadmium(II).,” *Colloids Surf B Biointerfaces*, vol. 86, no. 2, pp. 364–9, Sep. 2011, doi: 10.1016/j.colsurfb.2011.04.021.

[19] Z. Liu *et al.*, “Influence of rhamnolipids and Triton X-100 on adsorption of phenol by Penicillium simplicissimum.,” *Bioresour Technol*, vol. 110, pp. 468–73, Apr. 2012, doi: 10.1016/j.biortech.2012.01.092.

[20] L. Tang, G.-M. Zeng, G.-L. Shen, Y.-P. Li, Y. Zhang, and D.-L. Huang, “Rapid detection of picloram in agricultural field samples using a disposable immunomembrane-based electrochemical sensor.,” *Environ Sci Technol*, vol. 42, no. 4, pp. 1207–12, Feb. 2008, doi: 10.1021/es7024593.

[21] I. Alemzadeh and S. Nejati, “Phenols removal by immobilized horseradish peroxidase.,” *J Hazard Mater*, vol. 166, no. 2–3, pp. 1082–6, Jul. 2009, doi: 10.1016/j.jhazmat.2008.12.026.

[22] E. Kalaiarasan and T. Palvannan, “Removal of phenols from acidic environment by horseradish peroxidase (HRP): Aqueous thermostabilization of HRP by polysaccharide additives,” *J Taiwan Inst Chem Eng*, vol. 45, no. 2, pp. 625–634, Mar. 2014, doi: 10.1016/j.jtice.2013.07.003.

[23] Y. Zhang *et al.*, "Enzyme-substrate binding landscapes in the process of nitrile biodegradation mediated by nitrile hydratase and amidase.," *Appl Biochem Biotechnol*, vol. 170, no. 7, pp. 1614–23, Aug. 2013, doi: 10.1007/s12010-013-0276-1.

[24] L. Peplowski, K. Kubiak, and W. Nowak, "Insights into catalytic activity of industrial enzyme Co-nitrile hydratase. Docking studies of nitriles and amides.," *J Mol Model*, vol. 13, no. 6–7, pp. 725–30, Jul. 2007, doi: 10.1007/s00894-007-0181-z.

[25] H. Yu, J. Liu, and Z. Shen, "Modeling catalytic mechanism of nitrile hydratase by semi-empirical quantum mechanical calculation.," *J Mol Graph Model*, vol. 27, no. 4, pp. 522–8, Nov. 2008, doi: 10.1016/j.jmgm.2008.09.003.

[26] L. Song, M. Wang, J. Shi, Z. Xue, M.-X. Wang, and S. Qian, "High resolution X-ray molecular structure of the nitrile hydratase from Rhodococcus erythropolis AJ270 reveals posttranslational oxidation of two cysteines into sulfinic acids and a novel biocatalytic nitrile hydration mechanism.," *Biochem Biophys Res Commun*, vol. 362, no. 2, pp. 319–24, Oct. 2007, doi: 10.1016/j.bbrc.2007.07.184.

[27] S. Matar and L. F. Hatch, "Chemicals Based on Benzene, Toluene, and Xylenes," in *Chemistry of Petrochemical Processes*, Elsevier, 2001, pp. 262–300. doi: 10.1016/B978-088415315-3/50011-0.

[28] S. Mitra and P. Roy, "BTEX : A Serious Ground-water Contaminant," *Res J Environ Sci*, vol. 5, no. 5, pp. 394–398, May 2011, doi: 10.3923/rjes.2011.394.398.

[29] Y. G. Chushak, R. R. Chapleau, J. S. Frey, C. A. Mauzy, and J. M. Gearhart, "Identifying potential protein targets for toluene using a molecular similarity search, in silico docking and in vitro validation," *Toxicol Res (Camb)*, vol. 4, no. 2, pp. 519–526, 2015, doi: 10.1039/C5TX00009B.

[30] D. Wojcieszyńska, K. Hupert-Kocurek, A. Jankowska, and U. Guzik, “Properties of catechol 2,3-dioxygenase from crude extract of Stenotrophomonas maltophilia strain KB2 immobilized in calcium alginate hydrogels,” *Biochem Eng J*, vol. 66, pp. 1–7, Jul. 2012, doi: 10.1016/j.bej.2012.04.008.

[31] S. S. Jirapongphan, J. Warzywoda, D. E. Budil, and A. Sacco, “Simulation of benzene adsorption in zeolite HY using supercage-based docking,” *Microporous and Mesoporous Materials*, vol. 94, no. 1–3, pp. 358–363, Sep. 2006, doi: 10.1016/j.micromeso.2006.04.011.

[32] J.-L. Gong *et al.*, “Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent.,” *J Hazard Mater*, vol. 164, no. 2–3, pp. 1517–22, May 2009, doi: 10.1016/j.jhazmat.2008.09.072.

[33] P. Xu *et al.*, “Use of iron oxide nanomaterials in wastewater treatment: a review.,” *Sci Total Environ*, vol. 424, pp. 1–10, May 2012, doi: 10.1016/j.scitotenv.2012.02.023.

[34] A. K. Haritash and C. P. Kaushik, “Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review.,” *J Hazard Mater*, vol. 169, no. 1–3, pp. 1–15, Sep. 2009, doi: 10.1016/j.jhazmat.2009.03.137.

[35] O. S. Obayori *et al.*, “Differential degradation of crude oil (Bonny Light) by four Pseudomonas strains.,” *J Environ Sci (China)*, vol. 21, no. 2, pp. 243–8, 2009, doi: 10.1016/s1001-0742(08)62258-5.

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I Swati Shandilay, Roll No. 2K21/MSCBIO/52 student of M.Sc. Biotechnology, hereby declare that the project Dissertation titled "**In-silico targeting of Wastewater pollutant with Laccase enzyme using Molecular Docking**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**In-silico targeting of Wastewater pollutant with Laccase enzyme using Molecular Docking**" which is submitted by Swati Shandilay, Roll No. 2K21/MSCBIO/52, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the students 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
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Prof. Jai Gopal Sharma
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30/05/2023

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