Makara Journal of Science

Volume 28
Issue 3 *September*

Article 2

9-27-2024

Preliminary Molecular Study of Chloramphenicol Anchoring on Laccase Enzyme from Trametes hirsuta

Hanzhola Gusman Riyanto
Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Pamulang, Banten 15417, Indonesia, dosen02880@unpam.ac.id

Afiten Rahmin Sanjaya
Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Indonesia, Jawa Barat 16424, Indonesia

Recommended Citation

Riyanto, Hanzhola Gusman and Sanjaya, Afiten Rahmin (2024) "Preliminary Molecular Study of Chloramphenicol Anchoring on Laccase Enzyme from Trametes hirsuta," *Makara Journal of Science*: Vol. 28: Iss. 3, Article 2.
DOI: 10.7454/mss.v28i3.2428
Available at: https://scholarhub.ui.ac.id/science/vol28/iss3/2

Makara Journal of Science, 28/3 (2024), 194-201
doi: 10.7454/mss.v28i3.2428

Preliminary Molecular Study of Chloramphenicol Anchoring on Laccase Enzyme from *Trametes hirsuta*

Hanzhola Gusman Riyanto[1,2*] and Afiten Rahmin Sanjaya[2]

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Pamulang, Banten 15417, Indonesia
2. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Indonesia, Jawa Barat 16424, Indonesia

[*] *E-mail: dosen02880@unpam.ac.id*

Received March 8, 2024 | Accepted July 29, 2024

Abstract

Antibiotics are one of emerging pollutants generally emitted from livestock production and the food industry to the environment. The presence of this pollutant could initiate the development of resistant bacteria that can be fatal to human health. The degradation of antibiotics using enzymes or microbe could be an alternative because the residue or intermediate product is less harmful than of the conventional method. This research aims to support a preliminary study of the degradation of antibiotics using enzyme through molecular docking via Molecular Operating Environment software and molecular dynamics (MD) study via CABSFLEX 2.0 and WebGro macromolecular simulations. The molecular docking of the laccase-chloramphenicol complex has low binding energies of approximately −8.1350 and −8.2290 kcal/mol for both rigid and flexible methods, respectively, indicating that the formation of the complex is advantegous. MD simulation further revealed a decrease in rigidity after the interaction with the ligand. Hydrogen bonding analysis indicated up to five hydrogen bonds in the complex, underscoring the robustness of the enzyme--ligand interaction. These results collectively contribute to our understanding of the efficacy of enzyme-mediated antibiotic degradation and emphasize the potential for this approach to mitigate environmental and health concerns associated with antibiotic pollution.

Keywords: antibiotic degradation, laccase enzyme, molecular dynamics, trametes hirsuta

Introduction

Natural or synthetic chemical are emerging pollutants that are not commonly monitored in the environment but have the potential to enter the environment, cause ecological damage and threaten human health. These pollutants include compounds of pharmaceuticals (drugs, antibiotics, and hormones), pesticides, plastics, dyes, personal care products, and industrial additives[1–3]. Antibiotics are drugs that are usually used to fight bacterial infection. Antibiotics are also usually used in livestock production (e.g., aquatic, veterinary) and the food industry. Because of their wide application, antibiotics are increasingly released into the environment with water, soil, and sediment as major sinks [4–6]. Their presence in the environment became an emerging concern which promoted the development and spread of resistant bacteria that could infect people and could be fatal if some infections cause severe illness that leads to death.

Based on a previous study, the concentration of antibiotics in the Jakarta canal ranged from 17 to 1,489 ng/L [7]. Moreover, in Indonesia, antibiotic usage for animals including livestock, aquaculture, and others, has dramatically increased and is predicted to double in 2030 [8, 9]. Approximately 30-90% of antibiotics used are discharged into the environment, such as soil, water, and sediments. Consequently, the contamination of the environment has drastically increased which raised concerns about the development of antibiotic-resistant pathogenic bacteria. Bacterial resistance renders the treatment process less effective [10]. For example, approximately 70% of *Staphylococcus aureus* isolates became resistant to erythromycin within only 6 months of their initial treatment. Another example is that approximately 81% *Escherichia coli* isolated in water samples from China have genes resistant to tetracycline antibiotics. This situation could be fatal if the pathogenic bacteria carried deadly diseases [11, 12]. One of the solutions to overcome this issue is the use of wastewater treatment plants (WWTP). However, this method is ineffective and leads to other problems, such as the accumulation of antibiotics, which becomes a breeding ground for antibiotic-resistant pathogenic bacteria [11, 13, 14]. Degradation using microbes and enzymes can be an alternative to replace WWTP because it can break down antibiotics more effectively and produce environmentally friendly residue or intermediate products. In addition, this method is more economical than other approaches [15, 16].

