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An *In-Silico* Approach to Unlock the Potential of Microbial Enzymes in the Degradation of Microplastics

Veena Vinod [1], Aiswarya[2], Amritha P. S[3], Dr. P. B. Harathi[4*]

[1]Department of Zoology, PSGR Krishnammal College for Women Coimbatore, 641004, Tamil Nadu, India
Email ID: veenavin123@gmail.com ORCID ID: 0000-0002-4168-1418
[2]Department of Zoology, PSGR Krishnammal College for Women Coimbatore, 641004, Tamil Nadu, India
Email ID: aiswarya.thomas10@gmail.com

[3]Department of Zoology, PSGR Krishnammal College for Women Coimbatore, 641004, Tamil Nadu, India
Email ID: amritha3097@gmail.com ORCID ID: 0000-0001-8632-2542
[4]Department of Zoology, PSGR Krishnammal College for Women Coimbatore, 641004, Tamil Nadu, India
Email ID: harathi@psgrkcw.ac.in ORCID ID: 0000-0002-9167-2886
Corresponding Author: [4*]Dr. P. B. Harathi

Department of Zoology, PSGR Krishnammal College for Women, Coimbatore, 641004Email ID: harathi@psgrkcw.ac.in
ORCID ID: 0000-0002-9167-2886

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Abstract

Molecular docking is an emerging field that can aid in tackling rising environmental issues such as microplastic (MP) pollution. MP poses a threat to both the environment and human health, which makes its degradation crucial. Biodegradation is the most efficient eco- friendly method that uses the ability of microbes to secrete enzymes that can break down polymers. However, the *in vitro* culturing of microorganisms and screening of efficient enzymes in biodegradation is laborious and time-consuming.

The application of molecular docking to identify the enzymes that bind to target polymers is a promising approach. Therefore, this study aims to identify the enzymes that bind the target MP through molecular docking and simulations. In this study, 14 enzymes were docked against plastic compounds using Auto Dock Vina software version 4.2. Results show that enzymes such as Copper-dependent laccase (- 5.65 kcal/mol), Lignin peroxidase (- 5.21 kcal/mol), and Lipase (-3.11 kcal/mol) had the highest binding affinity to plastic compounds such as Polystyrene, Polyurethane, and Polyvinyl carbon respectively. Additionally, the amino acids involved in binding were discussed of the three highest binding affinity of each polymer along with the interactions such as Van der Waals, hydrogen bonding, and pi-pi interactions.

Keywords: *Microplastics*, *Microbial degradation*, *Enzymes*, *Docking*.

1. INTRODUCTION

Molecular docking (MD) is a computational technique that predicts the non-covalent binding of a macromolecule (receptor) and a small molecule (ligand), starting from those molecules' unbound structures, obtained from MD simulations, and homology modelling. By using the *in-silico* method virtual screening can be done and it finds hits and leads through library enrichment for screening and enriching the library of ligands for MD, it is very useful in analysing the molecular descriptors and physicochemical properties of activeligands. An *in-silico* approach can serve to be the first step in mitigating emerging pollutants such as MP. Conducting experimental studies on plastic degradation can be time-consuming, resource-intensive, and expensive. *In silico* is generally more cost-effective than performing laboratory experiments. Simulations can be run simultaneously, allowing researchers to explore various scenarios and obtain results quickly This approach enables researchers to explore and analyze different hypotheses and scenarios without any harm to living organisms. This helps in understanding the degradation mechanisms and designing more effective strategies for plastic waste management.

Plastic pollution is one of the tremendous problems faced today. Plastics are widely used because of their durability, flexibility, and feasibility. They are inexpensive, lightweight, strong, long-lasting, and corrosion-resistant (W.C. et al., 2016). The majority of plastics are decomposed into microscopic pieces known as “microplastics” due to weathering. PS, PE, and PP make up the majority of microplastics (MPs), which account for 92.4% of plastic waste. The hydrophobic surface of MP can adsorb organic and inorganic contaminants, thus prolonged contact with contaminant-laden MPs can harm plants, animals, and human health (Harmita Golwala et al., 2021). MPs are particularly harmful to the soil and have an impact on plant growth and development (Riling et al., 2019). MPs in the soil migrate and accumulate in plants which alters the biophysical characteristics of the soil and consequently leads to changes in biomass, tissue element composition, plant root traits, and soil microbial activity.

