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Research Article

IN-SILICO MOLECULAR MODELLING AND DOCKING STUDIES OF SELECTED TEXTILE DYES AND LACCASE TO INVESTIGATE THE DYE DEGRADATION MECHANISM OF THE ENZYME

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ABSTRACT

Among the fungal enzymes, laccases are of great interest, because laccase offer more stability and the possibility to utilize the enzyme in its immobilized form and are able to degrade numerous textile dyes. The aim of this investigation was to characterize the interaction of enzyme laccase from white rot fungus Trametes hirsuta with textile dyes and establish its ability to decolorize textile dyes in industrial effluent. Here the activity of laccase was determined by *in-silico* method where, the enzyme laccase from Trametes hirsuta was docked using Autodock Vina with dyes such as Reactive blue160, Congo red, Bromophenol blue, Disperse blue 1, Malachite green to characterized the degradation of the dyes by the enzymes using insilico method. The dyes were found to bind to the copper containing T1, T2, and T3 active site bridging with (GLN 237, GLN 242, TYR 244, ASN 262, ARG 423, SER 427, PHE 162, GLN 264, LEU 459, GLU 460, LEU 355) of laccase. Laccase are capable of degrading a wide variety of compounds, and they are commercially significant. This study provides useful information on the mechanism of dye degradation.

Keywords: Laccase, Molecular modelling, Docking, Textile dyes, Degradation

1. INTRODUCTION

There is manifold demand for textiles dyes, leading to their spillage in the wastewater and environment [1]. It is estimated that, nearly 10,000 types of synthetic dyes are commercially available today and 7 lakh tons of dyes are produced every year [2]. Globally 280,000 tons of textile dyes are discarded in textile effluent annually [3]. If this kind of environmental pollution persists in future pollution resistant living species will dominate the earth and sensitive species including the human may have to face the serious problems of survival and existence. Therefore for our betterment, we have to minimize our interference in the complex and sensitive network of environmental processes. More precisely we have to find all the possible ways of reducing each and every type of pollution from our environment, to sustain our life supporting earth [4]. The use of enzymes in the diverse fields of industrial application has been increased in recent years; fungal enzymes particularly, are of interest in biotechnological processes. Among the fungal enzymes, laccases are of great interest [5]. Laccase are glycosylated blue multi copper oxidoreductase (BMCO) enzymes, and are widely distributed in higher plants and fungi and have also been found in insects and bacteria. Since Laccases are multi-copper oxidases, they catalyze the oxidation of various organic and inorganic compounds [6]. Laccases contain three types of copper atoms, one of which is responsible for their characteristic blue colour. The enzymes lacking a blue copper atom are called yellow or white laccases [7]. Due to the ability of fungal laccases to oxidize phenolic and nonphenolic aromatic compounds, there is anincreased interest in the application of these enzymes for various industrial applications, including food, pulping, textile, wastewater treatment, and bioremediation, Laccase catalysis occurs in three steps: (1) type I Cu reduction by substrate; (2) electron transfer from type I Cu to the type II Cu and type III Cu trinuclear cluster; (3) reduction of oxygen to water at the trinuclear cluster [8, 9].

Ligands:

(A) Reactive blue 160 (RB160)

Reactive blue 160 (RB160) is one of the reactive azo dye which is being dispelled into the river. It is navy blue color powder with 50℃ solubility of 100 g/l. RB160 is a synthetic dye and mainly used for cotton, hemp, viscose, paint/cotton and polyester/stick blended fabric dyeing and printing. Extensive use of RB160 has drawbacks for

human life. More exposer to this dye may cause allergic reactions of respiratory tract, so this dye should be treated and removed from wastewater in an economical and efficient way [10].

(B) Congo red

Congo red is an organic compound, the sodium salt of 3,3′-bis4-aminonaphthalene-1-sulfonic acid. It is an azo dye. Congo red is water-soluble, yielding a red colloidal solution; its solubility is greater in organic solvents [11, 12].

(C)Bromophenol blue

Bromophenol blue (3′,3″,5′,5″-tetrabromophenol sulfonphthalein, BPB, albutes) is used as a pH indicator, a color marker, and a dye. It can be prepared by slowly adding excess bromine to a hot solution of phenolsulfonphthalein in glacial acetic acid [13].

