IN SILICO SCREENING OF VERSATILE PEROXIDASE : A COMPARATIVE ANALYSIS OF BINDING AFFINITY WITH A MIXTURE OF POLLUTANTS USING MOLECULAR DOCKING SIMULATION

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Submitted by:

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CANDIDATE'S DECLARATION

The work presented in my dissertation, "**In silico screening of Versatile peroxidase: A comparative study of the binding affinity using molecular docking simulation**," which I submitted to the Department of Biotechnology at Delhi Technological University, Delhi, in partially satisfying of the criteria for the award of a Master of Science in Biotechnology degree, is the outcome of my own distinctive and independent research. I, Deepali Dhyani, roll no. 2K23/MSCBIO/18, a student pursuing an M.Sc. Biotechnology, hereby verify to this. I declare that every bit of data and material used in my thesis has been correctly cited. This work, in whole or in part, has not been given to any other organization or educational institutions for the purpose of granting any degree, diploma, or professional title**.**

Place: Delhi **DEEPALI DHYANI**

Date: 5/June/2025

DEPARTMENTOF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)

CERTIFICATE

I certify that the project work completed by the students under my supervision is documented in the Project Dissertation, "In-silico screening of Versatile peroxidase: A comparative analysis of binding affinity with a mixture of pollutants using molecular docking simulations," submitted by Deepali Dhyani, Roll No. 2K23/MSCBIO/18, Department of Biotechnology, Delhi Technological University, Delhi, in partially satisfying of the requirement for the award of the degree of Master of Science. This work has never, as far as I know, been submitted in whole or in part for a degree or diploma at this institution or elsewhere.

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ABSTRACT

The infestation of the environment have become an issue across the worldwide due to the mass dissemination of polycyclic aromatic hydrocarbons (PAHs), herbicides, dyes, and endocrine-disrupting compounds, causing sustained environmental impact. Versatile peroxidase is a heme-based ligninolytic biocatalyst secreted by white rot fungi. They have multi-substrate compatibility and assist in the degradation of tenacious environmental toxins. This research employs in-silico molecular docking to broadly analyze the binding affinity of VP and a sample of multiple toxic pollutants, each characterized by their resistance to degradation and ecotoxicity. The three-dimensional structure of VP was designed and assessed followed by computational docking analysis with a provided industrial effluent using PyRx and Vina. Binding affinities, interaction dynamics, and essential catalytic residues were examined to analyze the stability and nature of enzyme-pollutant interactions. This comparative analysis also introduces the concept of competitive binding—how the ligands behave with versatile peroxidase in docking of a mixture of pollutants compared to individual docking. This in silico evaluation provides a fundamental understanding of enzyme substrate specificity, promoting ongoing research in biocatalyst development and pollutant degradation.

Keywords: Versatile peroxidase, molecular docking, binding affinity, polycyclic aromatic hydrocarbons, in silico inspection, comparative analysis, competitive binding, multi-ligand docking, enzymatic degradation.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

VP	Versatile Peroxidase
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
PAC	Polycyclic Aromatic Compounds
HEM	Haemoglobin
Mn	Manganese
PDB	Protein Data Bank
ALA	Alanine
PRO	Proline
VAL	Valine
LYS	Lysine
GLU	Glutamine
ARG	Arginine
HIS	Histidine
ILE	Isoleucine

CHAPTER 1

INTRODUCTION

The toxic consequences, long-term persistence, and bio accumulative potential of persistent organic pollutants (POPs) have made environmental pollution from these pollutants an important concern [1], [2]. Due to their ongoing accumulation and persistent existence, they pose adverse impacts on human health and ecology. The phenolic compounds such as bisphenol A, guaiacol, and 4-chlorophenol; the polycyclic aromatic hydrocarbons (PAHs) anthracene and naphthalene; and other contaminants like toluene, phenol, and atrazine are often observed in industrial effluents and are widely known for their toxicology and resistance to environmental degradation [3], [4].

These intricate pollution combinations are rarely adequately degraded by conventional remediation techniques. Physical and chemical remedies are two examples of conventional pollutant elimination strategies that are often expensive, futile, and could result in subsequent waste. An environmentally friendly and sustainable alternative in this context is biological remediation, which utilizes biological agents to purify areas with contamination.

Renowned for possessing ligninolytic enzyme mechanisms, especially the versatile peroxidase (VP), white-rot fungi (*Pleurotus eryngii*) have proven an exceptional capacity to break down complex aromatic contaminants [5]. A diverse variety of recalcitrant pollutants, including lignin derivatives in addition various xenobiotics, can be oxidized by VP, a heme-containing oxidoreductase produced by white-rot fungi [6]. It is a promising option for bioremediation processes applications due to its exceptional ability to oxidize a broad range of aromatic and non aromatic compounds including pollutants that have significant oxidative potential.

Molecular docking, along with other in silico approaches, has proved indispensable for predicting the dynamics and binding capacity between pollutants and enzymes. The significance of docking simulations in anticipating the biodegradation ability of enzymes like laccases, lignin peroxidases, and versatile peroxidases for a wide range of contaminants has been demonstrated in various studies [7], [8].

In natural environments, contaminants often exist as complex mixtures, and their potential for enzyme decomposition can be altered significantly by cooperative or competitive binding effects[9], [10].For example, the way that PAHs and phenolic chemicals interact in mixtures might impact how well enzymes like VP and laccase bind to their active sites and catalyze reactions [11].

Hence, it is essential to analyze pollutant-enzyme interactions in combination with simulations that better reflect environmental complexity and assess the potential for bioremediation. Rather than examining contaminants individually, we bridge this gap in this study by conducting a comparative in silico docking investigation of a pollutant mixture with versatile peroxidase. Compounds commonly found in contaminated industrial and agricultural runoff, such as anthracene, naphthalene, bisphenol A, guaiacol, 4-chlorophenol, phenol, toluene, and atrazine, were identified in our mixture[12], [13]. A binding energy shift was observed in the results when compared to individual docking investigations. Recent computational studies that highlight the relevance of docking in understanding mixture-based bioremediation provide additional support for our strategy [14].

LITERATURE REVIEW

Versatile peroxidase (VP), which is composed of heme, can oxidize a variety of substrates, including both hydroxybenzene and aliphatic substances. Its distinct catalytic adaptability makes it a viable option for bioremediation projects. Recent research has investigated VP's structural features in an effort to improve its activity and stability. To illustrate its potential in the bioconversion of β-naphthol, a model pollutant, Hoque et al. examined a novel plant-derived VP from Citrus sinensis, examining its active site [15].

Comprehending the interactions between VP and various pollutants has been made achievable via molecular docking. In an extensive docking research, Singh et al. evaluated the binding affinities of VP with priority pollutants such as phenol, naphthalene, bisphenol A, toluene, atrazine, guaiacol, and 4-chlorophenol. Significant interactions have been identified in their studies, suggesting that the enzyme may be able to fragment down these chemical compounds [16]. Similarly, Mishra et al. used molecular docking and dynamics computations to analyse the requisite interactions of bacterial laccases and peroxidases with commercial colours. Their studies highlighted how essential it is to fully understand the interactions among enzymes and their substrates in order to develop effective bioremediation techniques [17].

Environmental contaminants known as polycyclic aromatic hydrocarbons (PAHs) include naphthalene and anthracene. Peroxidases like VP have been found in studies to oxidize these compounds, thereby leading them to break down. Using molecular docking examinations, Singh et al. demonstrated that VP has the ability to degrade anthracene [16]. Using molecular docking with receptors for adrenergic stimuli, Verma et al. also assessed the toxicity of anthracene, providing data regarding interaction processes [19]. Industrial contaminants are frequently phenolic chemicals. It has been shown that VP effectively oxidizes phenol and bisphenol A, thereby rendering it easy to eliminate them from areas of contamination. The applicability of plant-derived VP in the degradation of such phenolic pollutants is supported by a study conducted by Hoque et al. [15]. Atrazine and toluene are renowned for their toxicity and persistence. Although there are few specific studies on how VP interacts with toluene and atrazine, the enzyme's wide variety of targets implies that it may be effective in degrading these

chemicals [16]. Two phenolic compounds that are frequently utilized as model targets in enzymatic analyses are guaiacol and 4-chlorophenol. VP's relevance for bioremediation applications is illustrated by its well-documented ability to oxidize such chemicals [16].

Finding efficient interactions between enzymes and pollutants has been made easier by the incorporation of molecular docking into bioremediation techniques.

For the mitigation of marine-based pollutants, Maqsood et al. also emphasized new developments in bioremediation techniques, such as bioaugmentation [20]. In a similar vein, microbially embedded enzyme biocatalysts have demonstrated promise for the removal of several pollutants in environmental applications [21].

Because of their high toxicity and resistant to degradation, chlorinated phenols are priority pollutants. Bilal et al.'s enzyme kinetic studies discovered that fungal VP effectively oxidized guaiacol, a prevalent substrate for peroxidases, observing over 60% degradation in 24 hours [22]. Singh et al.'s docking results also confirmed stable VP binding to 4-chlorophenol, indicating the enzyme's potential for halogenated aromatic degradation [16].

Bisphenol A (BPA) and phenol are prevalent water contaminants, particularly found in the effluents of the plastic and petrochemical industries. According to studies, VP effectively converts phenolic rings into quinones, which lessens the toxicity of the compounds. According to Singh et al.'s computational analysis, stable enzyme-ligand complexes were indicated by docking scores of −8.7 and −7.5 kcal/mol for BPA and phenol, respectively, when docked with VP [16].

Anthracene and naphthalene did had significant binding affinities to VP, according to Singh et al. [16], but they only verified the possibility of interaction rather than actualdegradation.

According to Verma et al. [19], anthracene binds well to biological receptors, but it also has a persistent toxicity that suggests stable connections.

Successful catalysis requires enzymatic flexibility and optimum alignment, which may not be possible with large, inflexible molecules like PAHs, according to other

research (e.g., [15], [17]). Compounds like toluene and atrazine bind to VP extremely poorly. Atrazine's triazine ring lacks in redox-active moieties, while the methyl group in toluene induces steric hindrance. Low binding affinity and little degradation potential are demonstrated by docking experiments [16], [23].

Despite the prevalence of individual docking research, little is understood regarding mixture docking, which entails docking multiple contaminants either sequentially or simultaneously. Multiple contaminants can influence the accessibility of the active site by cooperative or competitive binding, which could differ from the data obtained with a single ligand. For example, steric advantages could enable guaiacol or phenol to outcompete larger PAHs like anthracene for access to the active site. This competition is thought to be an unusual characteristic in computational enzymology and may affect degradation order [17],[18].

VP's use in treatment of wastewater, soil biological remediation, and biosensor creation all depend on an understanding of its molecular interactions with environmental contaminants. A significant anticipating method prior to experimental validation is computational docking.

CHAPTER 3

MATERIALS AND METHODS

3.1.1. PDB (Protein Data Bank)

The PDB (Protein Data Bank) is a renowned and often accessed resource in the discipline of structural biology. Users of the database can access biological macromolecules such as DNA, proteins, nucleic acids, and other complicated assemblies whose three-dimensional structures have been determined through experimental approaches. The PDB is maintained by the Worldwide Protein Data Bank (wwPDB), an organization that is a collaboration among many institutions throughout the globe.

The fundamental purpose of the PDB is to provide a standardized, organized, and publicly accessible platform for storing experimentally determined macromolecular structures. Each structure in the PDB is allocated a unique four-character numeric identity known as the PDB ID, which facilitates easy referencing and retrieval. A distinct PDB ID is given to every deposited structure, along with a wealth of metadata such as atomic coordinates, resolution, refinement statistics, experimental conditions, structure validation reports, and thorough annotations regarding the macromolecule's biological role and place of origin. The meticulous curation and validation of this data upholds high standards of quality, which is essential for researchers who depend on precise molecular data for computational studies.