They can persist for a very long time in the environment, which causes various effects in animals such as chronic pain, swelling, and mortality, as well as immune cell impairment. (Smith et al., 2018). From soil it enters aquatic ecosystems, fish that have been subjected to MPs experience neurotoxicity and abnormal behavior. Due to their non-selective feeding habits, filters and deposit-eating fish are more susceptible to MPs ingestion than predator fish (Wesch et al., 2016; Lusher et al., 2020). The fish gill is a very sensitive organ that performs many different tasks, including nitrogenous excretion, osmoregulation, and acid-base regulation. Any disruption of these systems could be fatal. Multiple studies have discovered MPs in edible fish, and they further indicate that MPs can penetrate human systems due to theeffects of biomagnifications (Alfaro-Nez et al., 2021; Goswami et al., 2020; James et al., 2020). The studies on the effects of MP on benthic marines and freshwater invertebrates, such as annelids, arthropods, ascidians, sea urchins, bivalve mollusks, and rotifers, were carefully reviewed by Haegerbaeumer et al., (2019).

MPs can affect human health through seafood, a vital component of the human diet. There is a major risk that intestinal contamination of MPs will spread to other parts of the body. MP produces a lot of toxic effects on humans, swelling and obstructions are caused asa result of the accumulation of MPs and nanoparticles in organs. Internalization of MPs in thecardiovascular system instigated an inflammatory response, blood cell cytotoxicity, vascular swelling obstructions, and respiratory problems, high blood pressure (Wright and Kelly,2017; Campanale et al., 2020). According to many investigations, it was found that MPs can translocate across living cells, including human intestinal follicle-associated epithelium and human lymphoid tissue macrophages.

Therefore, the degradation of plastics is a significant issue that demands sustainable and innovative solutions. Previously many abiotic methods have been applied for plastic degradation. Abiotic degradation includes processes like photodegradation and thermo-oxidation, whereas biotic degradation includes the activity of microorganisms. There are various methods for plastic degradation such as Ozone-induced degradation, Mechanochemical degradation, and Catalytic degradation. Although several chemical reprocessing methods for MP have been developed, the viability of these technologies is still constrained by high process costs, which are primarily caused by high energy consumption and the use of expensive and hazardous chemicals. Biodegradation is often favoured over other degradations due to its environmentally beneficial and pollution-free mechanism. In biodegradation, microbes break down polymers with both organic and inorganic materials like lignin, starch, cellulose, and hemicelluloses (Munuru Srikanth et al., 2022).

Enzymes are proteins that catalyze biochemical reactions and are highly specific to their substrates. The interactions between plastic compounds and enzymes can be mediated by specific residues in the enzyme's active site. By analysing the residues involved in ligand-protein interaction by using the computational method, it is possible to predict the ligand binding and its strength, and the residues can form various interactions with ligands such as hydrogen bonds, van der Walls forces, and hydrophobic interactions. Understanding these interactions is important for the development of sustainable plastic materials that can be safely used in various applications without causing harm to the environment or human health. Enzymes increase the hydrophilicity of polymers through oxidation/hydrolysis, which leads to the degradation of high molecular weight polymers into low molecular weight polymers.

According to studies, a variety of natural enzymes can hydrolyze and depolymerize these MPs to produce the end products CO_2 and H_2O. However, finding microorganisms and enzymes that can successfully break down these aliphatic polyesters is difficult due to the arduous and time-consuming nature of the process (Xiaodan Wang et al., 2019). With the increasing difficulty of isolating and culturing a multitude of microorganisms, extracting their enzymes, and evaluating their degrading effectiveness, *in-silico* approaches have shown to be an increasingly useful tool. Recent studies have demonstrated that a wide range of microbial enzymes are capable of breaking down synthetic polymers. An *in-silico* analysis of the binding affinity of common plastic molecules to various enzymes has been previously reported (Enyoh et al., 2022). Here, we elucidate the enzyme-plastic binding affinity to identify enzymes with a higher binding affinity. This study investigates the role of microbial enzymes in the degradation of MP through *in-silico* approaches