(D)Malachite green

Malachite green is an organic compound that is used as a dyestuff as well as an antimicrobial in aquaculture. Malachite green dye is mainly used for materials such as silk, leather, and paper. Microscopic analysis of cell biology & tissue samples is possible by using malachite green as biological stain [14].

(E) Disperse Blue 1

Disperse Blue 1 is a blue to black colored amino anthraquinone dye. Disperse blue 1 is primarily used in hair color formulations and in coloring fabrics and plastics, but has also been used as a fabric dye. Exposure to disperse blue 1 irritates the eye and skin. Disperse blue 1 is a mutagen and is reasonably anticipated to be a human carcinogen. Disperse Blue 1 has been prepared by acylation of 1,5-diaminoanthraquinone with oxalic acid, then nitration in sulfuric acid, followed by hydrolysis and reduction to the tetraamino compound, and by the reduction of mixed 1,5- and 1,8- dinitroanthraquinone to the corresponding diamino compounds, followed by acetylation, nitration, reduction, and hydrolysis [15].

2. MATERIAL AND METHODS

2.1.Receptor protein

The 3D structure of laccase (PDB ID: 3FPX) was downloaded from universal source PDB in .pdb format [16].

2.2.Sequences analysis for laccase protein

The sequence of laccase from Trametes hirsuta was obtained from UniProtKB database (UNIPROT: B2L9C1). Multiple sequence alignment is an activity performed when three or more proteins (or nucleic acid) sequence are present and are to be aligned partially or completely [17]. The amino acid sequence of the target 3FPX.pdb was aligned with the sequence of 2HRG.pdbcomplexed with p-methylbenzoate, respectively for identifying the active site on the laccase from Trametes hirsute [16, 18].

2.3.Sketching and optimization of Ligand molecules

The selected ligands were sketched using Chemsketch and transformed into the 3D structure, intermolecular interactions of these ligands were optimized to attain a local minimum energy structure using Argus Lab [19].

2.4.Active site

The amino acid sequences obtained after multiple sequence alignment between enzyme laccase from Trametes trogii (2HRG) and Trametes hirsuta (3FPX) were considered as active site for protein laccase from Trametes hirsute [16, 18].

2.5.Molecular docking

Docking attempts to find the best binding mode between two molecules. Molecular docking is a key tool in structural molecular biology and computer-assisted drug design [20-23]. AutoDock is molecular modeling simulation software. It is especially effective for Protein-ligand docking [21]. AutoDock is one of the most cited docking software in the research community.

Dock is currently maintained by The Scripps Research Institute and Olson Laboratory. For its input and output, vina uses the same pdbqt molecular structure file format used by Autodock [24].To predict the predominant position and orientation of a ligand (Reactive blue 160, Congo red, Disperse blue 1, Malachite green, and Bromophenol blue from PubChem) with a protein laccase of known three-dimensional structure, molecular docking was performed by using software Autodock Vina.

3. RESULTS AND DISCUSSION

The optimization of ligands were significantly changed the conformations of ligands with respect to its energies Table 1 shows the release of constraints and its final energy level.

Table 1: Ligand optimized energy using Argus lab tool

Dyes	RB160		Congo red		BPB		Malachite green		Disperse blue 1	
Parameters	Initial energy	Final energy	Initial energy	Final energy	Initial energy	Final energy	Initial energy	final energy	Initial energy	Final energy
MM Bond	1.482	0.022	0.769	0.013	0.008	0.008	0.168	0.018	0.113	0.01
MM Angle	3.162	2.989	1.381	1.075	0.224	0.222	0	0.01	0	0
MM Diheral	0.062	0.062	0.025	0.025	0.011	0.011	0	0	0	0
MM Improper torsion	0	0	0	0	0	0	0	0	0	0
MM vdW	6.644	0.141	3.608	0.106	0.05	0.05	0.436	0.084	0.204	0.054
MM Coulomb	0	0	0	0	0	0	0	0	0	0
Total Energy Kcal/mol	7123.9	2018.2	3629.5	766.32	185.2	184.03	379.98	71.08	199.41	44.1

