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Binding interaction of laccases from *Bacillus Subtilis* after industrial dyes exposure: Molecular docking and molecular dynamics simulation studies

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Dyes are becoming more widely used around the world wide, but there is no effective bioremediation approach for removing them completely from the environment. Several dyes are mentioned to be degraded through bacteria; however, it's still unknown how the particular enzymes act throughout the dye degradation. The behavior and function of these enzymes in the biodegradation of azo dyes (Textile dyes) had been investigated experimentally by the numbers of the researchers, however, the molecular mechanisms remain unclear. Therefore, the interaction mechanisms of textile dye (methyl orange) with laccase from *B. subtilis* were explored through molecular docking and molecular dynamics simulations, the three selected dyes (methyl orange, malachite green, and acid blue 62) that interact positively with laccase on the basis of their maximum binding energy, molecular docking results indicate that one of the three dyes is more stable as a target for degradation through *Bacillus subtilis* laccase. Therefore, subsequent research focused solely on one substrate: methyl orange. Molecular Dynamics simulation study was applied after the molecular docking to determine the interaction between laccases and methyl orange dyes. The trajectory was proved with root mean square deviation and root mean square fluctuation analysis. According to the molecular dynamics simulation results, laccase-methyl orange complexes remain stable during the catalytic reaction. So, this study demonstrates how laccase is involved in methyl orange bioremediation.

Keywords: Homology modelling, Laccase, Methyl orange, Molecular docking, Molecular dynamics simulations, Textile dyes

The significance of water in our lives is self-evident. It is an essential part of our daily routine, particularly in quenching our thirst. The purity of water is equally important, as contaminated water can lead to waterborne illnesses that can be deadly[1]. Human activities and industrialization have resulted in environmental contamination through improper waste disposal[2]. The wastewater generated by the textile industry contains high levels of dyes, as well as elevated levels of COD, BOD, pH, color, and heavy metals[3]. Synthetic dyes have been employed in many different fields, from the food and textile industries to printing, cosmetics, polymers, and even pharmaceuticals[4]. Azo dyes are widely used in the textile industry because they are affordable, versatile, give a vast array of colour options, and are easy to produce. These dyes are stable in many pH ranges, temperatures, and light conditions[5]. The textile industry's effluents pose a significant challenge at the present time. These industries are vital to the world economy, but they also consume a large quantity of water and are responsible for 20% of all industrial water pollution [6]. In recent years, biological waste treatment has gained popularity as a viable alternative to physicochemical approaches, which are unable to fully mineralize hazardous pigments[7,8]. To treat hazardous effluents, a biological approach such as microbial bioremediation is more effective [9] Biological methods, such as microbial bioremediation, are preferable to chemical ones for treating harmful effluents. Bacterial oxidoreductases are a class of bacterial enzymes that play a key role in the degradation and decolorization of hazardous dyes[10,11]. Laccases (EC 1.10.3.2), a multi-copper enzyme and one of the most important oxidoreductases, used in commercial dye degradation processes due to their ability to decolorize chromophore complexes such azo and triphenylmethane dyes[12,13]. Laccase from bacteria has not yet been thoroughly studied, as it is typically isolated from fungi and plants. Bacterial laccases are distinguished from fungal laccases by a number of characteristics, including temperature and pH stability. This enzyme is generated extracellularly or intracellularly by bacteria and is active over a broad temperature and pH range[14].

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Abbreviations: Molecular dynamics, MD; Molecular docking, MolDock; Root mean square deviation, RMSD; Root mean square fluctuation, RMSF; Radius of gyrations, Rg; Surface accessible to solvents; SASA; Molecular mechanics Poisson–Boltzmann surface area, MMPBSA

Bioinformatics and Molecular Docking (MolDock) techniques are widely available to provide insights on bioremediation mechanisms predicting dye degradation susceptibility using the laccase enzyme[15]. More precise dye degradation experiments using MolDock and Molecular dynamics (MD)and simulation will help researchers better understand the processes involved in its bioremediation. Numerous studies have already been conducted to look at the potential use of MolDock and MD simulations of protein-ligand complex stability in bioremediation[16]. In this study, Homology modelling of *Bacillus subtilis* laccase was done. Further docking investigations of three textile dyes, namely methyl orange, malachite green, and acid blue 62, belonging to the chemical classes azo, anthraquinones, and triphenylmethane respectively was performed. As per the results obtained, MD simulation, molecular mechanics poisson–boltzmann surface area (MMPBSA) analysis, and hydrogen bond interaction analysis were used further to evaluate the binding between laccase and methyl orange dyes. The research of laccase and methyl orange dye binding mechanisms using MD simulation led to a number of valuable conclusions. These findings provide us with a more comprehensive understanding of the biodegradation of methyl orange dye as a viable biological treatment approach.