The PDB is an essential tool for functional genome research, protein modification, the development of drugs, and molecular simulation. In the context of molecular docking, a crucial computational method for predicting how proteins will interact with tiny molecules (ligands), the PDB provides the structural information needed to create realistic receptor models. These receptor structures save time and money in the early phases of development and research by enabling scientists to evaluate potential contaminants or possible therapies in silico. For instance, the capacity of enzymes such as Versatile peroxidase and other essential enzymes to break down toxic contaminants is currently being studied in environmental biotechnology.

Scientists are able to predict which enzymes are most effective for bioremediation by using their 3D structures from the PDB and docking them with contaminants

like naphthalene, bisphenol A, or anthracene. This enables them to construct more effective environmental cleanup tactics.

Additionally, a variety of visualization and statistical techniques are provided by the PDB to aid with molecular understanding. Users can examine molecular surfaces, assess binding sites, display complex structures in atomic detail, and even model mutations to study their effects on protein stability and function utilising platforms such as PyMOL, Chimera, and the RCSB PDB's own 3D viewer. Because they enable intuitive investigation and more comprehensive understanding into molecular systems, these tools serve as essential for both novice and specialist researchers.

By encouraging flexibility, reliability, and collaboration in the biological fields, the PDB's broad availability and open-access design democratize scientific knowledge. Across every discipline, from fundamental studies to applied sciences, from academia to the pharmaceutical sector, the impact is undeniable. The database continues to grows quickly, with numerous new structures added each year to represent advances in experimental technology and contributions to biological research from throughout the world. The PDB provides an abundance of tutorials, examples, and resources for instruction in addition to raw data, making it a valuable learning resource for computational biology researchers and students.

Eventually, the Protein Data Bank is a crucial and flexible platform that promotes current biosciences and is much more than a mere collection of molecular structures. The PDB offers the fundamental structure information needed for innovation and discovery, whether medicine efficacy prediction, enzyme function comprehension, or pollution degradation strategy creation. Its importance in molecular docking underscores its vital role in solving real-world problems through in silico methods, especially in research domains such as environmental pollution degradation employing microbial enzymes. The PDB will only become more significant in promoting study, instruction, and real-world applications as scientific techniques and equipment progress.

3.1.2. PubChem

PubChem is an extensive database used by scientists in the disciplines of biology, chemistry, and bioinformatics will benefit from it. PubChem offers details on the characteristics, chemical makeup, and physiological functions that involve tiny organic molecules.

Drugs, chemicals, and biological assays constitute the database's three main sections. The substances section lists unique chemical substances and provides details about them, including their chemical compositions, analogies, and links to related sites. The compounds section offers extensive details on certain chemical compounds, including facts on their physical and chemical qualities, experimental and expected features, and references for educational journals.

The bioassays section contains information on the biological properties of substances. Assay descriptions, consequences, and bioactivity ratings are all comprised of this data. PubChem offers a range of search and analytical tools to facilitate the retrieval and exploration of data. Users can search the database using chemical names, IDs, molecular algorithms, and chemical structures. Additionally, PubChem provides tools for chemical structure similarity searches, compound grouping, and substructure searching. Researchers can use PubChem data for a number of reasons. It provides an investigation of the chemical world in seeking new pharmaceuticals, the determination of possible therapeutic targets, and prediction of the biological processes and toxicological profiles of substances. It is also simple to create structure-activity relationship (SAR) models.

Moreover, users may obtain additional information about the biological and medical implications of the 27 chemicals and substances that are intriguing by linking PubChem to other well-known databases, such as PubMed. For scientists working on drug discovery, smaller-molecule research, and chemical biology, it is a significant tool.

3.1.3. BIOVIA Discovery Studio Visualizer

Significant amounts of biological data can be examined and understood thanks to bioinformatics, which is crucial to modern medicines development and research.The comprehensive toolkit known as BIOVIA Discovery was developed by Dassault Systèmes for assisting bioinformatics procedures. It offers researchers with an array of features for data management, evaluation, visualization, and modeling predictions.

Data administration, analysis, presentation, and predictive modeling applications are made available to bioinformatics researchers through an extensive suite of programs called BIOVIA Discovery. With the help of its many tools and capabilities, researchers may prioritize drug candidates, speed up the drug discovery process, and get important understanding into biological systems. By combining many data formats, enabling sophisticated analysis techniques, and encouraging collaboration, BIOVIA development helps to encourage bioinformatics research and the development of new therapeutics.

Strong data management features in BIOVIA Discovery empower researchers to effectively organize, store, and have access to biological data. Merging many data kinds, include chemical structures, the field of proteomics genomes, and experimental consequences, is made easier by it. Strong visualization features are offered by the software that allow researchers to examine and analyze complex biological datasets. It equips scholars with dynamic visualizations that help them understand relationships, patterns, and trends in the data.

Protein structures can be investigated and represented with the help of BIOVIA Discovery, which simplifies comprehension of how peptides fold, maintain equilibrium, and interact with one another. Protein-ligand docking, protein structure prediction, and molecular dynamics computational tools are among the assets it offers to assist with structure-based drug discovery and protein modification. Program features involve the ability to identify conserved sections, detect motifs, and align genomes. These features facilitate the contrasting and annotation of DNA and protein sequences, enabling for the easier recognition of functional elements and evolutionary connections.

3.1.4. PyRx and Vina

A popular free and open-source virtual screening program that combines numerous computational techniques for drug discovery and molecular docking is called PyRx (Python Prescription). For conducting complicated docking experiments, it provides a user-friendly graphical user interface (GUI), which makes it especially beneficial for researchers and students who may not have been accustomed to command-line-based computational chemistry tools. PyRx, which includes additional features for ligand preparation and molecular visualization, relies on the AutoDock suite, particularly AutoDock Vina. This connection accelerates the otherwise complicated and challenging technological process of digital screening.

The powerful and efficient molecular docking engine AutoDock Vina, created by the Scripps National Institute, is an essential component of PyRx. Known for its speed, preciseness, and convenience for users, Vina is an enhanced AutoDock functionality. In order to maintain realistic binding affinity estimates and significantly improve docking performance, it employs a gradient-based conformational search technique. By evaluating the molecular binding energy between a ligand and a macromolecular target (usually a protein or enzyme), AutoDock Vina uses a scoring technique that enables researchers to rank various ligands according to their binding affinities. Structure-based drug design and enzyme-substrate relationship research, including environmentally friendly biotechnology applications such microbial enzymes' breakdown of pollutants, benefit significantly from this.

By integrating numerous parts into a single interface, PyRx optimizes the docking process. Ligand processing allows for the import of molecules in various formats (such as SDF, MOL2, and PDB) and their conversion to the necessary PDBQT format. Protein processing allows users to add hydrogens and charges while removing water molecules and unneeded chains from receptor files. The active site region where docking takes occur is indicated by the grid box layout. In order to focus the docking analysis on a specific binding site or cavity, this step is critical. High-throughput capabilities virtual screening involving multiple ligands against an individual receptor is made achievable by batch processing.

PyRx is renowned for its association with Open Babel, an open-source chemical toolbox for enhancing molecular structures and transcoding molecular file formats. Using force fields like MMFF94 or UFF (Universal Force Field), Open Babel in PyRx enables users to energy minimise the ligand structures. This guarantees that ligands start in a realistic conformation, enhancing docking accuracy. Upon

finishing docking, PyRx displays a well-organized results table that comprises:

1.Binding affinity values for each ligand-receptor complex, stated in kcal/mol.

2. Position rankings, with the most effective (lowest energy) stance shown first.

3. 3D visualization applications that let users examine the interactions between ligands and the receptor, such as steric complementarity, hydrophobic interactions, and bonding through hydrogen.

Using external tools like PyMOL or Chimera, the docking results generated by PyRx can be examined or exported for further investigation. This allows for an in-depth analysis of interactions, including the recognition of important sites involved in binding. When it comes to environmental molecular docking investigation, PyRx is an ideal tool for examining how pollutants like phenols, endocrine disruptors, and polycyclic aromatic hydrocarbons (PAHs) interact with microbial enzymes such versatile peroxidase, laccase, or dioxygenases. For assessing an enzyme's binding energy, compatibility with an active site, and possible degradation routes, it is feasible to dock it with different environmental pollutants using the Protein Data Bank (PDB). By spotting possible enzyme-substrate combination for bioremediation applications, these computer predictions assist in directing laboratory evaluations.

Furthermore, PyRx is a freely accessible software, and it may run on Linux, macOS, and Windows. PyRx is one of the most commonly utilized instruments in academic research, pharmacological screening, and computational education because of its accessibility and user-friendliness. A complete virtual screening platform is provided by PyRx, a versatile and successful molecular docking tool that is powered by AutoDock Vina. It is especially appropriate for researchers and students conducting in silico docking investigations since it combines ligand preparation, docking, and visualization tools into a single graphical user interface. PyRx enables greater understanding of molecular interactions with little experimental expenditure by spanning the gap between structural evidence and functional anticipating, whether it is used for environmental biotechnology or for drug development.

3.2. Workflow

3.2.1. Sample and Pollutant Selection :A contaminated industrial effluent sample is obtained and analyzed chemically to confirm the presence of the given pollutant compounds, each of a different chemical nature: phenol (a simple aromatic compound), naphthalene (PAH), anthracene (a tricyclic aromatic hydrocarbon), toluene (an aromatic hydrocarbon), atrazine (a herbicide), chlorophenol (a chlorinated aromatic compound), bisphenol (an endocrine disruptor), and guaiacol (a lignin-derived phenolic compound) using HPLC and GC. As our study is based on the in-silico analysis, we must obtain a standard 3D structure of each compound.

3.2.2. B. Retrieval of the receptor molecule and ligand structures : The structure of the target protein (versatile peroxidase) PDB ID: 3FJW is supplied by the Protein Data Bank (PDB). (https://www.rcsb.org/), a renowned protein database, and is extracted in PDB format from the PDB database for further docking studies. The tertiary structures of the eight ligand compounds are taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and extracted in the PubChem's SDF format for molecular docking.