Trametes hirsuta D7 is a new isolated fungus and one of the native varieties in Indonesia that produces oxireductase, including the laccase enzyme [17, 18]. Laccase enzymes are multicopper oxidases that catalyze the oxidation of substituted phenols, aromatic thiols and anilines in the presence of molecular oxygen [19,20]. Laccase enzymes have the following advantages the residue or intermediate product of its metabolite is less harmful [21], and it has a wide range of applications, for example, in pharmaceuticals, dyes degradation and biofuel production [22–27]. Laccase enzymes not only be used in several fields but also have good economic viability [28]. However, the potential for the degradation of microplastic and antibiotic is not fully known. In this study, we observe the potential of laccase enzyme for the degradation of antibiotics, such as chloramphenicol using molecular dynamics (MD) simulation which can provide insight into how antibiotics "move" in interacting with enzymes quickly and accurately.

Materials and Methods

Molecular docking simulations. Before conducting MD simulations structured data for both antibiotics and laccase enzyme was collected. Laccase enzymes structure were collected from rscb.org with PDB ID 3FPX [29,30]. All structures and ligands within the laccase enzyme, were removed, leaving only the first sequence and Cu ion. Furthermore, the preparation was conducted using the Molecular Operating Environment (MOE) software. First, protonation was conducted using the LigX method with a strength setting of 100,000 and energy minimization by adjusting the gradient to 0.05 kcal/mol.A^2. The result was saved in .pdb format. Subsequently, the enzyme active site was determined by conducting a site finder analysis and selecting the appropriate site, as referenced, PHE463 [31].

The molecular docking process was conducted using rigid and flexible docking methods. The rigid docking method was implemented using the MMFF94x force field. The ligand molecule (i.e., chloramphenicol) was downloaded from PubChem (http://pubchem.ncbi.nlm.nih.gov) and underwent two nearly identical stages, including washing, partial charge assignment, and energy minimization before conducting molecular docking. Then, the force field was changed to MMFF94X force field, and the structures were saved in .mdb format. Solvation for the protein and ligand structures was set to gas phase. Docking was performed with various retention ratios, namely 10:1, 30:1, and 100:1. The best conformations from the docking test were selected based on the lowest Gibbs free energy of binding ($\Delta G_{binding}$) and simulations with root-mean-square-deviation (RMSD) values < 2 Å were further processed using the flexible docking method.

In the subsequent stage, flexible docking was implemented using the AMBER10:EHT force field. Molecular docking was performed for 100 poses to obtain the Top 5 poses with the lowest Gibbs free energy of binding ($\Delta G_{binding}$) and RMSD values < 2 Å.

Interference test. The interference test was performed by simulating other emerging pollutants including, polyethylene terephthalate (PET), polystyrene, polyvinyl chloride, Nylon 66 and Nylon 6/66 which used the flexible molecular docking method. The structures of PET, polystyrene and polyvinyl chloride were obtained from Chemical Entities of Biological Interest (https://www.ebi.ac.uk/chebi/) whereas the structures of Nylon 66 and Nylon 6/66 were downloaded from PubChem. Furthermore, the docking results were compared with the previously obtained results.

MD simulation using the CABSFLEX 2.0 web server. CABSFLEX 2.0 was applied for MD simulations of the laccase-chloramphenicol complex. CABSFLEX 2.0 is a web server for fast simulation of protein structure flexibility based on a coarse-grained protein modelling method. MD simulation was conducted using the CABSFLEX 2.0 web-server-based program. (http://biocomp.chem.uw.edu.pl/CABSflex2). Laccase enzyme and laccase enzyme-ligand structure files in .pdb format were submitted to the CABSFLEX2 server using the SS2 mode and the protein residue was set as semirigid. This MD simulation was applied at 1.4 K with 50 cycles where the trajectory frames are adjusted to 50 per cycle.