Literature Review

Laccase enzyme, a glycoprotein which is able to oxidize both phenolic and non-phenolic substrates through either direct or indirect oxidation Alsaiari *et al*. (2021). This enzyme has a low substrate specificity, and does not require a cofactor for its activity, making it highly versatile[17]. Additionally, the possibility of expanding its range of oxidation through the use of redox mediators is a great advantage[18] reported by Alba *et al*.(2019).

Herkommerová et al. (2018) and Jamal et al. (2022) have both reported that laccase enzymes are very effective when it comes to the bleaching of cellulosic materials and color pigments. This process provides several advantages, such as stability, resistance to external conditions, better half-life and recoverability, improved pH levels, enhanced thermal stability, and reduced sensitivity to external influences[19,20]. This process is widely used in the bio-bleaching of natural pigments from cellulosic materials.

As reported by Pande *et al*. (2022) found that laccase plays a vital role in bioremediation, and MolDock was used to identify the dyes with the highest binding energy. From the ten dyes identified, three – pigment red 23, fuchsin base and Sudan IV – were further analysed in MD simulation. The MD simulation revealed that laccase forms strong bonds with these dyes through hydrogen bonds and hydrophobic bonds. This information is critical in understanding the stability of the enzyme-dye complex, and the role of laccase in catalytic reactions [21]. Moreover, this research provides valuable insight into the expression of laccase and its significance in bioremediation, both of which are essential for efficient and effective clean-up operations.

Study conducted by Bhatt *et al*. (2023) revealed that the laccase enzyme interacts well with pollutants such as glyphosate, lignin polymer, isoproturon and parathion, with binding energies ranging from −2.5 to −8.7 kcal/mol. Notably, the lowest binding energy was observed for the interaction of laccase and glyphosate, indicating laccase could play a role in degrading this pollutant [22]. Similarly, recent study has shown that the strain environment-friendly bacterial *Bacillus pseudomycoides* has been used as an effective agent for wastewater treatment. In a MolDock analysis, it was observed that the binding energy between the enzyme and pollutants such as methylene green, crystal violet and acid blue was in the range of −6.3 to −6.8 kcal/mol. Furthermore, the solvent accessible surface area, root mean square deviation (RMSD) and radius of gyration (Rg) were all indicative of the system stability, *i.e*. the enzyme to the pollutants - suggesting it could feasibly be used to decolorize single and mixed dyes[23].

Materials and Methods

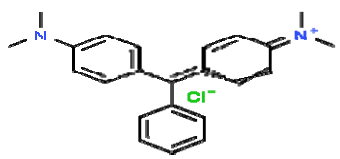
Dyes

Three industrial dyes, such as: methyl orange, malachite green, acid blue 62 were considered for this study. The dyes structures were obtained as spatial data files (SDFs) from National Center for Biotechnology Information (NCBI) PubChem database. Their chemical structures were listed in (Table 1).

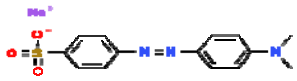
Homology modelling and validation

The three-dimensional structure of laccase from *B. subtilis* were unavailable in the Protein Data Bank (PDB), homology modelling was used to generate the laccase enzyme's structure. The NCBI Protein database has been used to find *B. subtilis* laccase protein sequences. (Accession number: ARO72333), allowing

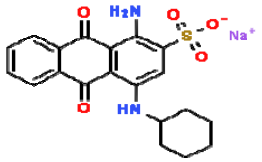
Table 1 — Ligands structure used in this study. (Source: ChemSpider web site)



malachite green



methyl orange



acid blue 62

secondary structure and physicochemical properties to be predicted[24]. BLASTp was used to identify the template structure. Based on the findings of protein BLAST, 1GSK (Chain-A endospore coat protein from *B. subtilis*) was selected as the template for laccase homology modelling. Structures of protein was retrieved from Protein Data Bank (PDB) and applied for homology modelling. Modeller 10.2(https://salilab.org/modeller/)[25] was used to model the homology of selected target sequences, On the basis of dope score predicted protein structure are selected further validation of predicted protein models was done by using UCLA-DOE LAB-SAVES v6.0 server (https://saves.mbi.ucla.edu/) to run ERRAT (program for verifying protein structures determined by crystallography)[26], VERIFY 3D (Determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar) and comparing the results to good structures.), PROCHECK (checks the stereo-chemical quality of a protein structure)[27], ProSA server (web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids)[28].

Binding sites identification

In this study, CASTp (CASTp 3.0: Computed Atlas of Surface Topography of proteins (uic.edu)[29] was used to locate, delineate, and calculate topological geometric and properties of protein structures. Three binding pockets were selected on the basis of prominent concave regions of proteins that are frequently associated with binding events based upon the alpha shape and the pocket algorithm developed in computational geometry.