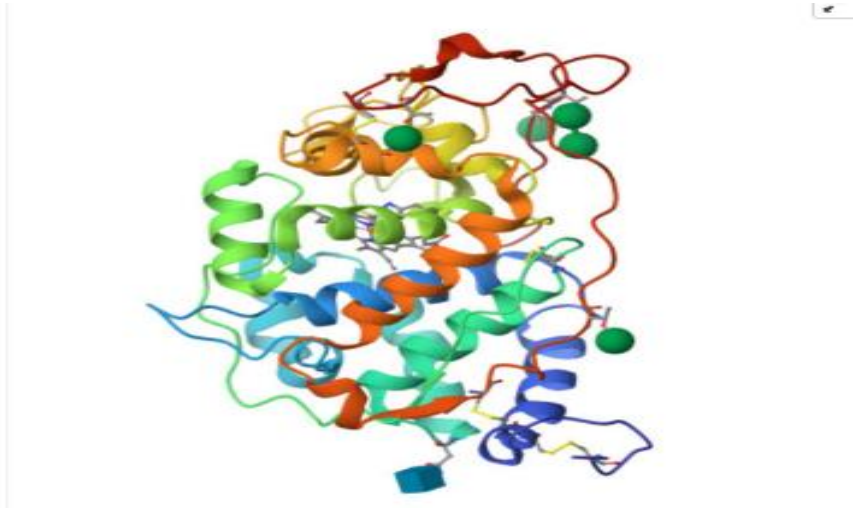
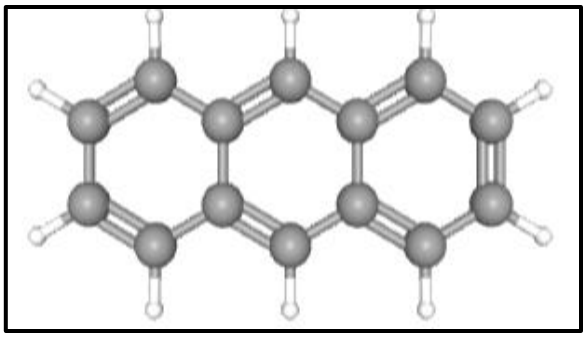
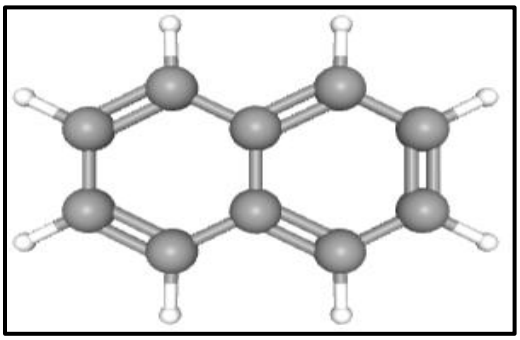


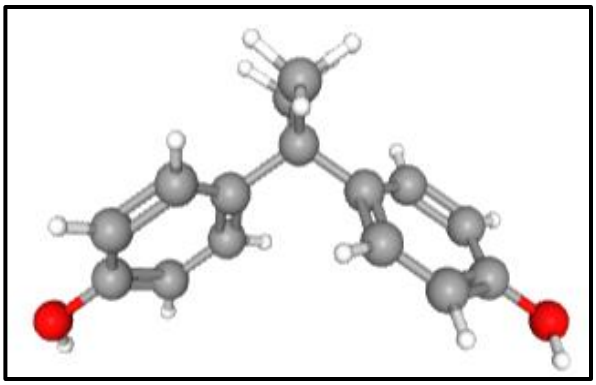
Figure 1: 3D structure of Versatile peroxidase obtained from Protein Data Bank PDB ID: 3FJW.



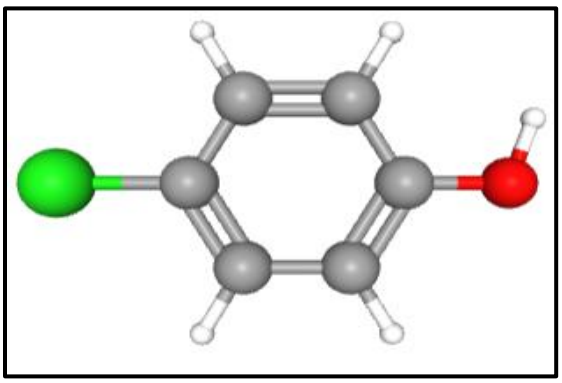
1.Anthracene



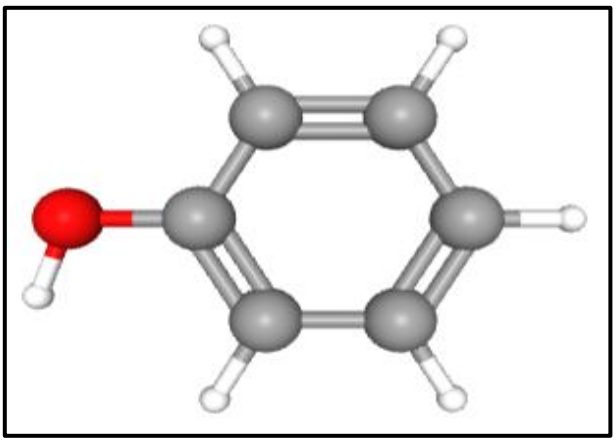
2. Naphthalene



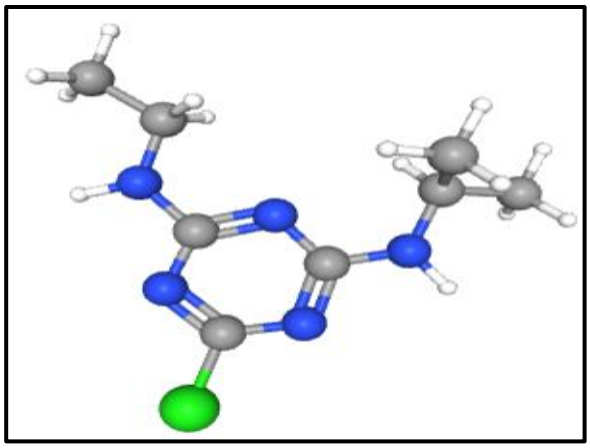
3. Bisphenol A



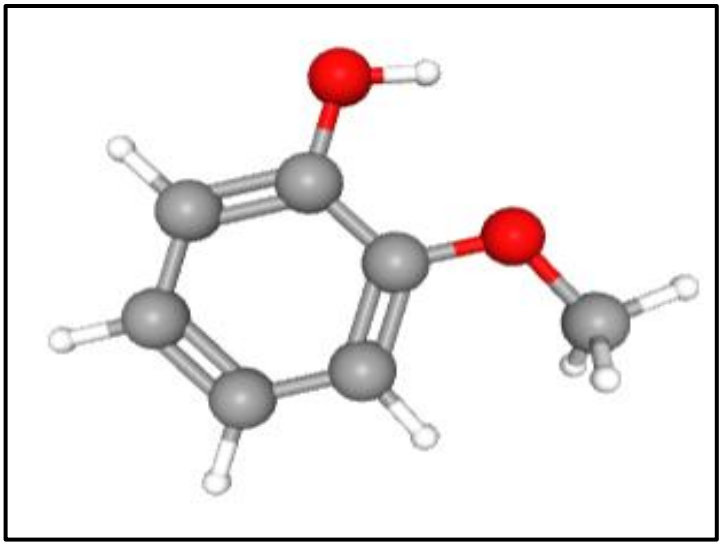
4. 4-Chlorophenol



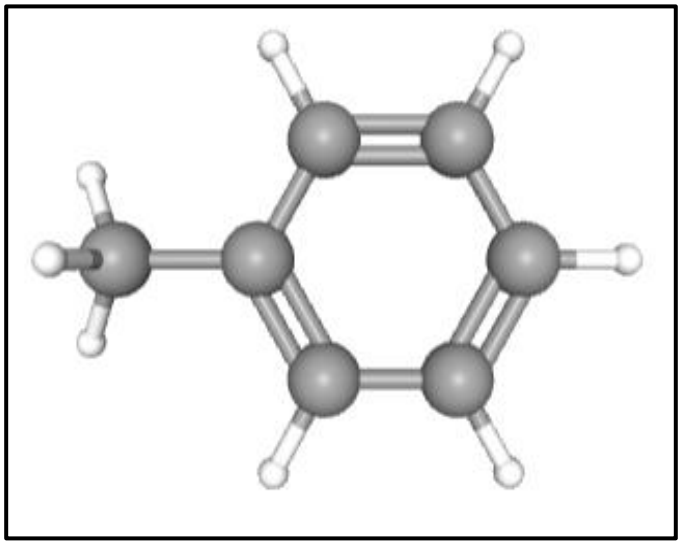
5. Phenol



6. Atrazine



7. Guaiacol



8. Toluene

Figure 2 : The 3D structures of organic pollutants present in the mixture used for docking analysis.

3.2.3. Preparation of Ligand : The structure of the ligand is prepared using PyRx, which is a virtual screening tool with open access to all. It is a very simple and quick ligand preparation tool that includes loading of the ligand (pollutant) 2D/3D files, which were previously extracted in the SDF format, and minimizing the energy of the ligand to ensure that the ligand is in a stable, low-energy state to be docked properly, which would otherwise give inaccurate results. The ligand file is then converted to PDBQT, required for docking with Vina and to enable flexible and accurate docking simulation.

3.2.4. Preparation of Protein : The structure of protein was prepared using molecular modeling and simulation software known as Discovery Studio, which is a critical step before molecular docking for accurate docking simulations. The protein preparation is needed to eliminate unwanted molecules like heteroatoms and water, add polar hydrogen atoms, minimize protein energy to remove unfavorable torsion, and predict and define the binding site. In the process of creating the versatile peroxidase structure, heteroatoms and unwanted chains (protein chain B was eliminated because it and chain A had the same structure). Along with the protein chain A, only the catalytically active essential heteroatoms such as HEM350 and Mn353 are kept which will act as the binding site for the ligands. Upon integration of polar hydrogens in protein structure, the ligand's binding site is identified.

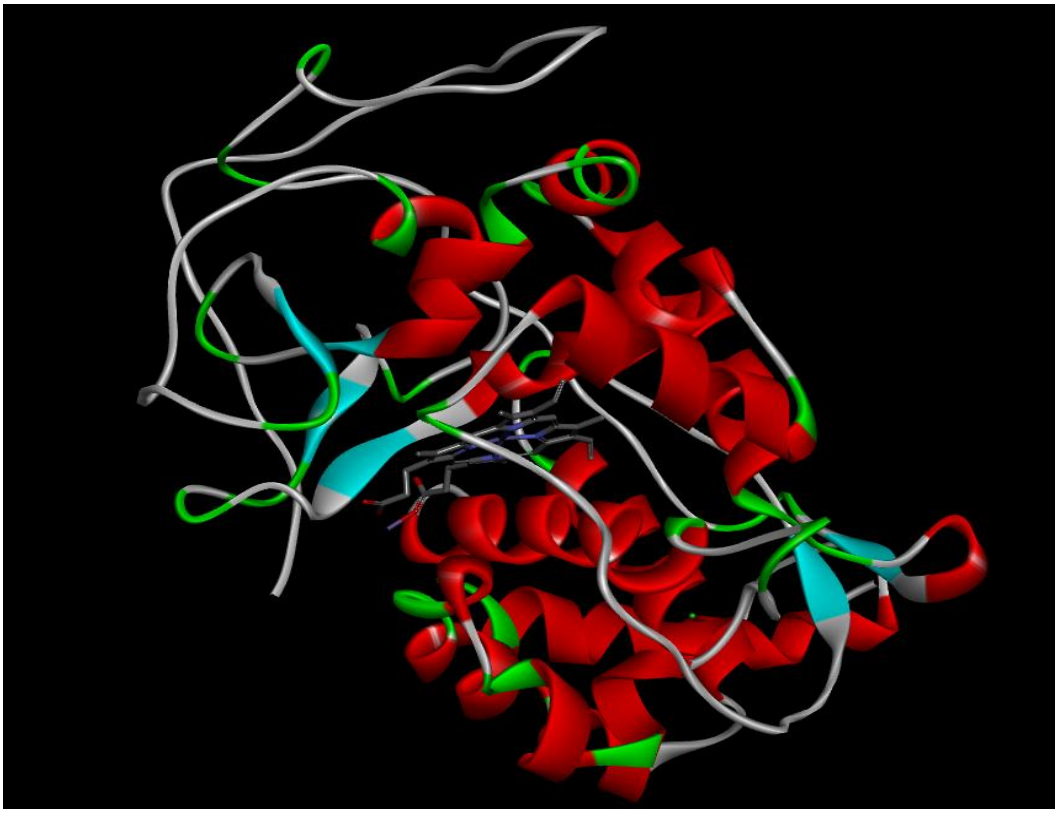


FIGURE 3: Prepared structure of Versatile peroxidase

3.2.5. Molecular Docking : Molecular docking, a type of in silico analysis, estimates the effectiveness of the ligands to bind with the receptor protein. The services of AutoDock Vina embedded with the PyRx virtual screen tool were used to perform molecular docking, predicting how well a ligand binds to a target protein. It has many advantages, e.g., being open source, supporting multiple platforms, etc. Docking simulations have been run using the tools to analyze docking scores, which indicate the ligand receptor strength. The ligand with a very high binding affinity will bind more strongly with the protein. The prepared protein (versatile peroxidase) structure along with the eight ligands (pollutants) is selected. Based on whether or not the active sites are previously known, we may opt for blind docking or specific docking by placing a grid box around the protein structure denoting the possible sites where the ligand would interact and bind to the protein. The docking experiment is run to analyze the docking scores, which denote the binding affinities of all the ligands (pollutants) of different chemical natures in a mixture of pollutants with the target protein (Versatile peroxidase). The docking scores for all the ligands were obtained and analyzed, each indicating a specific binding affinity of that ligand with the protein. The molecules with the highest binding energy are determined followed by moderate binding energy and the least binding energy.