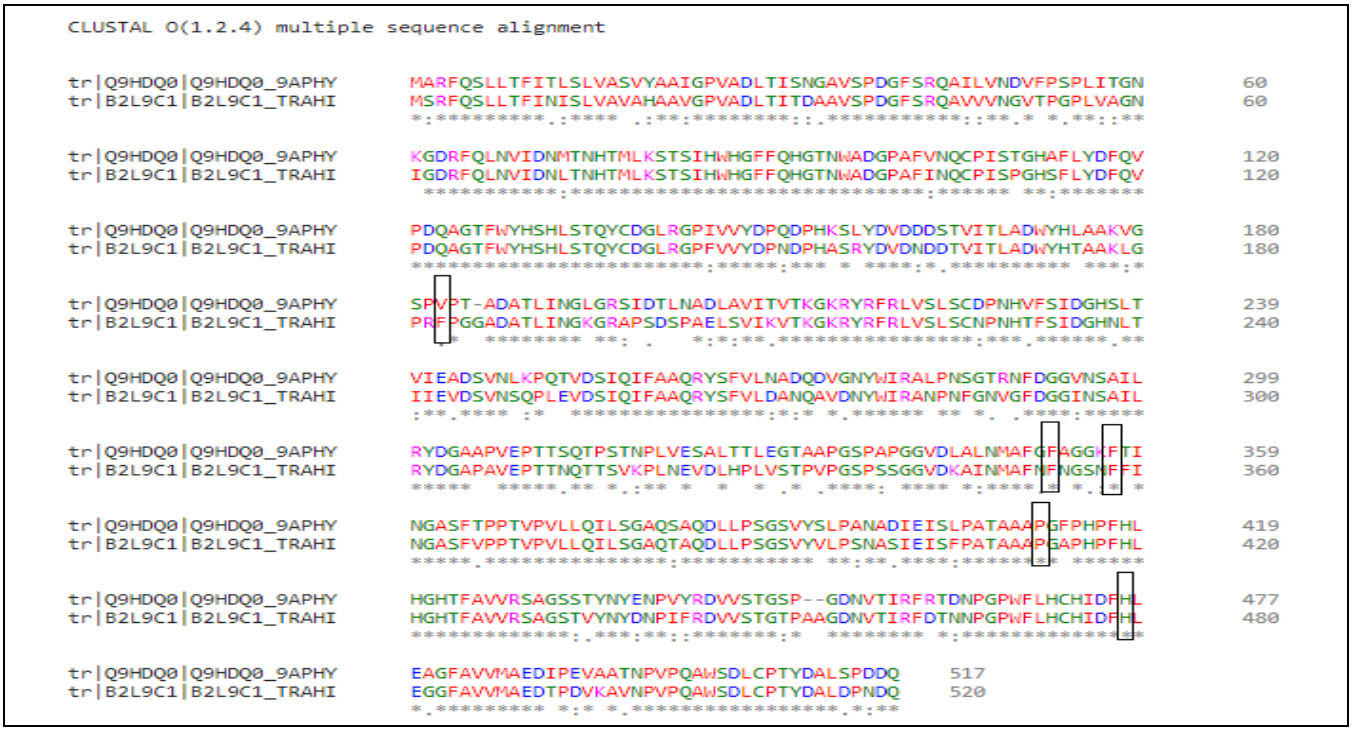


Fig. 1: ClustalO sequence alignment of laccase from Trametes hirsuta (PDB – 3FPX) with template 2HRG from Trametes trogii sp.

3.1. Active site

The active site region for laccase from Trametes hirsuta was obtained by MSA (Multiple Sequence Alignment) result of laccase from Trametes hirsuta (3FPX) and Trametes trogii (2HRG) [16]. The cavity obtained after MSA result were, PHE – 162, PHE – 332, PHE – 337, PRO-391, HIS-458 (Fig 1, & 2a). The dimensions obtained for selected cavity were, Center -X = 11.532, Y - 17.398, Z - 14.721, Size - X = 50, Y = 50, Z = 50 respectively. (Fig 2b, 2c).