Molecular docking

MolDock was done with PyRx (https://pyrx.sourceforge.io/)[30] open-source software Autodock Vina (https://vina.scripps.edu/)[31] is to determine the ligand's potential orientations and conformations at the binding location. The docking study's grid centre was set to X = 105.7, Y= 34.4, and Z= -2.8, X =68.87, Y=41.17, and Z=-3.57, X = 77.2, Y= 43.8, and Z= -8.1 with grid box dimensions of X= 28.0, Y= 29.1, Z= 27.7, and X=25.0, Y=24.91, Z=25.0, and X= 35.2, Y= 31.6, and Z= 34.8 coordinates, respectively.

Dye-degrading amino acids visualization

Ligplot.1.4.5 software (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) was used to analyze the two-dimensional interactions of enzyme-substrate structure, as well as hydrogen bonds and bond lengths[32]. PyMol (https://pymol.org/2/) a molecular visualization programme, was used to examine the three-dimensional enzyme-ligand structure[33].

Molecular Dynamics Simulation

The most efficient protein-ligand combination with the lowest binding free energy (obtained after docking) was then applied to 100 ns MD simulation using Gromacs 2021 (https://ftp.gromacs.org/gromacs/gromacs-2021)[34]. At the initial point, it is started by separating the protein and ligand from their respective complex form, to generate the topology file for an individual; protein and ligand. Here we constructed the protein topology with the help of the parameters implemented in CHARMM-36–feb2021[35]. The protein topology is constructed with "charmm36-feb2021.ff" and the water model “TIP3-Point, TIP3-point, recommended, by standard uses CHARMM TIP3”. While the topologies for ligand were constructed using the CHARMM-based online server - CGenFF server[36]. The protein-ligand complex is regenerated by manually fitting the residues from complex_processed.gro and unk.gro file in a new file complex.gro. The next step is followed by the solvation of a complex in dodecahedron form and is carried out by using the water model. The system is neutralized with appropriate positive (Na) and negative (Cl) ions. The generated complex with the solvent system was neutralized by the addition of 14 Na (+ve) ions.

To minimize the energy of the generated complex-solvent system, the steepest descent minimization was

used; the energy minimization was processed for 50000 numbers of maximum steps. The ligand position restrained index file is generated andgenerated.itp file was incorporated into the main topology file. By using the leap-frog integrator algorithm, the NVT (ensemble maintaining the constant number of particles, volume, and temperature) and NPT (ensemble maintaining the constant number of particles, pressure, and temperature) are subjected to 50000 steps, equal to 100 picoseconds, and temperature 300-K.

The generated equilibrated system was finally subjected to MD simulation for 50000000; 100000 ps (100 ns). Particle Mesh Ewald (PME)[37] was used to retrain the system with the LINCS algorithm[38] for long-range electrostatics, like Coulomb and Lennard Jones. For covalent bonds, the cut-off value was set to 12 Å. In the post-MD simulation analysis, deviations in the protein and ligand atoms were measured as RMSD. The graphs were generated using the XM grace Linux-based tool.

MMPBSA

MMPBSA (using MD simulation) is used to predict binding free energy. The relative binding affinities of methyl orange dye-laccase complex were calculated using the MMPBSA models.

Results and Discussion

Homology modeling and validation

Based on the dope score, Modeler10.2 generates five distinct models for each enzyme. To determine the structure for further research, the lowest Dope score was used. For a protein or enzyme to perform its function, its structure is critical.3D structure of the protein (Laccase) Visualization with PyMOL (Fig. 1A). The modelled protein was validated by UCLA-DOE LAB — SAVES v6.0 server having molecular weight of 58.51 kDa, the residuals from the developed model were found to be in a favorable area, and the overall structural quality was good. (Table 2), Ramachandran Plot (Fig. 1B)

Binding sites identification

CASTp was used to predict the possible binding pockets of laccase's 3D structure. CASTp evaluates, using the solvent-accessible surface mode and a molecular surface model, calculates the area and volume of expected pockets and voids. Top-ranking binding pocket 1 with a surface area of 127.3 $Å^2$ and volume of 270.7 $Å^3$, Binding pocket 2 with a surface area of 174.1 $Å^2$ and volume of 263.4 $Å^3$, and Binding pocket 3 surface area of 643.2 $Å^2$ and volume of 723 $Å^3$ were shortlisted. The first, second, and third binding pockets residues have been anticipated by CASTp. References 198T, 207F, 209P, 211A, 212P, 213E, 225V, 227A, 228F, 229C and 183K, 184L, 185P, 186S, 187D, 188E, 189Y, 248R, 344A, 345Q, 347E, 348S and 12I, 13P, 15T, 56P, 57G, 59T, 61E, 147G, 148A, 149I, 170G, 171A, 173I, 174I, 175H, 176D, 177P, 179E, 180K, 183K, 184L, 185P, 186S, 187D, 190D, 191V, 192P, 194L, 251R, 253R, 177G, 176S, 281G, 282L, 299R, 300Y, 301D, respectively, shown in (Table 3, Fig. 1C & 1D).