3.2.6. Protein Ligand Complex Analysis : Understanding the molecular relationships that determine binding preference, specificity, and potential biological functioning requires an understanding of complexes of protein and ligand. The present investigation used BIOVIA Discovery Studio Visualizer, an efficient software tool for examining and interpreting molecular interactions, to further analyze the docked complexes that originated from molecular docking between versatile peroxidase and a mixture of naturally occurring contaminantsThe visualization tools, such as Discovery Studio Visualizer, are used to view the tertiary structures of the docked complexes (the receptor molecule and the ligands). It also enables us to visualize the protein-ligand interactions, such as non-polar interactions, intermolecular forces, interactions with residues, dipole-dipole interactions, and pi-pi stacking, by generating a 2D diagram. This study validates if the ligand is efficiently accommodated within the active site and whether it determines favorable interactions that promote strong binding affinities, which

helps to validate the docking data. Additionally, BIOVIA's 2D interaction diagrams provide a concise depiction of complex chemical reactions, which is especially valuable when demonstrating and comparing diverse pollutant-ligand mixtures.

For determining which pollutants bind to versatile peroxidase the best, indicating a greater probability of enzymatic disintegration, interaction profiling is crucial in the context of environmental bioremediation. The BIOVIA Discovery Studio Visualizer is therefore essential to correlating docking data with structural understanding and supporting the logical assessment of enzyme-substrate interaction for the detoxification of toxins.

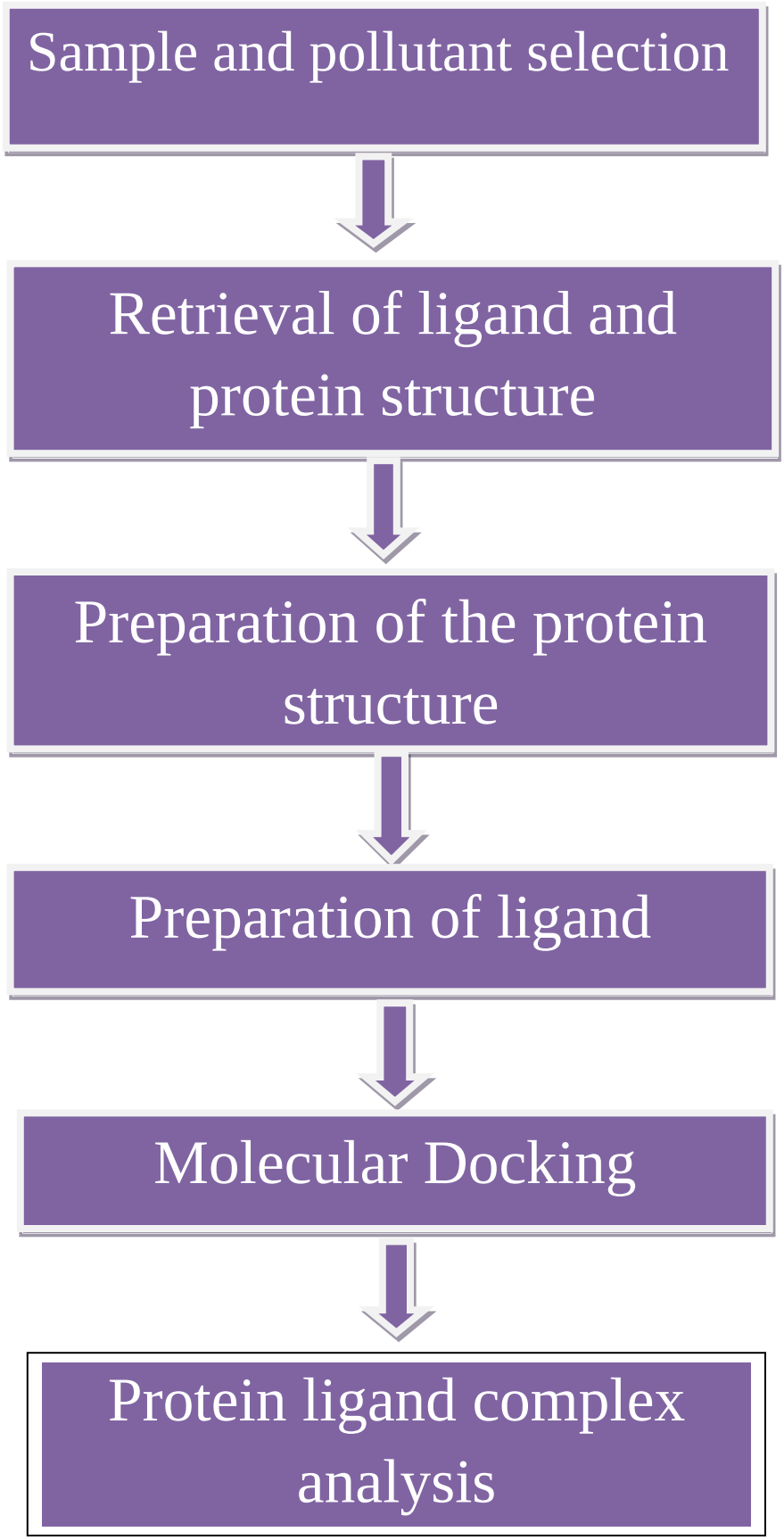


Figure 4: The flowchart of the protocols followed

CHAPTER 4

RESULTS AND DISCUSSIONS

Comparative data of the binding energies of various pollutants (ligands) was obtained. From the above docking analysis, it is concluded that the compounds like anthracene, naphthalene, and bisphenol are reported to have high negative binding energies with the values of -6.8 kcal/mole, -6.5 kcal/mole, and -6.1 kcal/mole, respectively. Among these, anthracene was found to have the highest value of negative binding energy. The high value of negative binding energy denotes a very strong affinity of the ligand to bind with the receptor protein. The compounds, like 4-chlorophenol, guaiacol, and toluene, were reported to have moderate negative binding energy values of -5.8 kcal/mole, -5.4 kcal/mole, and -4.9 kcal/mole, followed by phenol and atrazine, which were reported to have the least negative binding energy values of -4.6 kcal/mole and -4.2 kcal/mole, respectively.

Thus, anthracene, followed by naphthalene and bisphenol A, possesses the strong binding affinity with the versatile peroxidase, with anthracene being the strongest among all. This suggests that these pollutants have a more efficient and stable interaction with versatile peroxidase, leading to a more effective enzymatic bioremediation. On the contrary, phenol and atrazine showed reduced binding affinity, suggesting a decreased possibility of efficient degradation by versatile peroxidase. The various interactions, including the hydrophobic interactions(pi-alkyl), pi-pi stacking, intermolecular forces, and residual interactions that contribute to stabilizing the protein-ligand complex, were also studied in detail. VPs are best to act on substrates that are aromatic in nature, are polar, bulky, and planar, can fit well into its pocket, and are hydrophobic compounds like PAHs and phenol. It contains a catalytic site around the heme group, which is known to interact with the aromatic pollutants, making oxidation and degradation of a broad range of pollutants possible.

Table1: Binding energy (in kcal/mole) of selected organic pollutants

Pollutants	PubChem ID	Binding Energy (ΔG) (kcal/mole)	Bond strength
Guaiacol	460	-5.4	Moderate binding
Bisphenol A	6623	-6.1	High binding
4-Chlorophenol	4684	-5.8	Moderate binding
Anthracene	8418	-6.8	Highest binding
Toluene	1140	-4.9	Slightly lower binding
Phenol	996	-4.6	Very low binding
Naphthalene	931	-6.5	High binding
Atrazine	2256	-4.2	Lowest binding

The binding of VP with the ligand does not solely depend upon aromaticity but also involves optimal shape, size, and binding requirements. As versatile peroxidase possesses the combined catalytic activity of manganese peroxidase and lignin peroxidase acts on those compounds that mimic the lignin structure and thus degrades them. The interaction of versatile peroxidase with each pollutant present in a given mixture and the order in which they degrade it is justified by studying docking of a mixture rather than individual docking.

Anthracene is preferred to interact the most with VP over other aromatic compounds like naphthalene, bisphenol A, and phenol due to its high negative docking score(which indicates a stronger binding affinity with the enzyme). Also, anthracene consists of three fused benzene rings and possesses a planar structure due to which its large hydrophobic surface area can fit well into the enzyme pocket and interact strongly with the enzyme's catalytic site(around heme group), leading to the formation of a more stable complex and stronger van der Waals stabilizations. Its binding affinity is very strong, but its degradation is slower due to its stability and hydrophobic nature. Naphthalene is the second most preferred after anthracene, as it is also a planar structure consisting of two fused benzene rings and has a lower docking score than anthracene.

Naphthalene being smaller may not fit well into the enzyme's catalytic site, thereby reducing its binding strength. If we compare naphthalene and bisphenol A, naphthalene has a preference over bisphenol A as it is nonpolar and has a planar structure, making it suitable to bind into an enzyme's hydrophobic pockets. On the other hand, bisphenol contains a hydroxyl (-OH) group and thus acquires polar group properties, making it less susceptible to the enzyme's hydrophobic active sites. Also, it has a lower negative binding energy than naphthalene.

Following naphthalene and bisphenol, 4-chlorophenol and guaiacol are preferred as they both have lower negative binding energies than the former compounds. Although guaiacol is naturally the most preferred substrate for versatile peroxidase, they compete for binding in a given mixture of pollutants, as the enzyme prefers to bind with the molecules having higher binding energy for VP for degradation. Due to the presence of non-natural pollutants like anthracene, it behaves as a secondary substrate for the enzyme, making it less accessible to be easily attacked by the enzyme. Hence, in this case, competitive binding for guaiacol was introduced by anthracene, making it less available to be directly acted upon by the enzyme.

Toluene is not much or least preferred by versatile peroxidases. This may be due to the presence of a single benzene ring with a single methyl group attached to it, which might not be enough to activate the ring for oxidation. Also, it has a small aromatic surface, which might not be enough to form pi-pi stacking interactions with the enzyme's aromatic residues, and also, due to its small size, it may not fit properly into the active site of the enzyme, thereby decreasing its binding strength.

Being a traditional peroxidase substrate, Phenol also often exhibits significant reactivity, but here in the results it was analyzed to have very little binding energy. This might be due to a decreased substrate specificity and modified catalytic rate.

Atrazine is analyzed to be the weakest among all the eight ligands, having a docking score of least binding affinity. It's a triazine herbicide and has a bulky, polar, non-aromatic structure that differs from the aromatic and hydrophobic lignin-like substrates that VP usually attacks and prefers the degradation of the compounds. Microbial hydrolases are predominantly accountable for their breakdown.