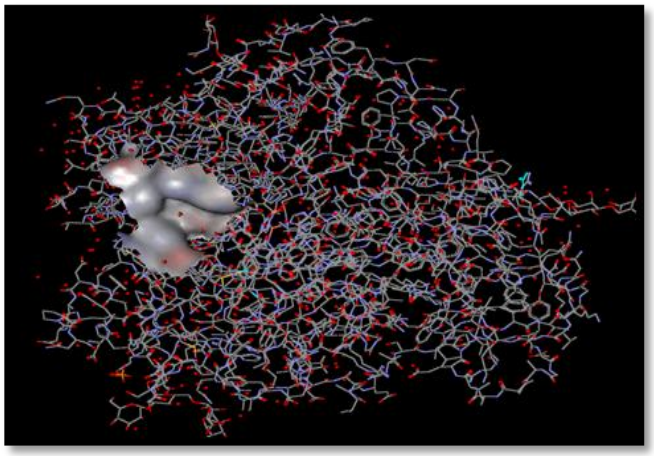


Fig. 2 (a) : Laccase active site

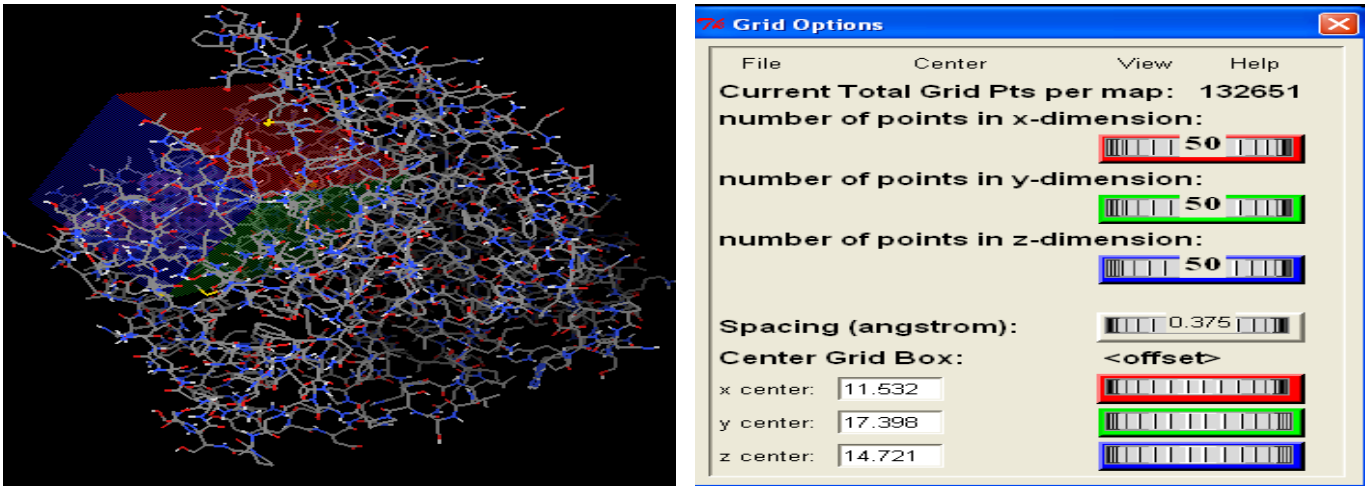


Fig. 2 (b & c): Grid box parameters for protein laccase

Table 2: Molecular interaction of different textile dyes with laccase

Molecule Name	H-Bond	Pi-Interaction	Binding Energy Kcal/mol	% of decolorization with Laccase enzyme [10]
Reactive Blue160	GUN:237, GLU: 242, TYR:244, ASN: 262, ARG:423, SER:427	PHE: 239	-10.7	100 %
Congo Red	PHE:162, GLN :237, ASN:264	PHE: 2	-8.1	80 %
Disperse Blue	SER 113, GLU 460, LEU 355	-	-8.0	-
Malachite Green	LEU:459	PHE: 81	-7.8	68 %
Bromophenol Blue	GLU 460	PHE: 81	-7.7	-

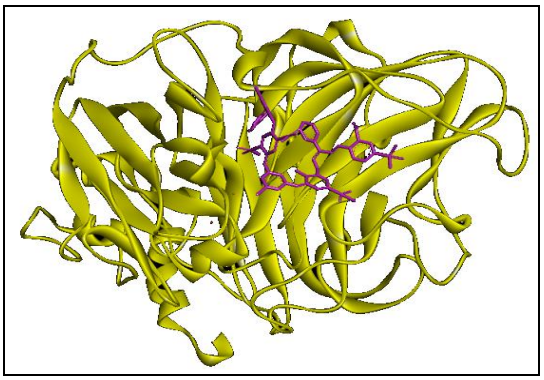
3.2. Molecular docking

The molecular docking experiment exhibited a good agreement of interaction between selected Laccase 3D structure and all the 05 dyes.