2. MATERIALS AND METHODS

2.1 Preparation of the enzymes used in the study

Enzymes that possess catalytic activity which is produced by fungi and bacteria were selected for docking studies. The Protein Data Bank (PDB) was used to acquire the three-dimensional (3D) structures of the enzymes used in the MD simulation. Enzymes with multiple chains were reduced into chain A after being retrieved from the PDB to enhance the binding efficiency between protein and target (Sasikala and Meena, 2016). Using PyMOL and Molecular Graphics Laboratory (MGl) techniques, interfering crystallographic water molecules and proteins were reduced. (Pettersen *et al.,* 2004; Duru *et a*l.,2021). The enzymes that are used in the study are listed in Table 1.

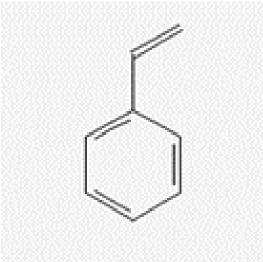
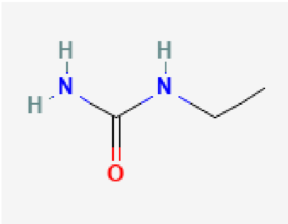
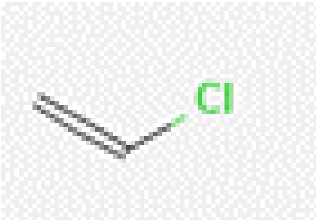
Table 1 List of enzymes produced by the MP-degrading microbes

SL. NO	ENZYMES	PDB ID	MICROBES
1	Laccase	5NQ7	Fungi (*Trametes sanguinea*)
2	Lipase	6L7N	Fungi (*Penicillium roqueforti* FM164)
3	PET hydrolase	7SH6	Bacteria (*Piscinibacter sakaiensis*)
4	Carboxylesterase	1AUO	Bacteria (*Pseudomonas fluorescens*)
5	Lignin peroxidase	1LLP	Fungi (*Phanerodontia chrysosporium*)
6	Manganese peroxidase	1MNP	Fungi (*Phanerodontia chrysosporium*)
7	Xylanase	7VC7	Fungi (*Phanerodontia chrysosporium*)
8	Copper dependent laccase	4JHV	Fungi (*Cerrena caperata*)
9	Lyases	1IDJ	Fungi (*Aspergillus niger)*
10	Cutinase	1XZA	Fungi (*Fusarium vanettenii*)
11	Alkane hydroxylases	2V3B	Bacteria (*Pseudomonas aeruginosa PAO1*)
12	PHB depolymerase	2VTV	Bacteria (*Paucimonas lemoignei*)
13	PMMO	3CHX	Bacteria (*Methylosinus trichosporium*)
14	Oxidase	1I19	Bacteria (Brevibacterium sterolicum)

2.2 Plastic compounds used in the study

Structures of plastic compounds were retrieved from the PubChem library. Plastic compounds such as PS, PVC, and PUR are taken for the study. The 3D Structure-Data Files (SDF) were downloaded and used for the docking study. Previous studies have reported that the enzyme action begins when the plastics have been reduced to an acceptable size (generally ranging from 10 to 50 carbons) (Restrepo-Florez *et al.,* 2014). The structures of plastic compounds used in the study have been depicted in Table 2.

Table 2.2 Structure of plastic compounds found in PubChem

Plastic compounds	Polystyrene (PS) 7501	Polyurethane (PUR) 12254	Polyvinyl chloride (PVC) 6338
Structure			

2.3 Molecular Docking Simulation

Auto dock software version 4.2 was used to perform the numerous ligands docking of the MP compounds on the enzyme targets. Blind docking of the MP compounds was carried out at the enzyme cavities to allow the ligands unrestricted access to engaging with sites where they expended the least amount of energy. The enzyme-ligand complexes of the substances were visualized using Biovia Discovery Studio