MD simulation using the WebGro web server. MD simulation was also conducted using WebGro Macromolecular Simulation Server (https://simlab.uams.edu/) to compare the results of the previously conducted MD simulation that used CABSFLEX 2.0. The force field applied for MD simulation was GROMOS96 43a1 for laccase enzyme with SPS water model for the ligand in the triclinic system and sodium chloride. Then, the energy of the laccase enzyme-ligand complex was minimized using the steepest descent with 5,000 steps. Furthermore, the equilibration type NVT/NPT was applied at 300 K with a pressure of 1 bar. Subsequently, the MD simulation was conducted using leapfrog integration with a simulation time of 50 ns, only 1,000 frames were captured during the simulations because of limitations [32–35].

Results and Discussion

Molecular docking with the MOE software. Flexible docking, which is conducted using an induced-fit model, ensures adaptability in predicting the binding pose of interaction such as protein-ligand complex. In contrast to rigid docking, this method considers the capability of ligands to induce modifications or changes in the orientation of side chains residues that located at the active binding site of the target [36, 37]. The $\Delta G_{binding}$ and RMSD values of the laccase-ligand complex were determined in this simulation. The results of molecular docking simulation are shown in Table 1. The binding energies of both rigid and flexible docking were determined to be −8.1350 and −8.2290 kcal/mol, respectively, which indicated that the formation of the complex was a favorable energy aspect. The RMSD value of both rigid and flexible docking were determined to be 0.9043 Å and 0.9316 Å, respectively. RMSD value is a parameter that indicates the stability and flexibility of protein/enzyme, as well as the distance between backbone and atoms of protein [38]. The low value of RMSD simply indicated that the formation the laccase-chloramphenicol complex was quite stable [39].

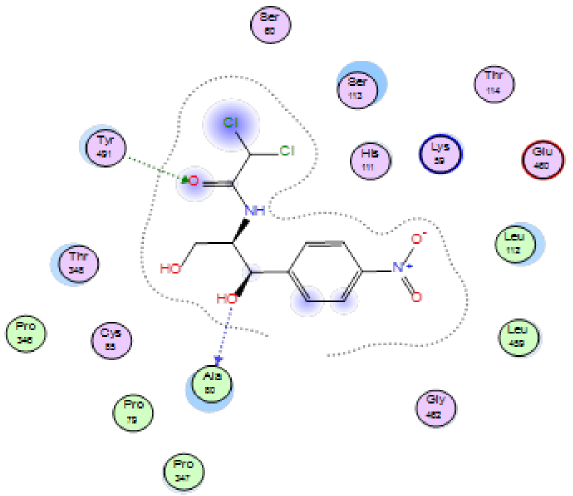
The binding site of the laccase enzyme exhibited a pronounced affinity for interacting with ligands containing phenolic and non-phenolic functional groups including hydroxide and amine. This interaction was facilitated by the presence of multicopper oxidases on the active site of the enzyme [5]. Specifically, the ligand occupied on the side chain of the Tyr491 amino acid through a polar bond and was anchored by the interaction of hydroxide with the Ala80 chain, further contributing to the stability of the laccase-enzyme-ligand complex. (Figure 1a). Although chloramphenicol does not contain the phenol, aromatic thiol and aniline functional groups, the laccase enzyme is included in a group of ligninolytic enzymes that have strong oxidizing properties; thus, it still can oxidize the hydroxy and halides functional group that are contained in chloramphenicol [40]. The proposed biodegradation mechanism of chloramphenicol by the laccase enzyme is shown in Figure 1b.

Interference test. Indonesia is one of the countries with the second largest microplastic pollution after China, with 1.29 million tonnes per year of microplastic waste thrown into the ocean or waters [40,41] . Therefore, we conducted this test to predict the selectivity of the laccase enzyme in the degradation of chloramphenicol when exposed to additional pollutant particles such as commonly used microplastics. The interference compound exhibited a lower energy binding affinity than chloramphenicol, indicating a reduced likelihood of interaction with the enzyme, as shown in Table 2. By contrast, PET and Nylon 66 exhibited similar binding energies to chloramphenicol [5].