Molecular dynamics and visualization

PyRx Software of Autodock Vina tools was used to investigate dye interactions with laccase. Dye structure that obtained from the NCBI PubChem database in the form of SDFs. The ligands energy minimization using Open Babel program tools under the uff force field, Conjugate gradient optimization algorithms method with 200 steps. Before docking, the Open Babel program tools were used to convert the data to PDBQT format. For protein minimization, all water molecules were eliminated and hydrogen atoms were affixed in BIOVIA Discovery Studio Visualizer2021 (https://discover.3ds.com/discovery-studio-visualizer). Based on data gathered by surrounding pocket active site residue, the docking grid box was chosen by the web server, and a CASTp of proteins calculation produced pockets of the modelled protein. The lowest Vina score, binding affinity (kcal/mol), and the maximum number of hydrogen bond interacting complexes were selected for further evaluation of the interactions of dyes and enzymes. This is shown in (Tables 4 and 5), the binding pocket-2 show all laccase-methyl orange complex show lower binding affinity and a maximum number of hydrogen bond formations than the other two binding pockets. So laccase-methyl orange complex was selected for further analysis of the interaction. The two-dimensional enzyme-substrate complex interactions, along with hydrogen bonds and bond lengths, had been studied by applying the Ligplot.1.4.5 software. This is shown in (Table 6 and Fig. 2).

Molecular Dynamics Simulation

The MD simulation provides greater observation into the binding and interactions of ligand and an understanding of pharmacophoric-based interaction within the binding site of the protein including the ligand dynamicity behavior. The conformational space acquired by the ligand in the binding site region