4. 3. RESULTS AND DISCUSSION

The capacity of microbes in biodegradation is attributed to their ability to secrete enzymes. It is important to validate their binding efficiency. These microorganisms release numerous enzymes and it is ardous to check the binding capacity of each enzyme *in*-vitro. MD simulation of multiple enzymes to multiple plastic fragments or monomers can aid in simplifying the tedious work. MD can be used to predict the interactions between the ligand and compound. Blind docking was performed as the active site pockets are unknown. The results also reveal parameters like hydrophobic interactions, hydrogen bonding and binding affinity. MD studies of fourteen enzymes (which include Laccase, Lipase, PET hydrolase, Carboxylesterase, Lignin peroxidase, Manganese peroxidase, Xylanase, copper-dependent laccase, Lyases, Cutinase, Alkane hydroxylases, PHB depolymerase, PMMO, Oxidase) was performed against the plastic compounds such as PS, PUR, PVC and the results of binding affinity has been presented in **Table.5.1**.

Table 3.1 Binding Affinities of Enzymes with Plastic Compounds

ENZYMES	PDB ID	BINDING AFFINITY		
		PS	PUR	PVC
Laccase	5NQ7	-5.09	-3.92	-2.87
Lipase	6L7N	-3.8	-4.67	-3.11
PET hydrolase	7SH6	-4.6	-3.59	-2.69
Carboxylesterase	1AUO	-4.18	-3.6	-3.01
Lignin peroxidase	1LLP	-4.53	-5.21	-2.52
Manganese peroxidase	1MNP	-4.51	-5	-2.78
Xylanase	7VC7	-5.55	-0.93	-2.68
Copper dependent laccase	4JHV	-5.65	-4.61	-2.8
Lyases	1IDJ	-4.13	-4.57	-2.52
Cutinase	1XZA	-3.69	-2.63	-2.42
Alkane hydroxylase	2V3B	-4.89	-3.92	-2.89
PHB depolymerase	2VTV	-4.93	-3.43	-2.78
PMMO	3CHX	-5.15	-3.4	-2.79
Oxidase	1I19	-5.38	-4.4	-2.78

The amino acid interaction between the plastic compounds and enzymes has been presented in Table 3. For each plastic compound, the highest binding affinity demonstrating enzymes have been listed along with the amino acid residues that participated in the interaction.

Table 3.2 Interactions between plastic compounds and enzymes

COMPOUNDS	PROTEIN-LIGAND INTERACTIONS		
PS (7501)	**Copper dependent laccase** VAL 303, PRO 420, ILE 421, VAL 414	**Xylanase** LEU 54, MET 326, TYR 330, TRP 59, LEU 329, TYR 57	**Oxidase** PHE 317, TRP 314, ILE 459, VAL 474, PHE 455

PUR (12254)	**Lignin peroxidase** ASN 182, ARG 43, ASP 183, GLY 86, GLN 189, GLU 89	**Manganese peroxidase** LYS 180, GLY 82, GLU 39, ASP 85, ARG 42, ASP 179	**Lipase** GLU 254, ASP 255, GLU 257, ASP 198 ALA 258
PVC (6338)	**Lipase** TRP 261, PRO 223, ASN 224, HIS 196, MET 264, ILE 220, ASN 225, VAL 228, PHE 263, TYR 262, VAL 226	**Carboxylesterase** SER 139, LEU 163, ALA 137, TRP 192, CYS 162, GLY 176, TYR 141, HIS 164, ALA 179, THR 140, PHE 121	**Laccase** PHE 90, GLN 119, PRO 121, PHE 89, GLN 123, PHE 118, ASP 122, GLN 91, VAL 120

3.1 Binding Affinity towards PS

PS (7501) is a commonly used synthetic polymer for packaging industries and also in daily use articles. Among the fourteen docked enzymes, Copper-dependent laccase, Xylanase, and Laccase show the highest binding affinity towards PS, and the binding interaction has been presented in Figure. 3.1.

Copper-dependent laccase shows the highest binding affinity (-5.65) against PS. The residues interacting with the enzyme are VAL 303, PRO 420, ILE 421, and VAL 414. There are three alkyl and π-alkyl bonds depicted in pink colour and one π-sigma interaction depicted in purple colour between laccase and PS. Xylanase exhibits the second most binding affinity (-5.55) against PS. Microbes such as *Agaricus bisporus*, and *Fusarium solani* (fungi) can secrete xylanase. The residues that interacted with the enzyme are LEU 54, MET 326, TYR 330, TRP 59, LEU 329, and TYR 57. There is one π-π standard bond depicted in dark pink and five alkyl and π-alkyl interactions depicted in light pink colour between xylanase and the ligand.