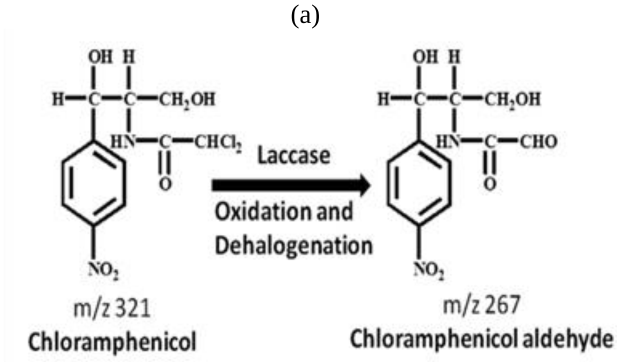
MD using CABSFLEX 2.0. MD simulation is a method to simulate the protein-ligand complex system over a specific time frame, enabling the analysis of stabilisation of the protein-ligand complex and the definition of their conformation changes [41–44]. In this study, we conduct MD simulation using CABSFLEX 2.0 to observe the conformation change of the laccase-chloramphenicol complex. As illustrated in Figure 2, the root‒mean square fluctuation (RMSF) values of both laccase enzyme and laccase-chloramphenicol complex were analyzed. The complex exhibited a more flexible conformation than raw laccase enzyme in the absence of chloramphenicol. Specifically, in certain residue area (around residue 315), the RMSF value increase drastically up to 8.41 Å after laccase is linked to the ligand indicating a notable transition from rigidity to increased flexibility in the residue area after the binding of the ligand.

Table 1. Molecular Docking Results of Both Rigid and Flexible Docking

Parameter	Rigid docking	Flexible docking
$\Delta G_{binding}$	-8.135 kcal/mol	-8.229 kcal/mol
RMSD	0.904 Å	0.931 Å



(a)



(b)

Figure 1. (a) Visualization of the Laccase-Chloramphenicol Interaction, (b) Proposed Biodegradation Mechanism of Chloramphenicol by the Laccase Enzyme [5]

Table 2. Docking Result of the Interference Compound

Compound	$\Delta G_{binding}$ (kcal/mol)	RMSD
Poly ethylene terephthalate (PET) (CHEBI : 53239)	-8.0146	0.898
Polystyrene (CHEBI : 53276)	-5.7965	0.890
Polyvinyl chloride (CHEBI : 53243)	-4.5318	0.891
Nylon 66 (CID 36070)	-7.7281	1.343
Nylon 6/66 (CID 168236)	-5.1164	0.709
Chloramphenicol	**-8.1350**	**0.904**