These findings show that a VP has a unique hierarchy of binding preferences in a competitive multiligand environment. Bulky, planar aromatic compounds like anthracene, naphthalene, and bisphenol A showed a greater extent of interaction due to their pi-pi stacking and hydrophobic nature and their ability to bind within or close to heme active site. 4-chlorophenol and guaiacol bind in a moderate manner,

whereas toluene, phenol and atrazine bind poorly. It might be due to enzyme saturation, competition for active sites and alteration of catalytic oxidation. This illustrates the real-life competition among various pollutants to be degraded by the VP and demonstrates which of the pollutants will degrade faster in the environment and which one will persist for a long time and may also require undergoing a degradation process with more efficient enzyme.

Although the potential substrates for versatile peroxidase and guaiacol and phenol due to their small size and presence of hydroxyl group. Although if a pollutant is binding very well to the active site of the protein(enzyme) that doesn't necessarily mean that it will be entirely degraded by that enzyme. Despite anthracene and naphthalene shows a very high affinity with the VP, they cannot be decomposed as they have a very high redox potential, forms a stable complex with the enzyme which is very difficult to degrade and hence they persist in the environment for a longer time and cause serious hazards also they can indirectly provide hindrance for the native substrates for VP like guaiacol and phenol and obstruct their decomposition process.

Hence a competitive binding environment can alter the entire degradation mechanisms provided the pollutants are present in a mixture and not individually. This concept is very important to study the bioremediation of industrial effluent in real life scenario along with the experimental analysis to confirm the actual enzymatic breakdown of these pollutants in a given mixture as the docking process only measures the binding affinity and not the reactivity factor. Hence this should also be supported with the substrate specificity while analyzing a pollutant mixture in the real bioremediation world. Hence along with this provided data laboratory testing is also important to validate this hypothesis.

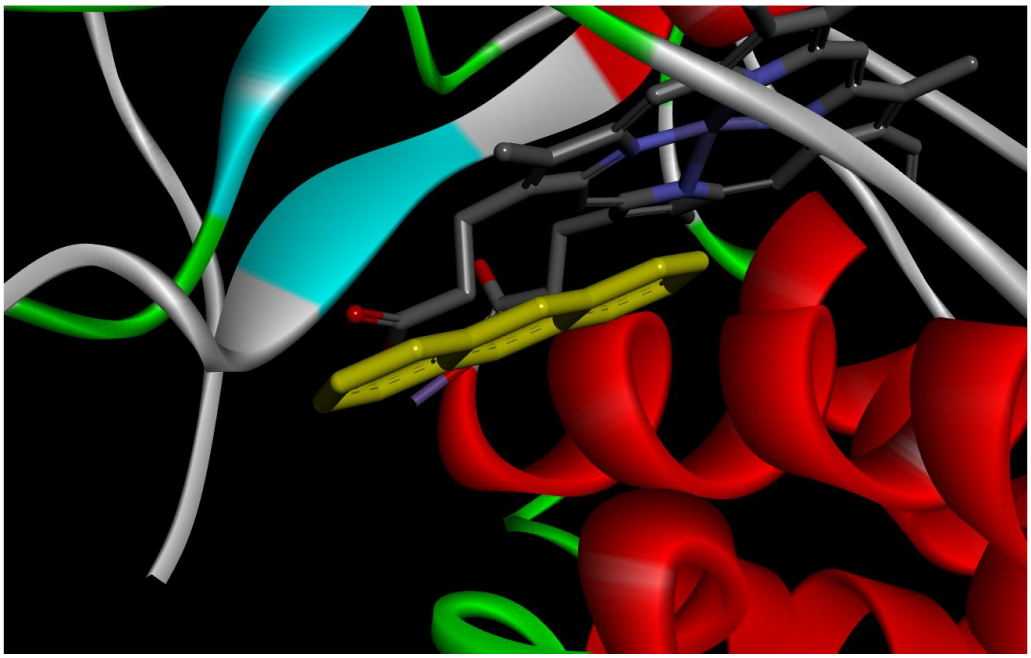
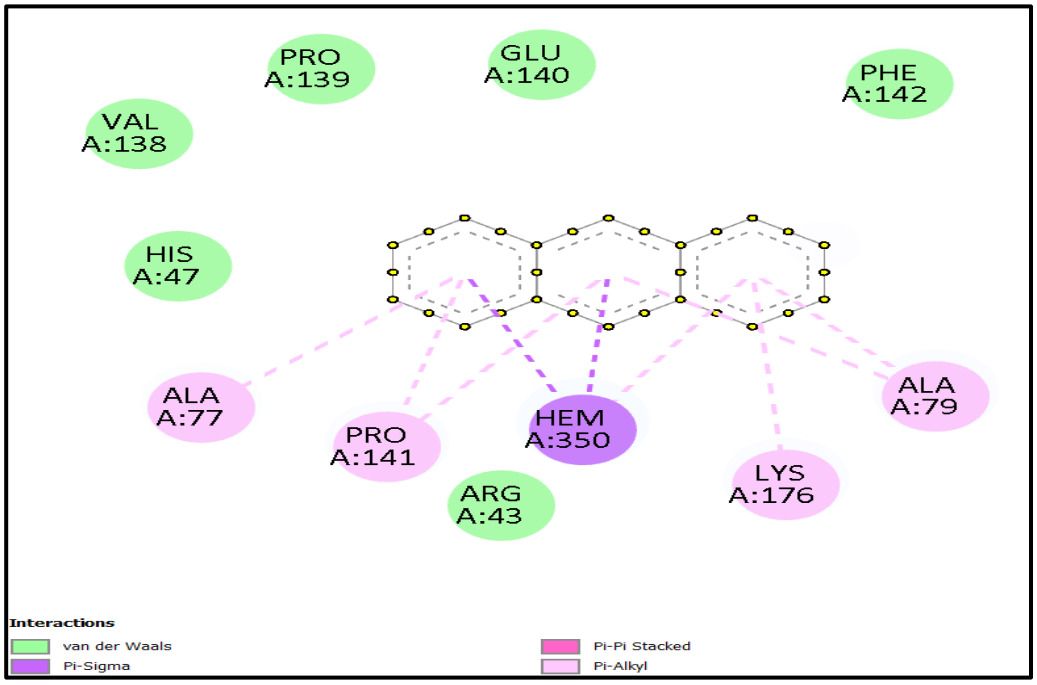


Figure 5: Docking of anthracene with Versatile peroxidase



Anthracene residual interactions

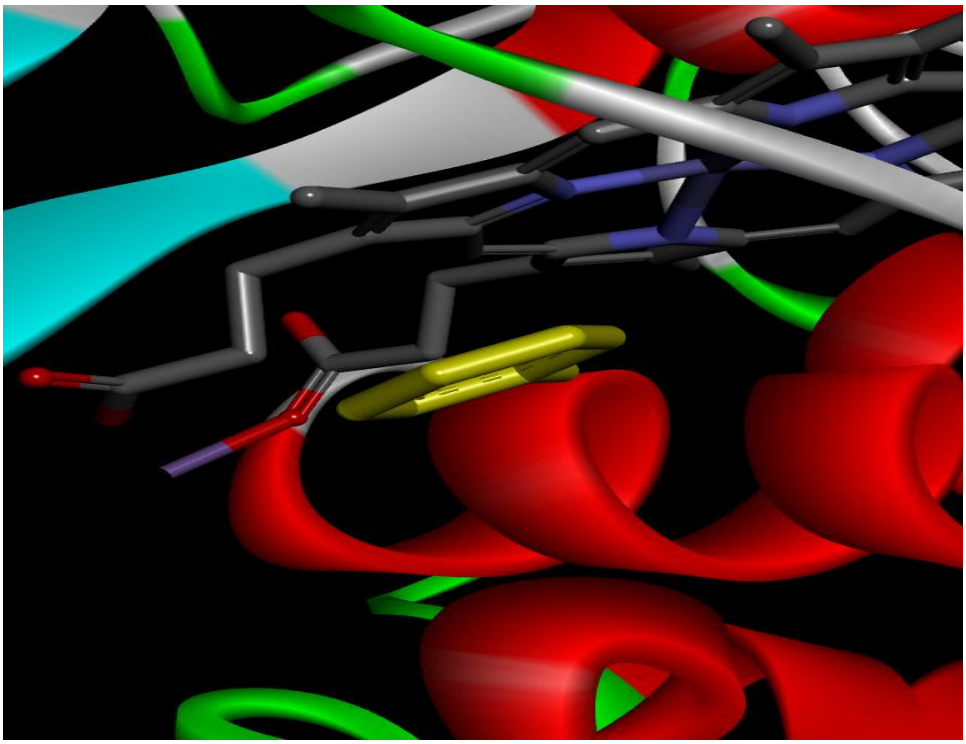
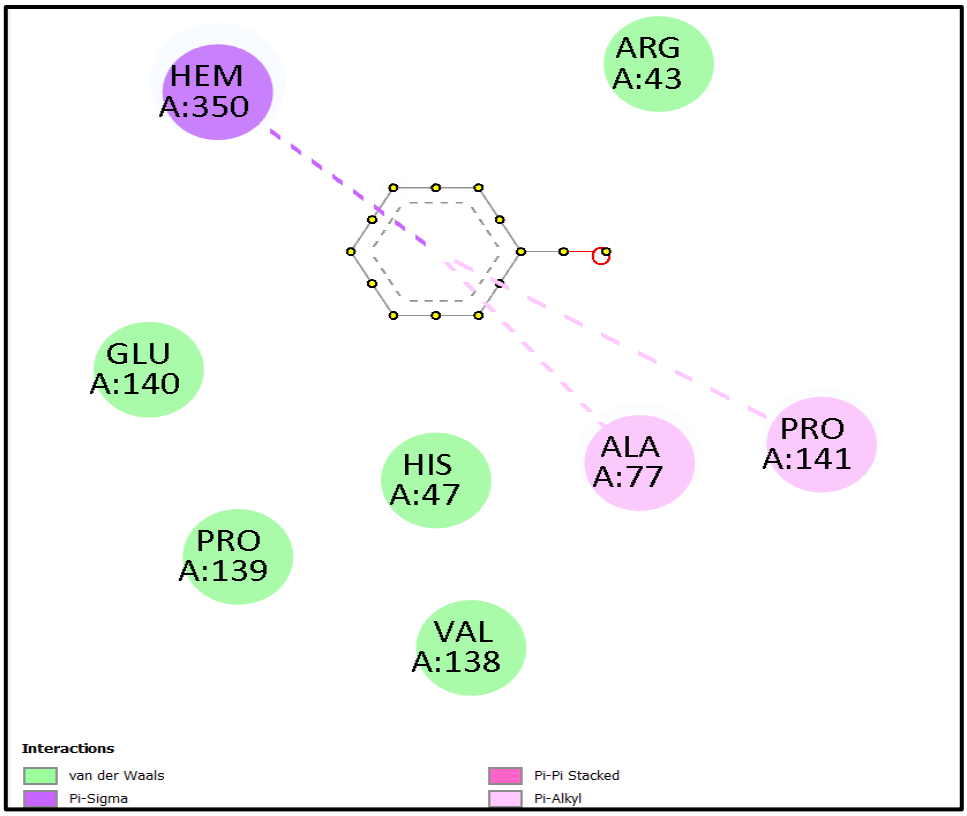