Oxidase showed the third-best binding affinity (-5.38) against PS. Microbes such as *Aspergillus niger van tieghem* F1119, *Heterobasidion parviporum* (fungi), and *Streptomyces setoni* (bacteria) secretes oxidase. The residues interacting with the enzyme are PHE 317, TRP 314, ILE 459, VAL 474, and PHE 455. There is one Pi donor hydrogen bond depicted in green colour and one π- π stacked interaction depicted in dark pink colour and three π-alkyl interactions depicted in light pink colour between oxidase and the ligand.

Similar findings were reported by Danso et al., (2019) that *Gloeophyllum striatum* DSM and *Gloeophyllum trabeum* DSM generated significant depolymerization of PS after 20 days of incubation. In the previous study, the white rot fungi *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Trametes versicolor*, and the brown rot fungus *Gloeophyllum trabeum* were linked to PS depolymerization when co-incubated with lignin. Similarly, various bacteria have been shown to develop biofilms on PS films and particles, either alone or as members of consortia, and thereby degrade the polymer. Weight loss was the primary focus of these investigations. Styrene degradation in bacteria is well-studied in Pseudomonas, Xanthobacter, Rhodococcus, Corynebacterium, and others.

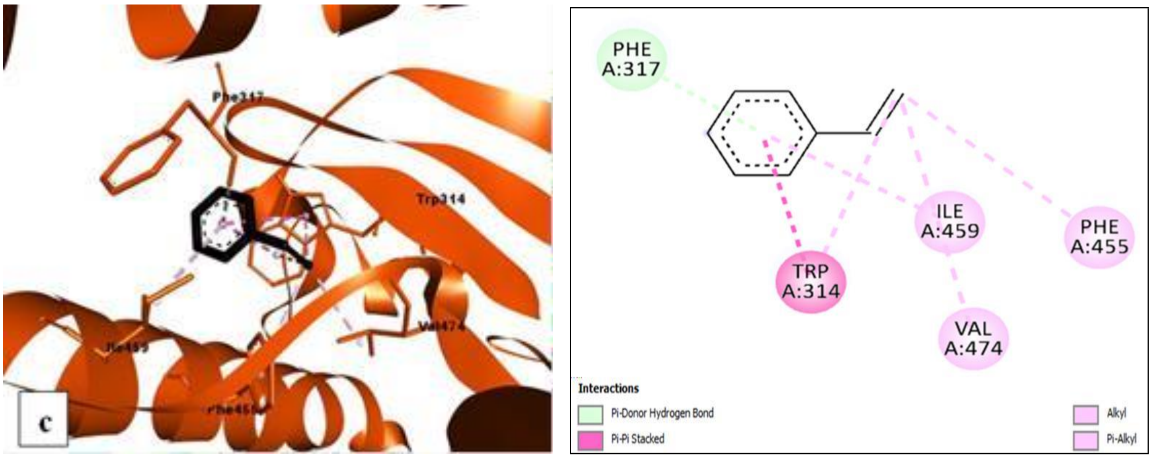


Figure. 3.1 3D (LHS) and 2D (RHS) MD images showing binding interactions with PS (a) Copper dependent Laccase (b) Xylan (c) Oxidase

3.2 Binding Affinity towards PUR

PUR (12,254) is a synthetic polymer that is commonly used in the creation of foams, insulating materials, textile coatings, and paint to prevent corrosion. PUR is docked against 14 enzymes. Enzymes such as Lignin peroxidase, Manganese peroxidase, and Lipase show the best binding affinity towards PUR, and the binding interaction has been presented in Figure. 3.2. Among these, Lignin peroxidase shows the highest binding affinity (-5.21) against PUR. Microbes such as *Phanerochaete chrysosporium*, and *Trametes versicolor* (fungi) secrete the enzyme. The residues that interacted with the enzyme are ASN 182, ARG 43, ASP 183, GLY 86, GLN 189, and GLU 89. There are five van der Waals interaction shown in light green colour in the figureureure and one hydrogen bond shown in dark green colour between the enzymes and ligand.