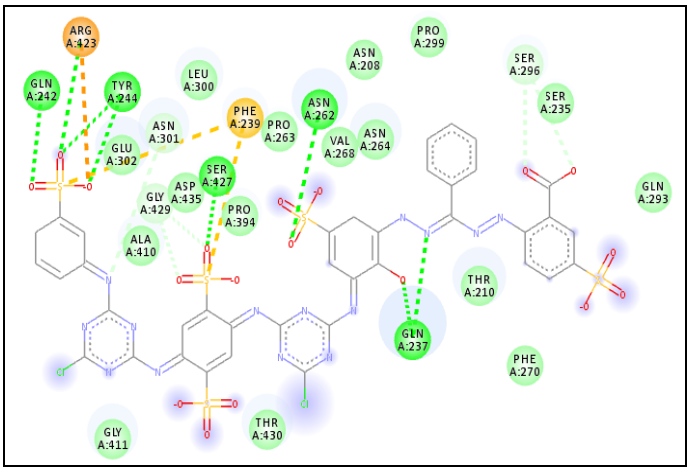
All the 05 dyes have successfully placed themselves within the active site pocket of the Laccase, acquired a minimum energy conformation by exhibiting a good pharmacophoric interactions and making hydrogen-bond and pi-interactions.

3.2.1. Laccase and Reactive blue 160

The binding energy is -10.7 Kcal/mol and lowest among the other dyes complexes. The stability of complex and fastest degradation of Reactive blue 160 is concluded by the 8 hydrogen bonds that are formed between Reactive Blue 160 and Laccase active site residues, Of the 8 hydrogen bonds formed, two H-bond are with GLN 237, other two with TYR 244, and each of one H-bond with GLN 242, ASN 262, ARG 423, SER 427 and 1 pi- bond formed with PHE 239. (Fig 3a, 3b & table 2).

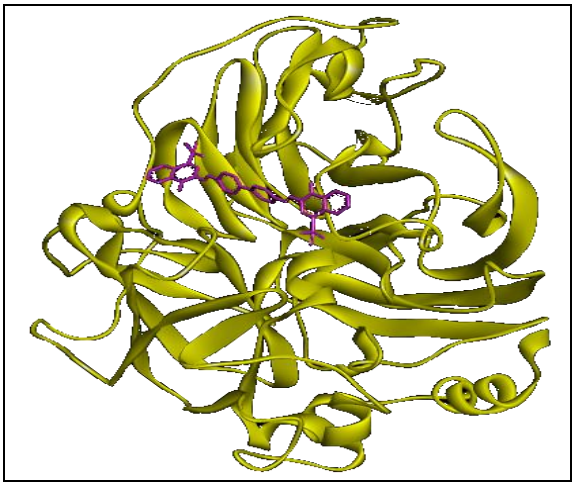


3(a)

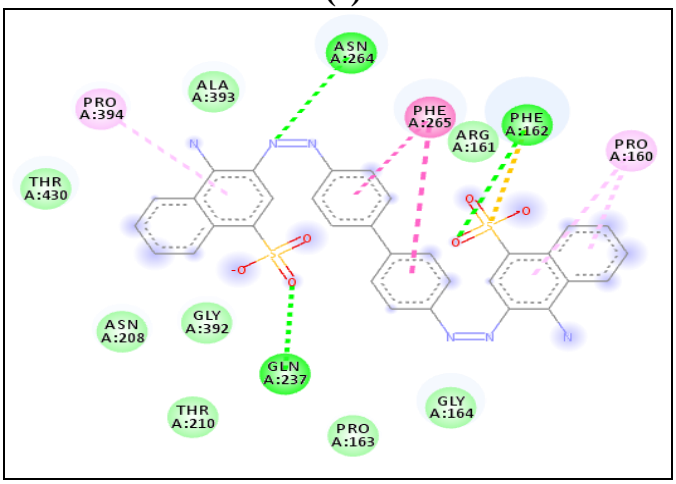


3(b)