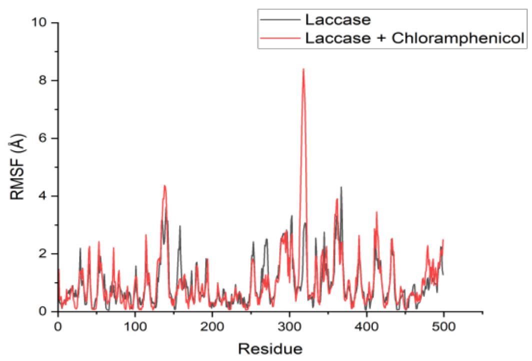


Figure 2. RMSF Values of the Laccase Enzyme and Laccase-Chloramphenicol Complex

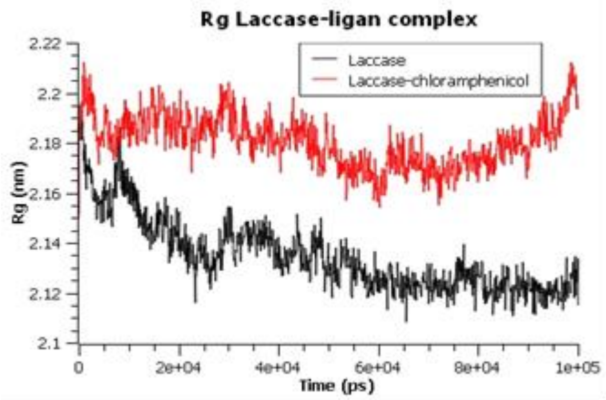


Figure 3. Rg Values of the Laccase Enzyme and Laccase-Chloramphenicol Complex

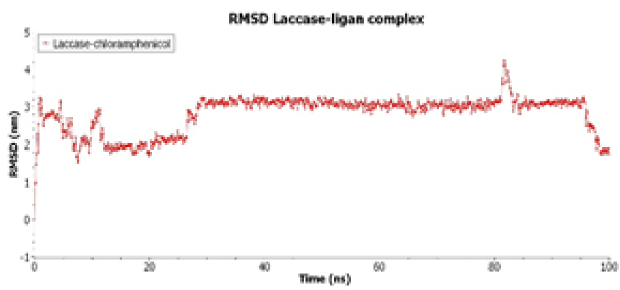


Figure 4. RMSD Value of the Laccase−Chloramphemicol Complex

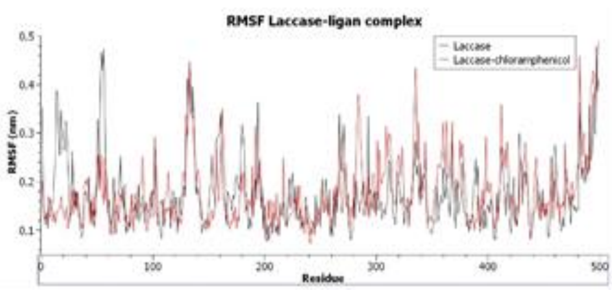


Figure 5. RMSF Values of the Laccase Enzyme and Laccase Chloramphenicol Complex

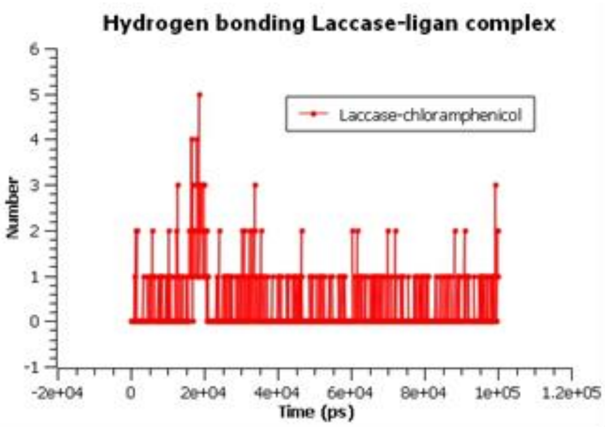


Figure 6. Hydrogen Bonding Value of the Laccase−Chloramphenicol Complex

MD using WEBGRO. In this study, we also used WebGro to conduct MD simulation on the laccase−chloramphenicol complex to confirm the previous results. WebGro is a fully automated online tool for performing MD simulation of macromolecules (i.e., proteins) alone or in complex with ligands (i.e., small molecules). The results of this method are expressed as the radius of gyration (Rg), RMSF, RMSD, and hydrogen bonding (Hb).

The Rg value is a unit of measurement for variations in the compactness of an enzyme-substrate complex and refers to the folding and unfolding of proteins. The folding of the enzyme indicates the stabilization of the Rg value, whereas variations in the Rg value indicate the unfolding of the enzymes [43]. The average Rg value of the laccase enzyme and laccase-chloramphenicol complex are 2.134 ± 0.014 and 2.180 ± 0.010 nm, respectively (Figure 3). The slightly higher value of the laccase−chloramphenicol complex infer that the enzyme−ligand complex is more flexible [42, 45].

Figure 4 shows that the RMSD value of the laccase-chloramphenicol complex was < 3.5 Å. However, at time frame approximately 80 ns, the RMSD value increased up to 4 Å. The high RMSD value indicates that the conformation of laccase-chloramphenicol complex is more flexible than that of the laccase enzyme [31].

RMSF analysis was used to estimate the movement of the location of an atom at a given temperature and pressure. The RMSF results showed the flexible regions of the protein and determined the net fluctuation of the protein during the MD simulation. A lesser RMSF value indicated that the enzyme-substrate complex was more stable, whereas a higher value indicated more flexibility during the MD simulation. As shown in Figure 5, the RMSF value of the laccase-chloramphenicol complex is higher than that laccase enzyme. This finding also confirm the previous result that also increase after the protein links with the ligand [46].