Manganese peroxidase exhibits the second most binding affinity (-5) against PUR. This enzyme is produced by microbes such as *Phanerochaete chrysosporium* (fungi). The residues that interacted with the enzyme are LYS 180, GLY 82, GLU 39, ASP 85, ARG 42, and ASP 179. There are two carbon-hydrogen bonds, two van der Waals interactions and two conventional hydrogen bonds between the ligand and enzymes. The third binding score is exhibited by Lipase (-4.67). It is secreted by the microbes *Aspergillus versicolor*, and *Bacillus subtilis*. The residues that interacted with the enzyme are GLU 254, ASP 255, GLU 257, ASP 198 ALA 258. There are two carbon-hydrogen interactions, two hydrogen bonds and one van der Waals interaction between lipase and ligand.

Howard et al., (2001) stated that Gram-negative ß-Proteobacteria from the genus Pseudomonas have been connected with PUR activities most frequently. PueB lipase from Pseudomonas chlororaphis was one of the first enzymes discovered to act on PUR. This organism encodes at least one more PUR-active enzyme, named PueA. Both enzymes are lipases, and PUR is destroyed by hydrolase secretion. P. protegens strain Pf -5 also degrades PUR through a similar method. However, it was discovered in this strain that PUR breakdown is tightly regulated by carbon catabolite regulatory mechanisms and that both lipase genes, named pueE and pueB, appear to be required for the development of PUR dispersions.