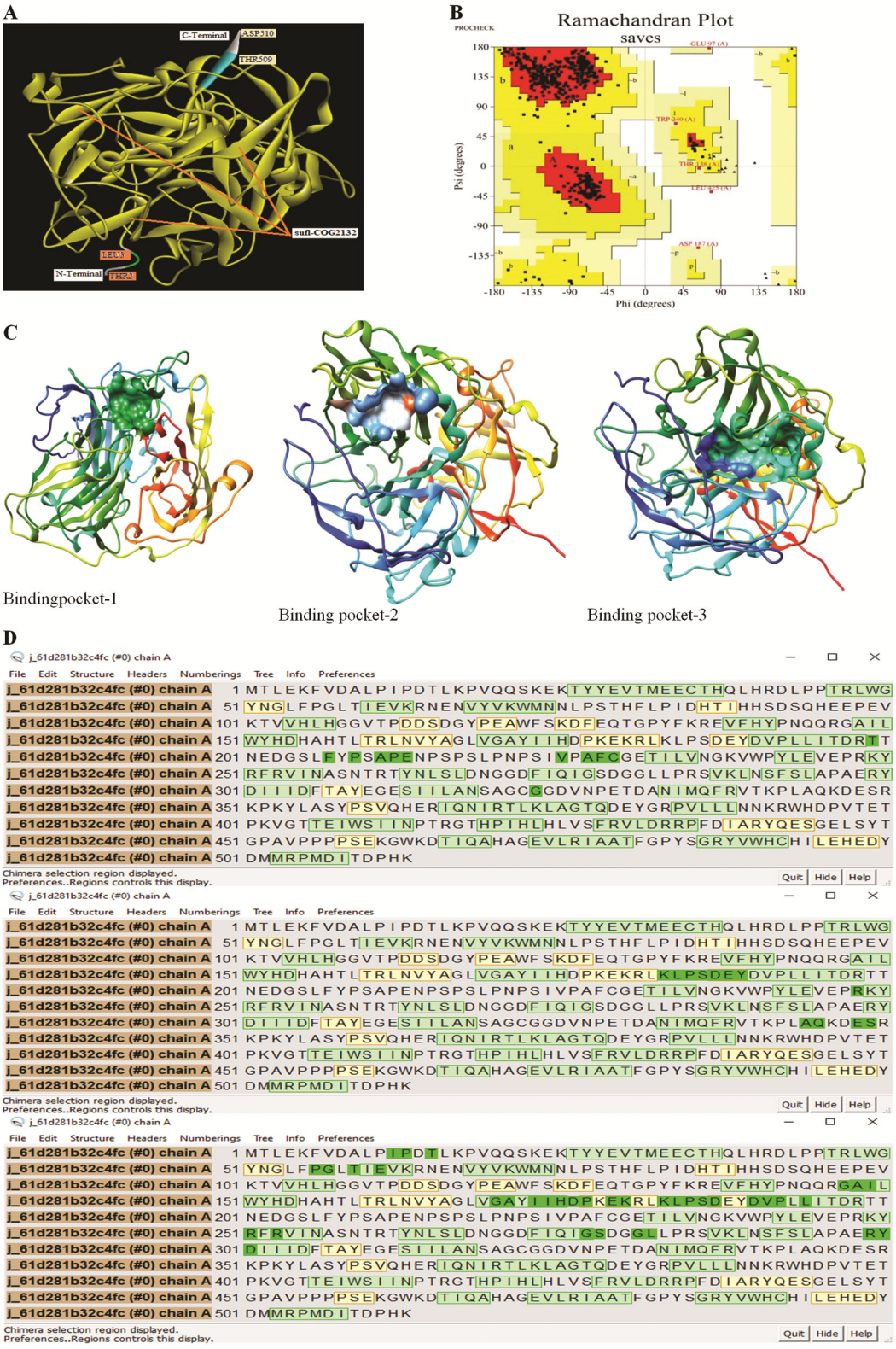


Fig. 1 — Three-dimensional structure of the protein (Laccase) showing N terminal, C terminal and one conserved domain (Sufl-COG2132) (A), Ramachandran plot (B), Active binding pockets in protein (C), residues (green displayed) involved in active binding pocket (D)

Table 2 — Parameters for evaluating predicted three-dimensional structures of laccase enzyme using UCLA. -DOE LAB— SAVES v6.0 server to run ERRAT and verify 3D, PROCHECK and by ProSA server.

Enzyme	PROCHECK Analysis	ERRAT analysis	VERIFY 3D	ProSA analysis
	RMFR RAR RDR	Overall quality factor		
Laccase	89.9% 9% 0.2%	71.82	Pass (92.79%)	-8.22

RMFR: Number of residues in most favored region, RAR: Number of residues in allowed region, RDR: Number of residues in disallowed region.

Table 3 — Binding pocket amino acid residues

Binding pocket	Binding pocket amino acid
1.	198T, 207F, 209P, 211A, 212P, 213E, 225V, 227A, 228F, 229C, 323G
2.	183K, 184L, 185P, 186S, 187D, 188E, 189Y, 248R, 344A, 345Q, 347E, 348S
3.	12I, 13P, 15T, 56P, 57G, 59T, 61E, 147G, 148A, 149I, 170G, 171A, 173I, 174I, 175H, 176D, 177P, 179E, 180K, 183K, 184L, 185P, 186S, 187D, 190D, 191V, 192P, 194L, 251R, 253R, 177G, 176S, 281G, 282L, 299R, 300Y, 301D

Table 4 — Molecular Docking scores and Number of Hydrogen bond of several screened dyes along with laccase.

S. No.	Enzyme-ligand Complex	Binding energy Kcal mol^{-1}	Number of Hydrogen bond
		Pocket-1 Pocket-2Pocket-3	Pocket-1 Pocket-2Pocket-3
1.	laccase -malachite green	-7.1 -7.3 - 7.3	0 4 0
2.	laccase -acid Blue 62	-6.9 -8.7 -8.7	1 0 4
3.	laccase -methyl orange	-6 -7.2 -6.4	3 7 3

Table 5 — Molecular Docking scores and Number of Hydrogen bond of several screened dyes along with laccase. (Binding Pocket-2)

S. No	Substrate's name	Binding energy Kcal mol^{-1}	Number of Hydrogen bond
1.	malachite green	-7.3	4
2.	acid blue 62	-8.7	0
3.	methyl orange	-7.2	7

Table 6 — The residues involved in the enzyme-substrate interaction

	Hydrogen bond interaction		Hydrophobic bond interaction	
Enzyme-substrate complex	No. of bond	Involved residues	No. of bond	Involved residues
laccase- malachite green	0	-	12	Glu188, Val191, Asp187, Asp190, His175, Pro13, Asp14, Thr15, Ile12, Glu61, Thr59, Ile173
laccase- acid blue 62	4	His175, Arg253, Glu179, Arg251	5	Ser186, Lys180, Lys183, lys63, Leu184
laccase- methyl orange	7	Arg25, Arg253, Leu184, His175, Asp176, Glu179	4	Gln348, Gln345, Ser186, Lys183

over the period specifies the stability of the protein-ligand complex[39]. The generated complex.gro file with all topology and constraints, after the neutralization and solvation, the file consists of 513-protein residues, 1- other residues (ligand), 16198-solvent (water residues), and 14-Ion's residue (NA^+ ions) was generated. The complex system converged to its lowest energy form < 1000 in 941 steps using the Steepest Descent algorithm, the potential energy was recorded with -7.00869e+05 Kj/mol. The averagebond energy stated 6.76775e+03 kj/mol, the average proper dihedral energy of 2.26978e+04 Kj/mol, and the average improper dihedral energy of 1.11754e+03 Kj/mol, all the analyses exhibited stability in the graphical representation. The NVT and NPT analysis exhibited total energy of - 5.54128e+05 Kj/mol and -5.61911e+05 Kj/mol, with an average temperature of 2.99938e+02 (K) and Pressure (bar) of -1.67901e+01 can be observed in the given graphical images. After the MD simulation of a subjected protein-ligand complex, the measurement of protein and ligand Root mean square deviation (RMSD) provides a good estimate of the conformational stability of the system.