Hydrogen bonding is the most type of intermolecular interaction in biochemical systems, especially on proteins and enzyme. Therefore, This bond can provide as insight into the stability and dynamics of the protein structure with the ligand [47] which are essential for the specificity, metabolism, and catalysis of the substrate. Figure 6 shows that the laccase−chloramphenicol complex has a maximum of five hydrogen bonds. The higher the number of hydrogen bonds is, the stronger will be its interaction of the enzyme−substrate complex [48, 49].

Both MD simulations show similar results for laccase−chloramphenicol complex stabilization. Both CABSFLEX 2.0 and WebGro show that the conformation of the laccase enzyme becomes less rigid after bonding with the ligand.

Conclusion

The molecular study of the laccase−chloramphenicol interaction was conducted using the molecular docking and MD simulation approach. The calculated binding energies of both both rigid and flexible docking were −8.1350 and −8.2290 kcal/mol, respectively. Furthermore, the RMSD value of the molecular docking approach < 2 Å. The MD study of the laccase-chloramphenicol complex was conducted via CABSFLEX 2.0 and WebGro macromolecular simulations. Based on the RMSF value obtained from both MD simulations, the complex was less rigid after interacting with the ligand than before interacting with ligand. This finding was confirmed by the Rg and RMSD value which indicated that the conformation is more flexible after bonding with the ligand. The laccase−chloramphenicol complex has up to five hydrogen bonds which indicates the interaction is strong.

References

[1] Krishnakumar, S., Singh, D.S.H., Godson, P.S., Thanga, S.G. 2022. Emerging pollutants: impact on environment, management, and challenges. Environ. Sci. Pollut. R. 29: 72309–72311, https://doi.org/10.1007/s11356-022-22859-3.

[2] Vasilachi, I.C., Asiminicesei, D.M., Fertu, D.I., Gavrilescu, M. 2021. Occurrence and fate of emerging pollutants in water environment and options for their removal. Water. 13(2): 181, https://doi.org/10.3390/w13020181.

[3] Teodosiu, C., Gilca, A.F., Barjoveanu, G., Fiore, S. 2018. Emerging pollutants removal through advanced drinking water treatment: A review on processes and environmental performances assessment. J. Clean. Prod. 197(1): 1210–1221, https://doi.org/10.1016/j.jclepro.2018.06.247.

[4] Becker, D., Giustina, S.V.D., Rodriguez-Mozaz, S., Schoevaart, R., Barceló, D., de Cazes, M., *et al*. 2016. Removal of antibiotics in wastewater by enzymatic treatment with fungal laccase–Degradation of compounds does not always eliminate toxicity. Bioresource Technol. 219: 500–509, https://doi.org/10.1016/j.biortech.2016.08.004.

[5] Navada, K.K., Kulal, A. 2019. Enzymatic degradation of chloramphenicol by laccase from *Trametes hirsuta* and comparison among mediators. Int. Biodeter. Biodegr. 138: 63–69, https://doi.org/10.1016/j.ibiod.2018.12.012.

[6] Sanjaya, A.R., Atan, D.P., Putra, G.A.M., Widodo, D.S. 2023. Titanium dioxide effect on the decolourisation of organic dyes Remazol Black B using electrocoagulation *in situ*-Fe_2O_3. Int. J. Environ. An. Ch. 1–16, https://doi.org/10.1080/03067319.2023.2206965.

[7] Anh, H.Q., Le, T.P.Q., Le N.D., Lu, X.X., Duong, T.T., Garnier, J., *et al*. 2021. Antibiotics in surface water of East and Southeast Asian countries: A

focused review on contamination status, pollution sources, potential risks, and future perspectives. Sci. Total Environ. 764: 142865, https://doi.org/10.1016/j.scitotenv.2020.142865.

[8] Boeckel, T.P.V., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., *et al*. 2015. Global trends in antimicrobial use in food animals. P. Natl. Acad. Sci. USA. 112(18): 5649–5654, https://doi.org/10.1073/pnas.1503141112.

[9] Tiseo, K., Huber, L., Gilbert, M., Robinson, T.P., Boeckel, T.P.V. 2020. Global trends in antimicrobial use in food animals from 2017 to 2030. Antibiotics. 9(12): 918, https://doi.org/10.3390/antibiotics9120918.