Figure 6: Docking of Phenol with Versatile peroxidase



Phenol residual interactions

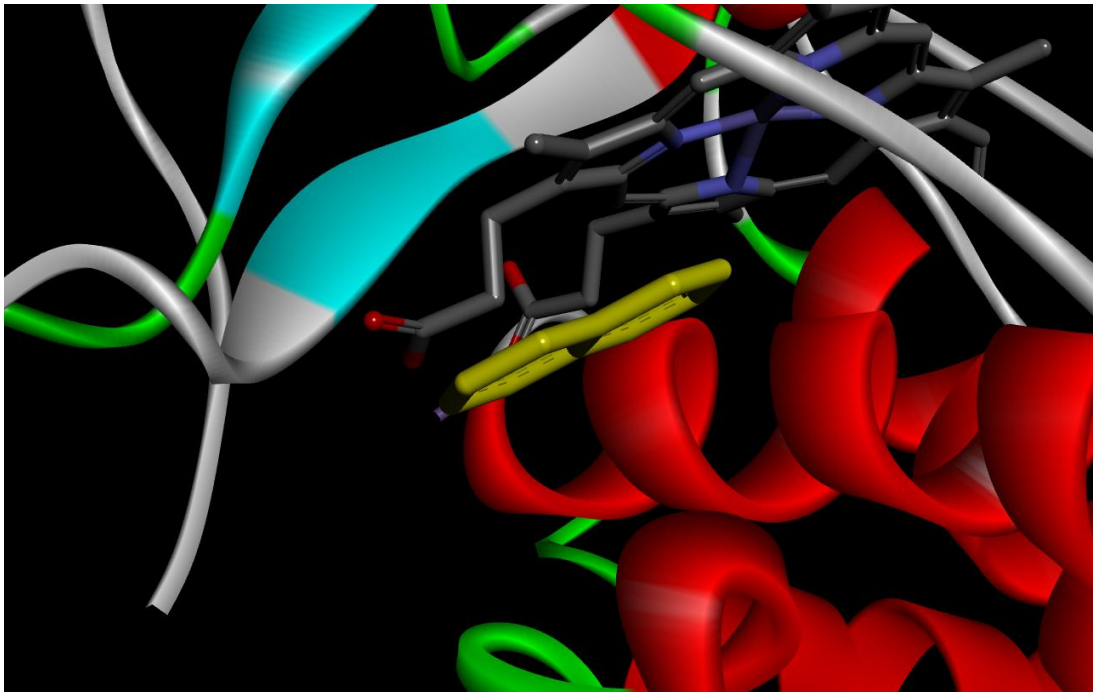
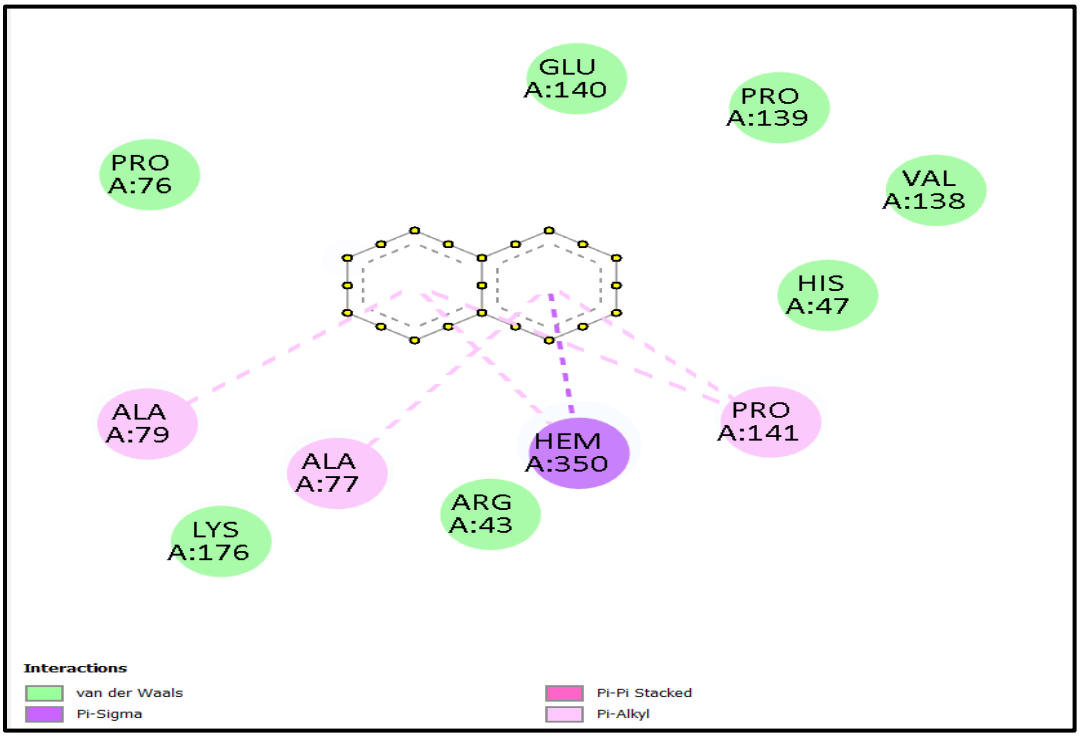


Figure 7: Docking of naphthalene with Versatile peroxidase



Naphthalene residual interactions

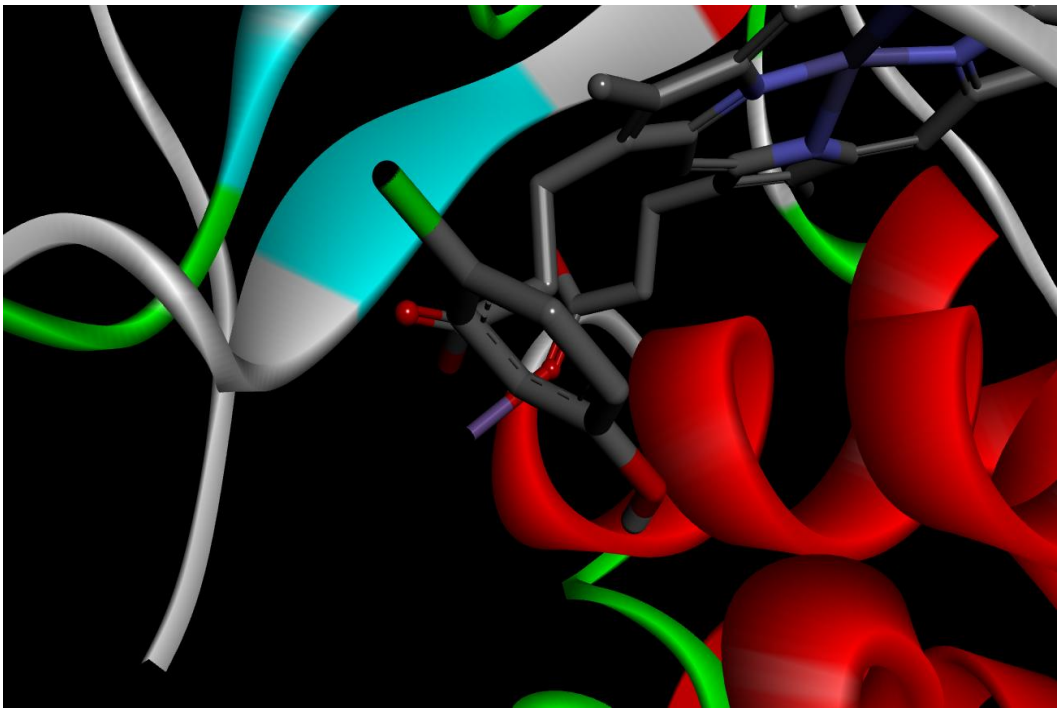
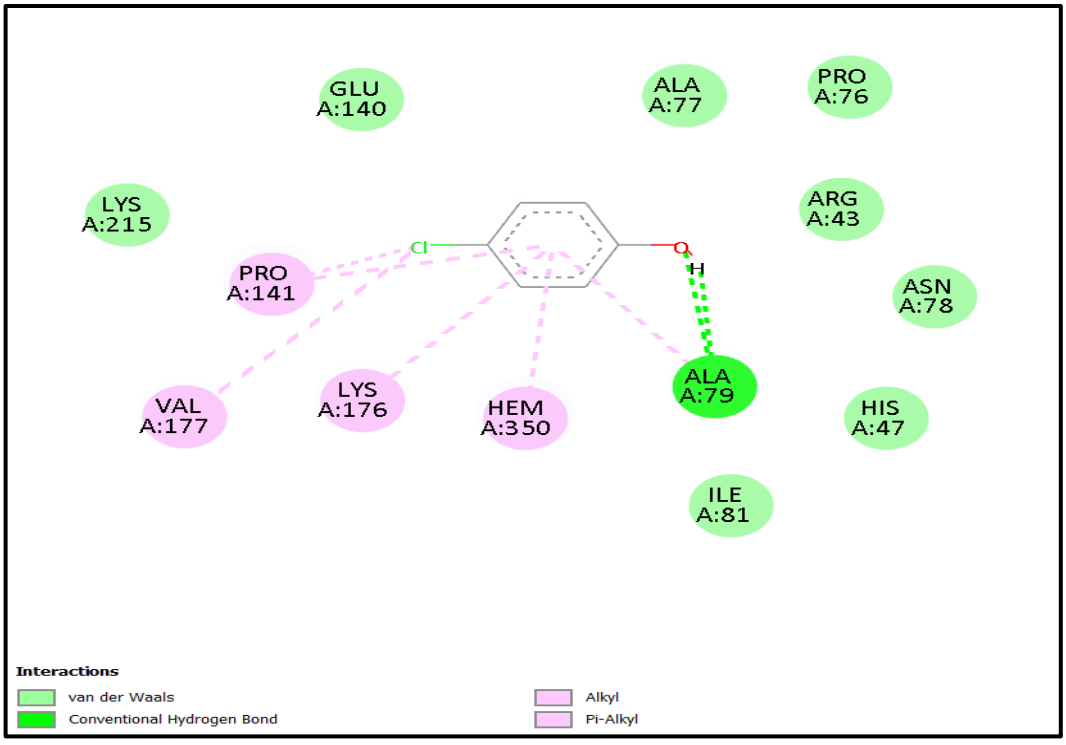