Fig. 3: (a) Molecular interaction of protein laccase and dye Reactive blue 160.
(b) Protein laccase and dye Reactive blue 160 residue interactions



4(a)



4(b)

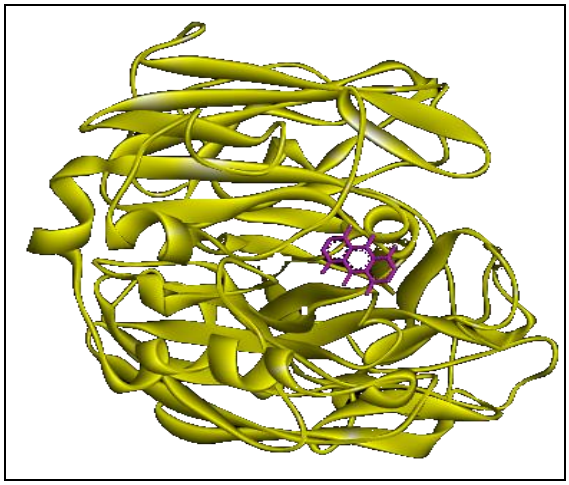
Fig. 4: (a) Molecular interaction of protein laccase and dye Congo red
(b) Protein laccase and dye Congo red residue interactions

3.2.2. Laccase and Congo red

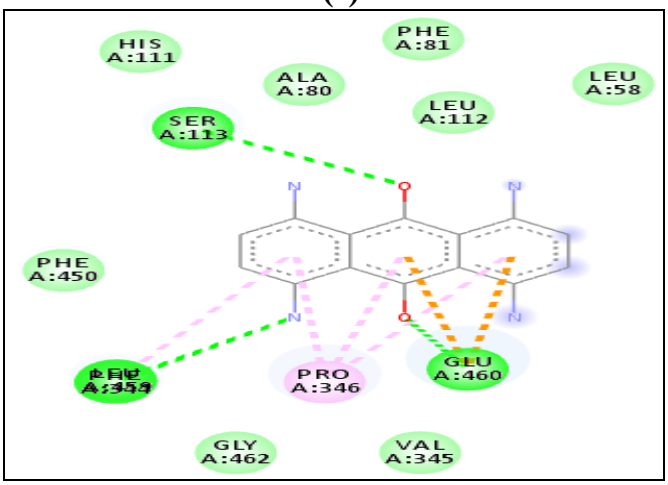
In case of Congo red, the computational study reveals a binding energy of -8.1 kcal/mol with three hydrogen bonds that are formed with PHE 162, GLN 237, AS 264 and one pi- bond between PHE 265. (Fig. 4a, 4b & table 2).

3.2.3. Laccase and Disperse blue1

Disperse Blue 1 exhibited a binding energy of -8.0 Kcal/mol, where 3 hydrogen bonds are formed between disperse blue and SER 113, GLU 460, LEU 355, no pi-interaction were reported in the complex. (Fig 5a, 5b & table 2).



5(a)

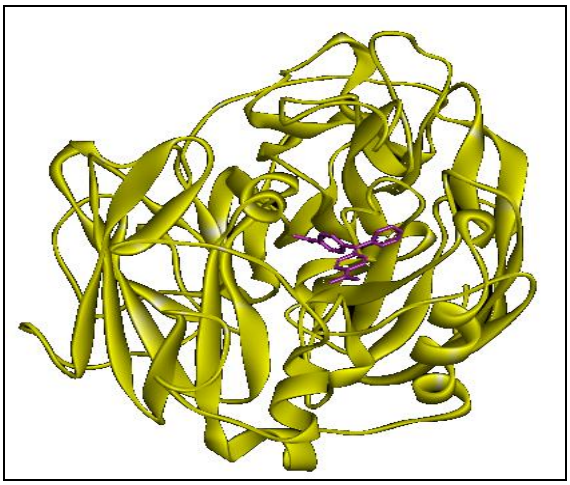


5(b)