The enzyme-substrate complex RMSD

The difference in the enzyme-substrate complex had been obtained by the RMSD at 100 ns. The deviations that a protein builds throughout simulation determine its conformational stability. Proteins with fewer variations are considered to be more stable. RMSD was calculated for the backbone of the methyl orange with the laccase (Fig. 3A). The RMSD values for laccase-methyl orange complexes were found to be 0.09 to

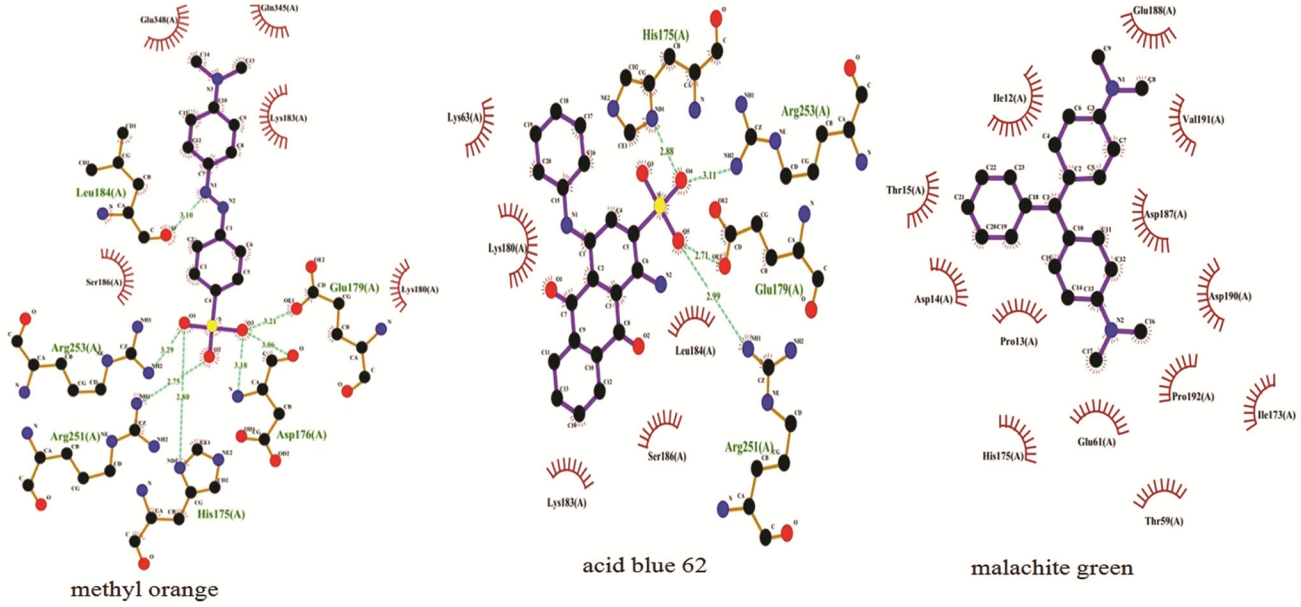


Fig. 2 — 2D Molecular interaction between top hit ligands and H-bonds, as well as hydrophobic bonds. (methyl orange, acid blue 62, malachite green) and laccase (Dotted green lines show hydrogen bonds, Brown ignited arcs show hydrophobic interactions)

0.4 nm. The enzyme-ligand complexes are stable for methyl orange biodegradation, as evidenced by minor fluctuations and a lower RMSD. Some research has evaluated the RMSD for enzyme-substrate complexes in order to better understand the variability within the complex all over biological processes[40,16].

The enzyme-substrate complex's RMSF

At a given temperature and pressure, the location of an atom was determined by Root Mean Square Fluctuation analysis (RMSF). The RMSF analysis shown the protein's flexibility sections and calculated the net variations of the protein during the molecular dynamic simulation. A lower RMSF value showed a more stable enzyme-substrate combination, whereas a higher value showed greater MD simulation flexibility. During the MD simulation, the methyl orange complex was stable, with less fluctuation and higher consistency according to the RMSF findings of the study.

For the enzyme-substrate complexes, fluctuations in the residue content were observed during the 100 ns trajectory time frame (Fig. 3B). The study's RMSF data demonstrated that methyl orange complexes were remained steady throughout the MD simulation, with less fluctuation and higher consistency. RMSF was already applied to investigate enzyme-substrate complexes in the past.The laccase-phenol complex of RMSF has been investigated and found to be useful in remediation[41,45]. Furthermore, Salicylaldehyde dehydrogenases have been evaluated using the RMSF method, that exhibited RMSF high scores, indicating moldable protein residues. The RMSF was already correlated to the bounden of cofactors and substrates[42]. These studies demonstrate as an enzyme's flexibility declines; it experiences conformational changes that alter substrate binding during catalysis.