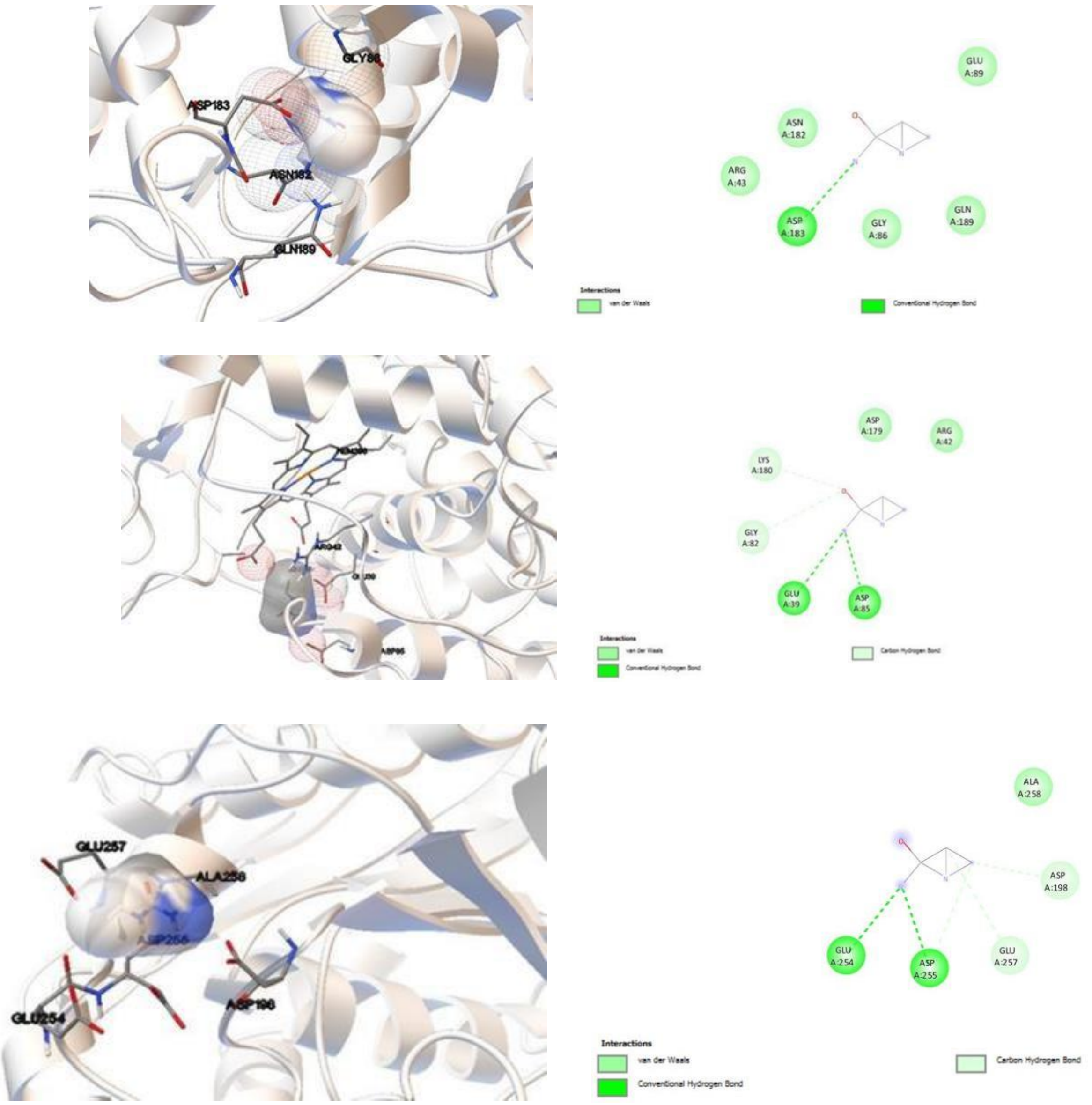


Figure. 3.2 3D (LHS) and 2D (RHS) MD images showing binding interactions with PUR (a) Lignin peroxidase (b) Manganese peroxidase (c) Lipase

5.3 Binding Affinity towards PVC

PVC (6338) is the third most frequently produced polymer. It is composed of repeating chloroethyl units. Among these Lipase, Carboxylesterase, and Laccase exhibit the highest binding affinity towards PVC, and the binding interaction has been presented in Figure. 3.3.

Lipase showed the highest binding affinity (-3.11) against PVC. Aspergillus versicolor and Bacillus subtilis can secrete this enzyme. The residues that interacted with the enzyme are TRP 261, PRO 223, ASN 224, HIS 196, MET 264, ILE 220, ASN 225, VAL 228, PHE 263, TYR 262, VAL 226. There are seven van der Waals interactions depicted in pink colour and 4 π-alkyl interactions depicted in green colour between the ligand and lipase. Carboxylesterase exhibits the second most binding affinity (-3.01) against PVC. The enzymes are produced by culturable microbes such as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* (bacteria), and *Archaeoglobus fulgidus*. The residues that interacted with the enzyme are SER 139, LEU 163, ALA 137, TRP 192, CYS 162, GLY 176, TYR 141, HIS 164, ALA 179, THR 140, PHE 121. These microorganisms offer potential solutions for the degradation and removal of MPs from the environment. There are two halogen bonds with Cl depicted in blue colour, four π-alkyl bonds depicted in light pink shade and five van der Waals interactions depicted in green colour between the carboxylesterase and the ligand. The third binding score is exhibited by Laccase (-2.87). Microbes such as *Trametes versicolor*, *Fomitopsis pinicola*, and *Streptomyces badius* produce these enzymes. The residues that interacted with the enzyme are PHE 90, GLN 119, PRO 121, PHE 89, GLN 123, PHE 118, ASP 122, GLN 91, and VAL 120. There are 4 van der Waals interactions shown in green colour and five π-alkyl bonds shown in pink colour between the enzyme and PVC monomer.

Ru et al., (2020) stated that PVC has the greatest percentage of plasticizers (up to 50%). Many fungi utilize plasticizers as a source of nutritional carbon, and plasticized PVC is usually prone to fungal or bacterial attacks. They found a variety of microbes having degrading abilities towards PVC such as *Poliporus versicolor*, and Mycobacterium sp. NK0301, *Acanthopleurobacter pedis*, and others.