Figure 8: Docking of 4-Chlorophenol with Versatile peroxidase



4-Chlorophenol residual interactions

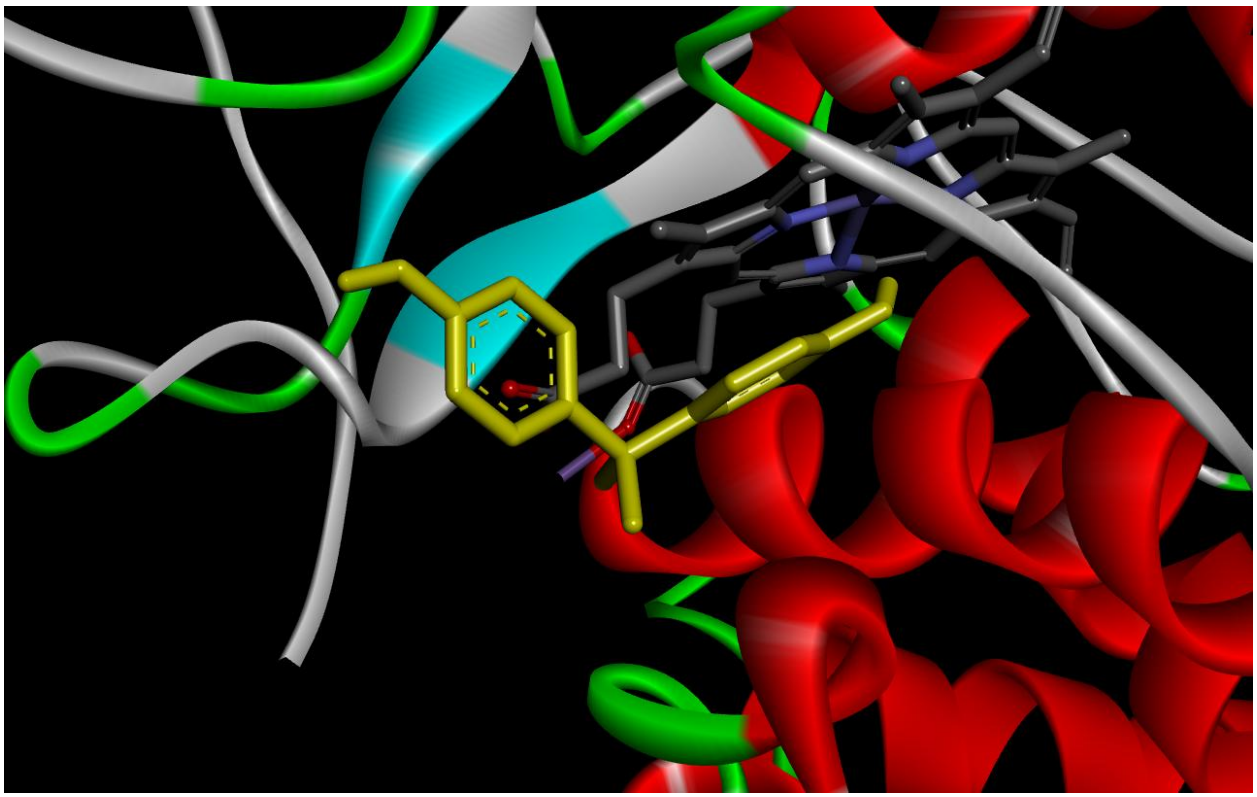
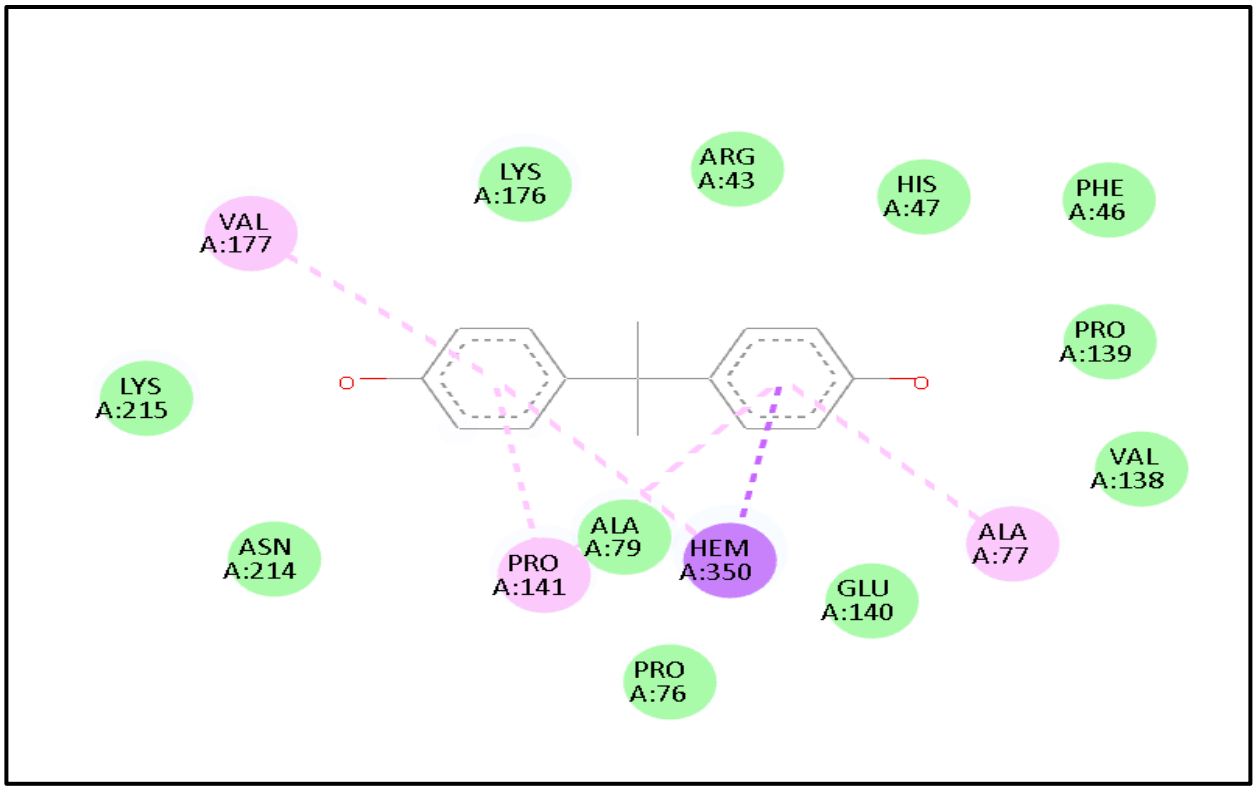
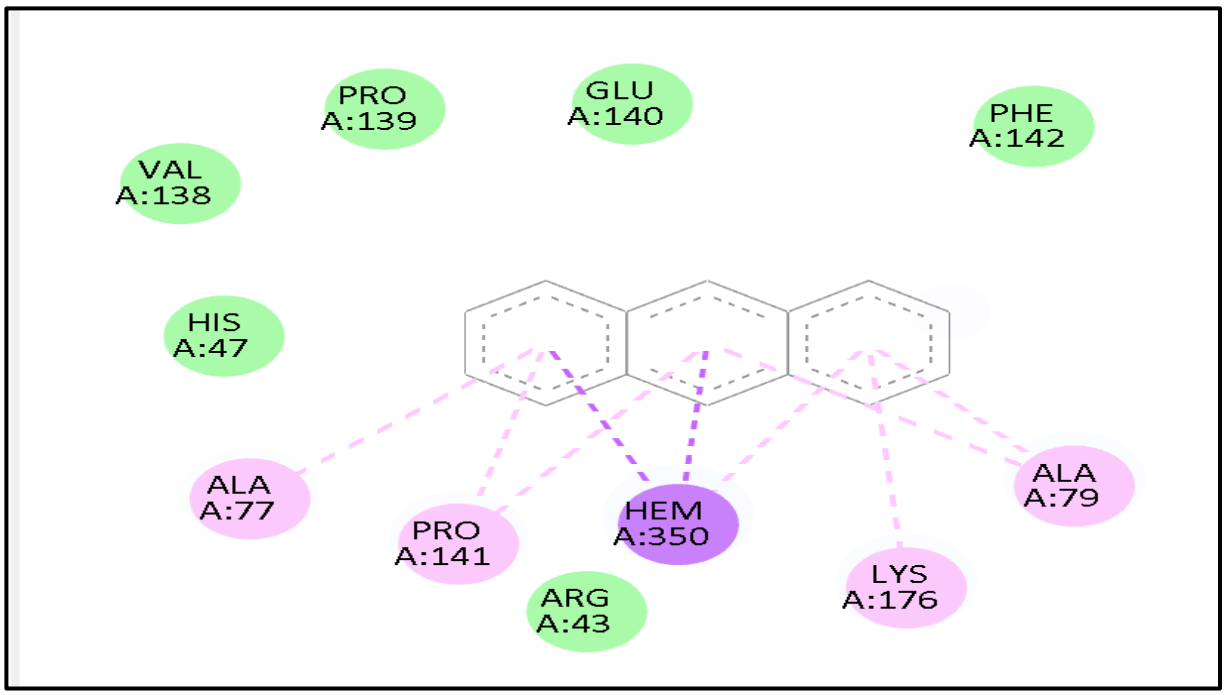


Figure 9 : Docking of bisphenol-A with Versatile peroxidase



Bisphenol-A residual interactions

Figure 10: Docking of atrazine with Versatile peroxidase



Atrazine residual interactions

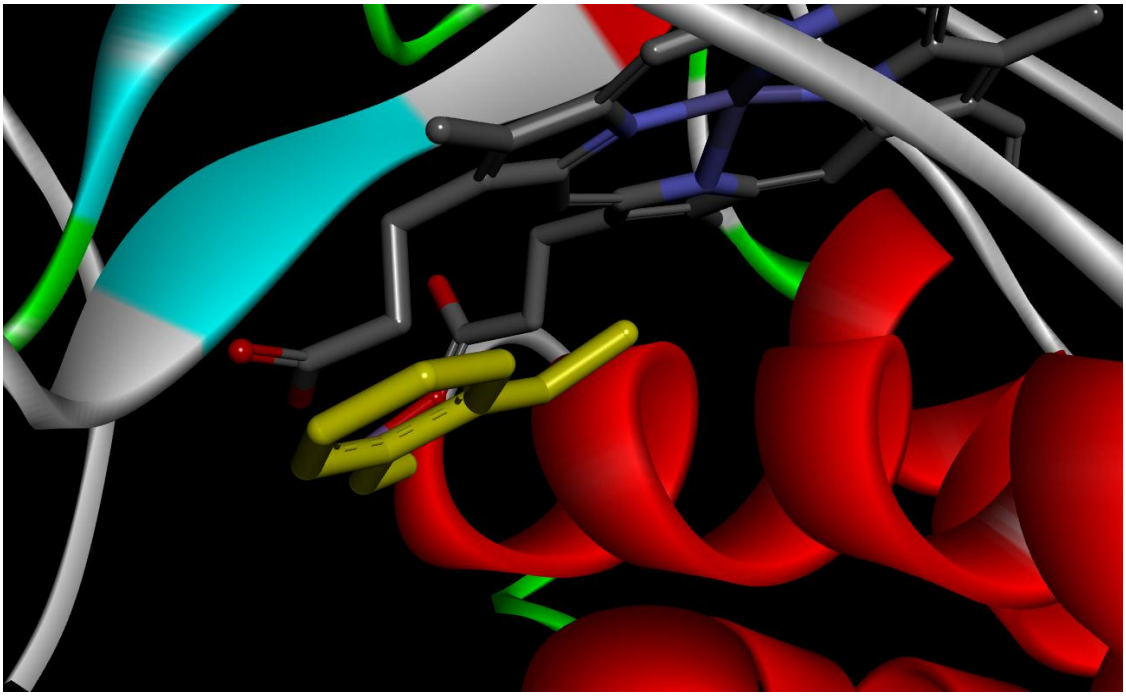
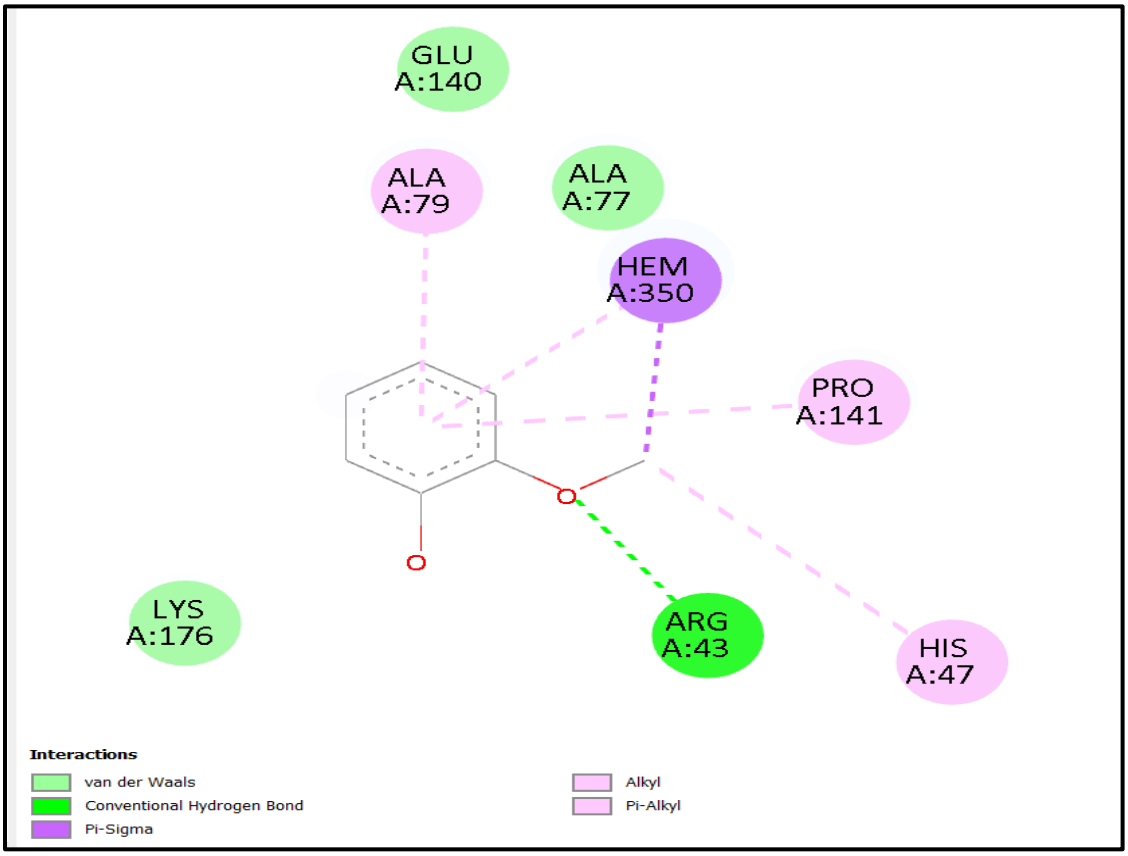