Radius of gyration

The radius of gyration (Rg) is a unit of parameters for differences in enzyme-ligand complex closeness. It simply links to protein folding and unfolding. For this analysis, the last 100 ns of trajectory data were used to calculate the radius of gyration. The overall Rg score for the laccase-methyl orange was 2.3 ± 0.005 nm. A stable Rg occurs when the enzyme is folded, and a fluctuating Rg indicates the unfolding of the enzyme [43]. The overall Rg score demonstrated that all enzyme-substrate combinations had a similar and consistent Rg value (Fig. 3C). This means they are perfectly overlaid over each other, resulting in the substrate-enzyme complex being compacted and stabilized[43]. A study found that the Rg value of phenol-laccase remained consistent under different situations, showing that the Rg importance is associated with the consistency of the enzyme-substrate complex[44].

Solvent accessible surface area

The surface accessible to solvents (SASA) using the MD simulation, we can determine how much of

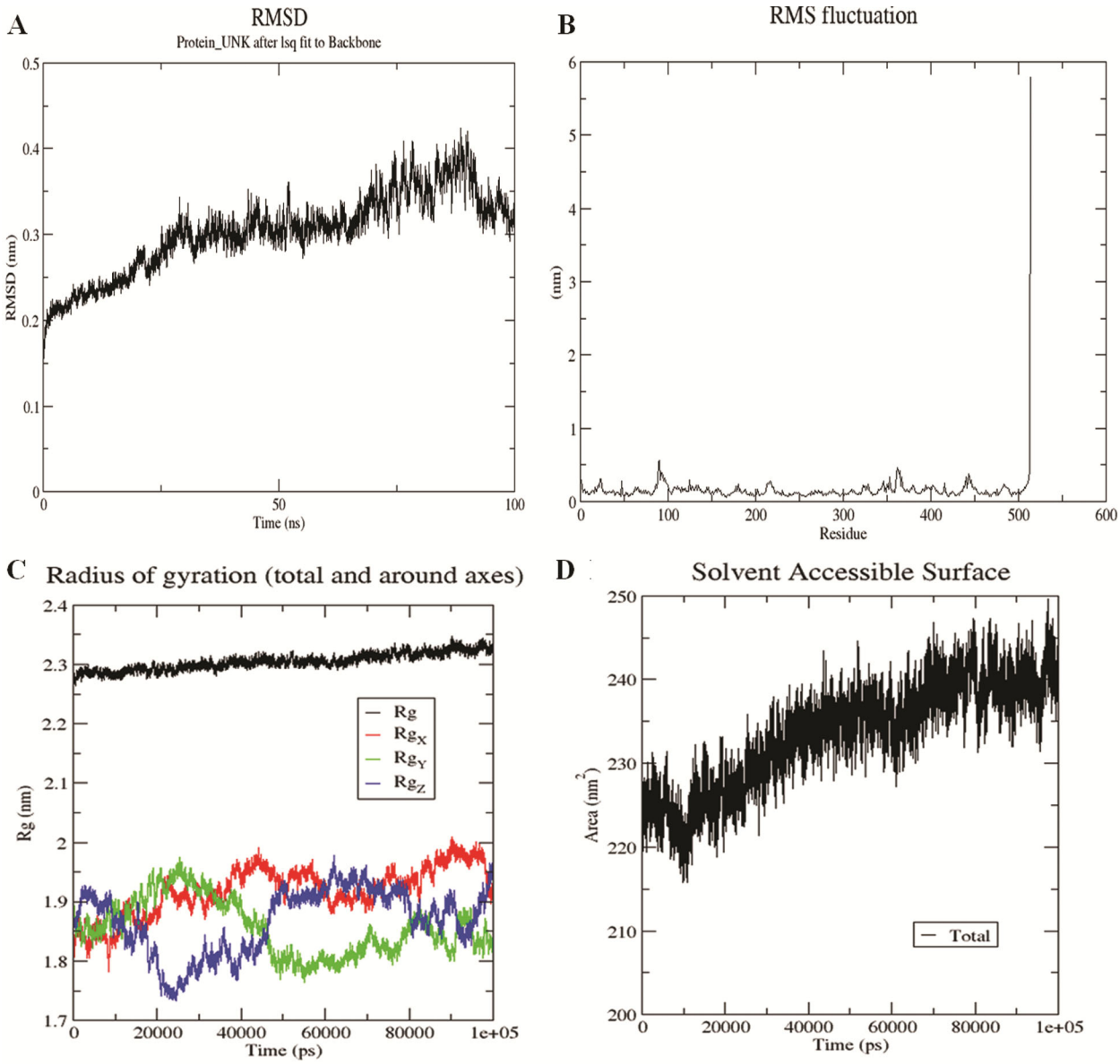


Fig. 3 — Graphic depiction of RMSD (A), RMSF (B), Rg (C) and SAS (D) profile of the enzyme-substrate complex for 100 ns MD simulation.