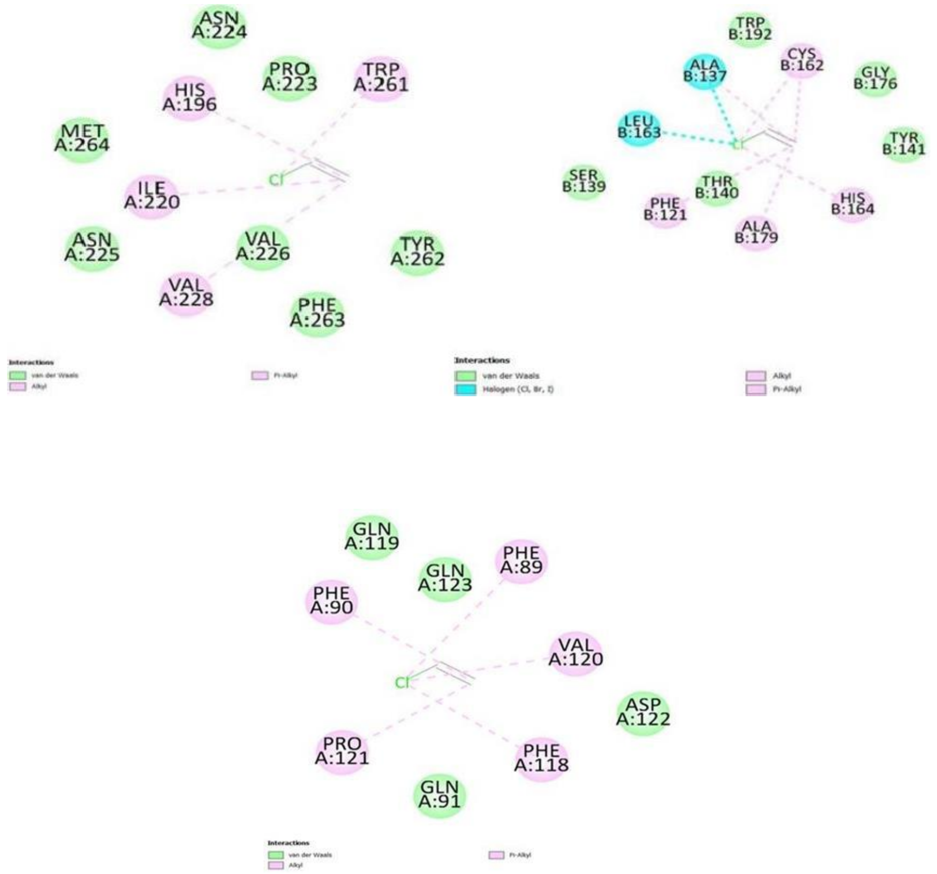


Figure. 3.3 2D MD images showing binding interactions with PVC (a) Lipase (b) Carboxylesterase (c) Laccase

4. CONCLUSION

Recently, MD studies have been applied to understand the mechanism behind the degradation of synthetic polymers by microbes. Microorganisms produce several enzymes that can depolymerise and degrade polymers. According to studies, many natural enzymes can hydrolyze and depolymerize MPs to produce the end products CO_2 and H_2O. However, finding microorganisms and enzymes that can successfully break down different polymers is difficult due to the arduous and time-consuming nature of the process. Thus, MD was employed to test the binding efficiency of multiple enzymes against multiple polymers. This study investigates the role of microbial enzymes in the degradation of MP through *in-silico* approaches. MD of fourteen enzymes against plastic materials that included PS, PUR, and PVC were performed. High-binding affinity enzymes were found. Copper-dependent laccase (-5.65), Xylan (-5.55), and Laccase (-5.38) had the highest affinity for PS. Lipase (-4.67), Manganese peroxidase (-5), and lignin peroxidase (-5.21) all show strong binding affinities in

PUR. Enzymes that exhibit a binding affinity towards PVC include Lipase (-3.11), Carboxylesterase (-3.01), and Laccase (-2.87). These findings can help in screening the microbes for degradation, based on their ability to produce the enzymes that has the highest binding efficiency.

DECLARATIONS

Author Contributions

Dr. P. B. Harathi had the conceptualized idea and critically reviewed the work. Aishwarya Thomas performed the literature search, and data analysis and drafted the manuscript. Veena Vinod and Amritha P S critically reviewed the work.

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Conflict of Interest

None of the Authors declare a conflict of Interest.

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