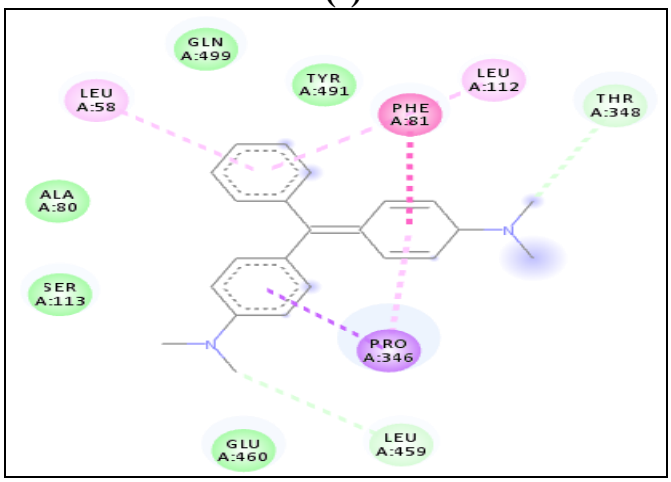
Fig. 5: (a) Molecular interaction of protein laccase and dye Disperse blue1
(b) Protein laccase and dye Disperse blue1 residue interactions

3.2.4. Laccase and Malachite green

Malachite green exhibited a binding energy of -7.8 Kcal/mol with one hydrogen bond with LEU 459 and 1 pi- bond with PHE 81. (Fig 6a, 6b & table 2).



6(a)

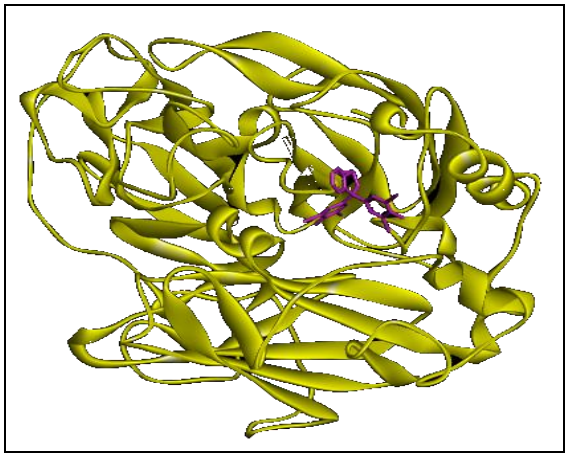


6(b)

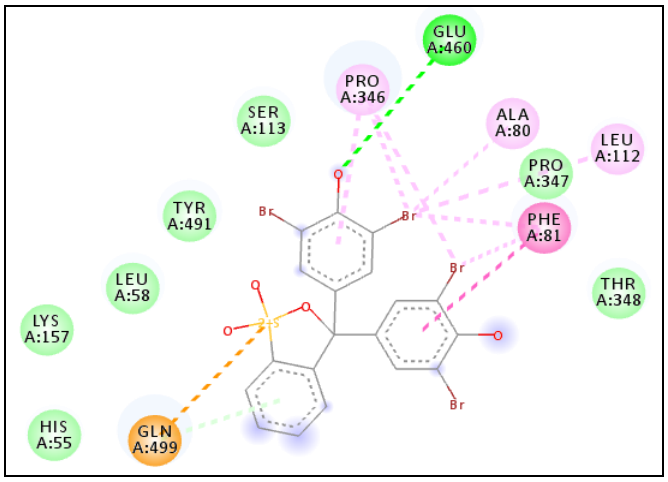
Fig. 6: (a) Molecular interaction of protein laccase and dye Malachite green
(b) Protein laccase and dye Malachite green residue interactions

3.2.5. Laccase and Bromophenol blue

However Bromophenol blue shows a binding energy of -7.7 Kcal/mol, and only one hydrogen bond formed with GLU 460 and 1 pi- bond with PHE 81. (Fig 7a, 7b & table 2).



7(a)



7(b)

Fig. 7: (a) Molecular interaction of protein laccase and dye Bromophenol blue
(b) Protein laccase and dye Bromophenol blue residue interactions

In conclusion it was found that Reactive blue 160 bound laccase with 05 hydrogen bonds and 01 pi-interaction. This stable complex between enzyme and dye may be responsible for efficient degradation. Whereas Congo red and Malachite green with 03 and 01 hydrogen bonds and 01 pi-interaction with Laccase results in less stable complex may lead to delay in degradation. In case of Disperse blue 1 and Bromophenol blue the computational modelling studies has exhibited 03 and 01 hydrogen bonds with no pi-interaction with Disperse blue 1. Further lab experiments for may validate the exact outcome.

4. ACKNOWLEDGMENT

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