Figure 11: Docking of guaiacol with Versatile peroxidase



Guaiacol residual interactions

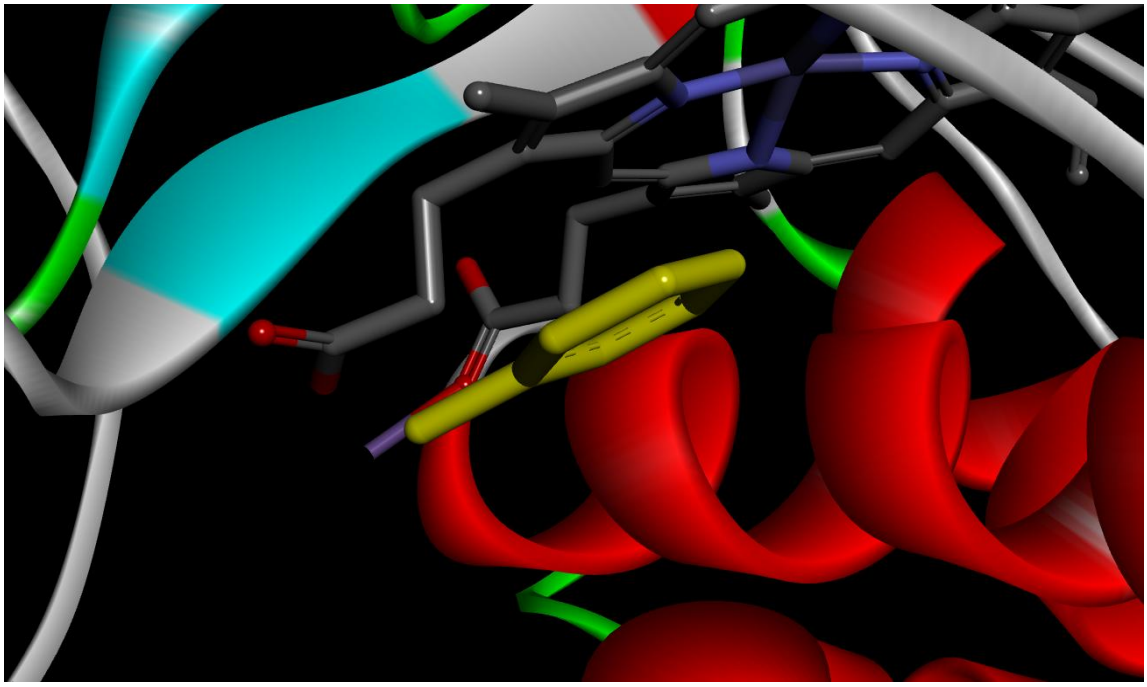
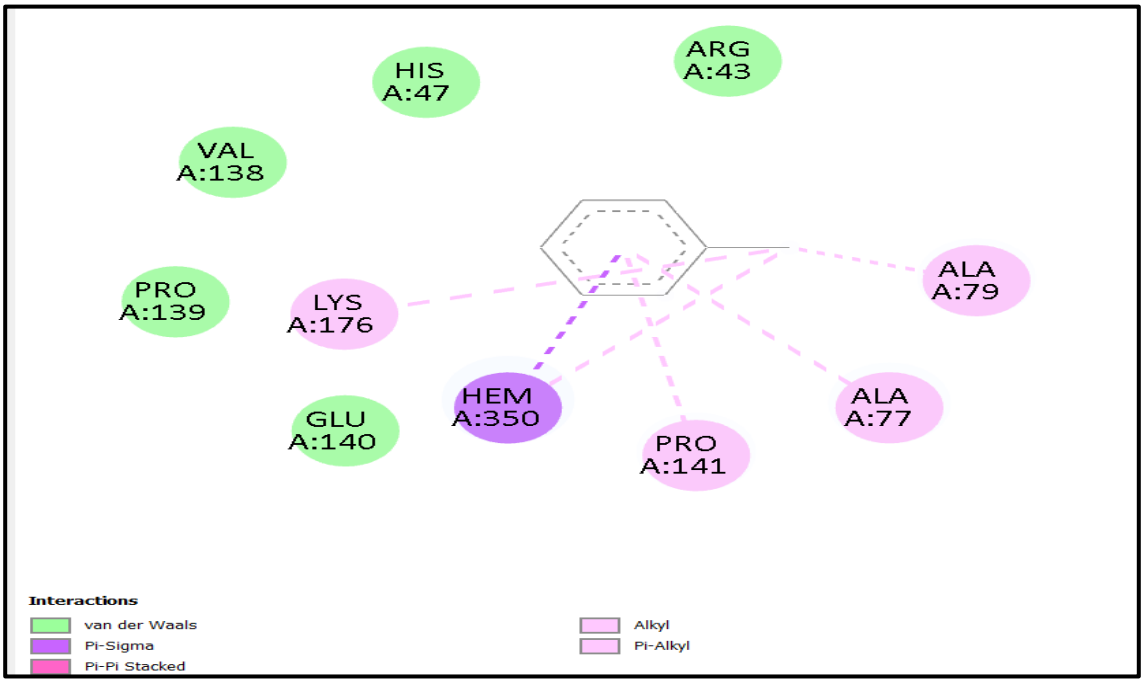


Figure 12: Docking of toluene with Versatile peroxidase



Toluene residual interactions

Table 2: Pollutants exhibiting maximum binding energies when interacting with versatile peroxidase, via the residues mentioned.

Compounds	PubChem ID	Binding Energy (kcal/mole)	Interacting Residues
Anthracene	8418	-6.8	ALA77, ALA79, PRO141
Naphthalene	931	-6.5	ALA79, ALA77, PRO141
Bisphenol A	6623	-6.1	VAL177, ALA177, PRO141
Guaiacol	460	-5.4	ALA79, PRO141, ARG43, HIS47
Toluene	1140	-4.9	ALA79, ALA77, PRO141, LYS176
Phenol	996	-4.6	ALA77, PRO141
4- Chlorophenol	4684	-5.8	ALA79, LYS176, VAL177, PRO141
Atrazine	2256	-4.2	ALA77, PRO141, ARG43, LYS176, ALA79

CHAPTER 4

CONCLUSION AND FUTURE PERSPECTIVE

Versatile peroxidase (VP) is a ligninolytic enzyme produced by lignin-degrading fungus, capable of oxidizing a broad spectrum of environmental pollutants [24]. Its unique catalytic versatility allows it to act on aliphatic and aromatic compounds, including persistent organic pollutants like bisphenol A, atrazine, and polyaromatic hydrocarbons [25].

The research finding in the paper, demonstrates novel insight into the transition observed in the binding behavior of the pollutants when comparing individual docking computations with the ones that include competing multiligand conditions. It is very obvious from the results that the binding sequence and selectivity of the pollutants with versatile peroxidase are not consistent but vary in a competitive binding environment. Among all the pollutants, anthracene was found to possess the highest binding energy, predominantly occupying the heme-containing active site. The naphthalene and bisphenol A were analyzed to have the second and the third highest binding energies. In contrast to previous research, guaiacol, which is considered to be an excellent natural substrate for versatile peroxidase, showed decreased binding when other high-binding-affinity ligands were present in the sample mixture. Toluene, phenol, and atrazine possessed the lesser binding energies, with atrazine ranking the lowest and least compatible. The mixture's revised binding hierarchy suggests that competitive binding and steric hindrance have a major impact on the accessibility in the enzyme's catalytic site, especially in the case of ligands that exhibit a stronger binding in individual docking.

However, catalytic efficiency does not always correlate to high docking. Along with docking scores it is also necessary to analyze the kinetic assays to evaluate the capacity of versatile peroxidase to degrade these chemicals in an actual bioremediation condition. This shift in the binding behaviour demonstrates the complexity of the enzyme pollutant interactions in real-life analysis where the various multiple contaminants are present in the environment, where the existence of one pollutant can influence the binding of the other pollutant.

The future prospects include proper assessment and validation of the results of the in-silico analysis regarding the actual binding behavior and degradation process in vitro under the real-life environmental scenario. Another factor that can be focused on is modifying the surface residues that might help reduce steric hindrance and enhance multi-ligand degradation. Understanding how competitive binding leads to the production of more toxic intermediates is also an area in which further studies can be conducted. Moreover, nanoparticles could be used to immobilize VP, leading to a gradual release of pollutants with time, allowing optimal destruction of pollutants with time, thus bridging the gap between theoretical docking simulations and practical implementations.

The following areas of investigation have been recommended for further research:

1. Experimental Validation: To verify the in silico docking results, a laboratory-based enzyme assays and biodegradation investigations are vital. This would verify the multipurpose peroxidase's true degrading efficiency against the chosen pollutants in a controlled setting.

2. Enzyme Engineering: The selective ability and catalytic performance of versatile peroxidase for target pollutants, especially those indicating lower binding affinity in this work, could be improved by adopting protein engineering methods like site-directed mutagenesis or directed evolution.

3. Microbial Expression Systems: To create scalable and affordable systems for environmental uses such soil bioremediation and wastewater treatment, future research may investigate the heterologous expression of customizable peroxidase in microbial hosts.

4. Mechanisms of the enzyme-substrate interaction and the possibility of practical applications might be better understood through the integration of kinetic modeling and thermodynamic analysis.

5. Future research should assess the complementary or adversarial effects of pollutant combinations on enzymatic degradation in order to more accurately replicate natural settings, as contamination of the environment rarely involves single chemicals.

6. Integrating the enzyme or organisms producing it in contaminated environments and carrying out field tests to evaluate its efficacy, structural stability, and security for the environment are long-term prospects.

Versatile peroxidase is a potential biocatalyst for the environmentally sustainable destruction of significantly hazardous environmental contaminants because of its exceptional catalytic flexibility and bonding capability to a wide variety of substrates. The integration of versatile peroxidase in pollutant degradation strategies offers a viable and environmentally friendly solution to address the growing concerns of chemical contamination.

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