the protein surface is exposed to the water solvent and investigate contacts between the complex and the solvent. The overall SASA of 238 ± 3.1 nm^2 was calculated for laccase-methyl orange. As a result, the SASA values for laccase-methyl orange compounds throughout 100 ns of MD simulation are impressively stable, indicating that the shape of the protein has not altered significantly (Fig. 3D). The effects of laccase-based phenol remediation additionally confirmed this[44]. Thus, here we determined that the pockets had formed on the laccase's surface. Based on a study, the intrusion of enzyme-catalyzed reactions is triggered using adjustments in the cavity[45,46]. Biosurfactants were found to improve the scale of enzymes in bioremediation in another investigation because of alters in cavities[44].

MMPBSA

Applying the MM-PBSA strategy installed in GROMACS, the binding free strength used to be calculated from MD simulation trajectories. It was observed that the overall Laccase-methyl orange complexes had sufficient binding energy. laccase-methyl orange had a binding free energy of –60.75 Kcal/mol in the complex, indicating that the ligand conformation is more stable.

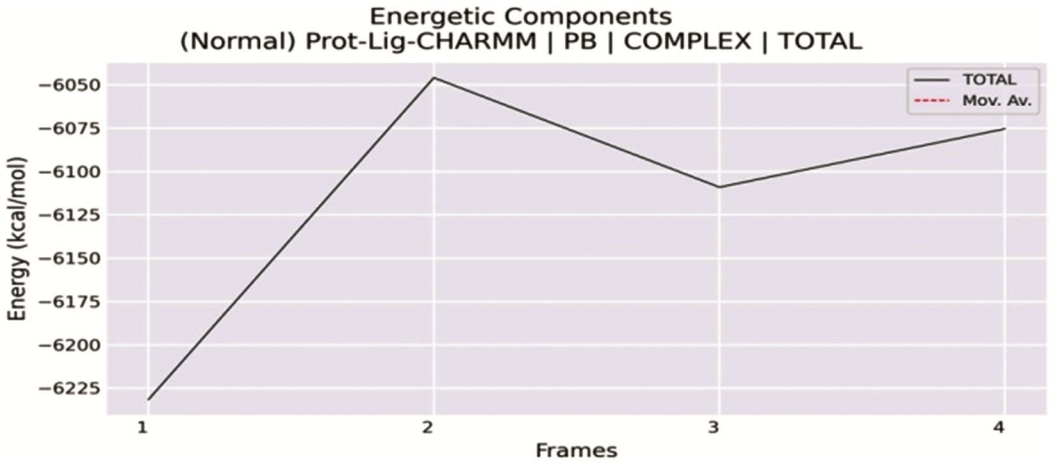


Fig. 4 — Total binding energy of complex (Frame Wise)

In this graph (Fig. 4), we can see that the binding energy is starting at −62.25 kcal/mol and gets stabilized at −60.75 kcal/mol at the end. So, we can say that there are not much of fluctuations in the graph hence the binding energy is staying stable throughout the process. The MM-PBSA model has been used already in phenol bioremediation and laccase-based MD simulation[44]. According to earlier research, the MM-PBSA study aids in determining the genuine efficacy of the enzyme-substrate compound in xenobiotic substance remediation[45,44]. The laccase-methyl orange complex had been applied for the MM-PBSA investigation in this study, and the results show that laccase can remediate a wide range of textile dyes.

Discussion

In this study the modelled protein was validated by UCLA-DOE LAB — SAVES v6.0 server, the results showed that the residues from the built model fell in favorable regions and the overall structure quality was good. The docking results illustrate the interactions between the dye and the investigated protein. The mainly polar amino acids of laccases have been interacting with textile dyes. The MD simulation was used to learn more about how methyl orange dye interacts with laccases. Based on the results from the overall MD simulation (including RMSD, RMSF, and Rg analyses), the Post-MD simulation (containing SASA and MMPBSA analyses), and the binding free energy analysis, determined that methyl orange is a fairly stable compound with excellent binding affinities with laccase. As a result, it was concluded that laccases from *B. subtilis* are found to be the most favorable in methyl-orange dye degradation and need further experimental characterization for their potential large-scale bio-remedial applications. This study also presents a pipeline for the screening of microbial enzymes for azo dye degrading abilities before the wet lab affirmation. Based on binding energies and H-bonding results, we propose that laccase from *B. subtilis* can efficiently bind with the methyl orange dye, and, in addition, induce its degradation. It was anticipated that this model can serve as a reliable *in silico* predictor for the potential of microbial enzymes for azo dye degradation. Further wet lab experiments are needed to enhance our understanding of the mechanisms underlying the degradation of dyes by microbial enzymes. This research serves as a basis for further exploration of laccases and other microbial enzymes responsible for dye degradation.

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