[10] Kraemer, S.A., Ramachandran, A., Perron, G.G. 2019. Antibiotic pollution in the environment: From microbial ecology to public policy. Microorganisms. 7(6): 180, https://doi.org/10.3390/microorganisms7060180.

[11] Chen, Z., Yu, D., He, S., Ye, H., Zhang, L., Wen, Y., *et al*. 2017. Prevalence of antibiotic-resistant *Escherichia coli* in drinking water sources in Hangzhou City. Front. Microbiol. 8: 1133, https://doi.org/10.3389/fmicb.2017.01133.

[12] Rahmawati, I., Fiorani, A., Sanjaya, A.R., Irkham, Du, J., Saepudin, E., *et al*. 2024. Modification of boron-doped diamond electrode with polyaniline and gold particles to enhance the electrochemiluminescence of luminol for the detection of reactive oxygen species (hydrogen peroxide and hypochlorite). Diam. Relat. Mater. 144: 110956, https://doi.org/10.1016/j.diamond.20 24.110956.

[13] Aristi, I., von Schiller, D., Arroita, M., Barceló, D., Ponsatí, L., García-Galán, M.J., *et al*. 2015. Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: Subsidy or stress? Freshwater Biol. 60(7): 1398–1410, https://doi.org/10.1111/fwb.12576.

[14] Gothwal, R., Shashidhar, T. 2015. Antibiotic pollution in the environment: A review. Clean-Soil Air Water. 43(4): 479–489, https://doi.org/10.1002/clen.201300989.

[15] Routoula, E., Patwardhan, S.V. 2020. Degradation of anthraquinone dyes from effluents: A review focusing on enzymatic dye degradation with industrial potential. Environ. Sci. Technol. 54(2): 647–664, https://doi.org/10.1021/acs.est.9b03737.

[16] Fatah, F.R.A.A., Sanjaya, A.R., Rahmawati, I., Syauqi, M.I., Gunlazuardi, J., Ivandini, T.A. 2024. Cathodic luminol electrochemiluminescence on TiO_2 nanotube array. Mater. Chem. Phys. 319: 129288, https://doi.org/10.1016/j.matchemphys.2024.129288.

[17] Hidayat, A., Yanto, D.H.Y. 2018. Biodegradation and metabolic pathway of phenanthrene by a new tropical fungus, *Trametes hirsuta* D7. J. Environ. Chem. Eng. 6(2): 2454–2460, https://doi.org/10.1016/j.jece.2018.03.051.

[18] Andriani, A., Maharani, A., Yanto, D.H.Y., Pratiwi, H., Astuti, D., Nuryana, I., *et al*. 2020. Sequential production of ligninolytic, xylanolytic, and cellulolytic enzymes by *Trametes hirsuta* AA-017 under different biomass of Indonesian sorghum accessions-induced cultures. Bioresource Technol. Rep. 12: 100562, https://doi.org/10.1016/j.biteb.2020.100562.

[19] Yang, J., Lin, Y., Yang, X., Ng, T.B., Ye, X., Lin, J. 2017. Degradation of tetracycline by immobilized laccase and the proposed transformation pathway. J. Hazard. Mater. 322(Part B): 525–531, https://doi.org/10.1016/j.jhazmat.2016.10.019.

[20] Arregui, L., Ayala, M., Gómez-Gil, X., Gutiérrez-Soto, G., Hernández-Luna, C.E., de los Santos, M.H., *et al*. 2019. Laccases: Structure, function, and potential application in water bioremediation. Microb. Cell Fact. 18: 200, https://doi.org/10.1186/s12934-019-1248-0.

[21] Yanto, D.H.Y., Krishanti, N.P.R.A., Ardiati, F.C., Anita, S.H., Nugraha, I.K., Sari, F.P., *et al*. 2019. Biodegradation of styrofoam waste by ligninolytic fungi and bacteria. IOP Conf. Ser. Earth Environ. Sci. 308: 012001, https://doi.org/10.1088/1755-1315/308/1/012001.

[22] Yanto, D.H.Y., Guntoro, M.A., Nurhayat, O.D., Anita, S.H., Oktaviani, M., Ramadhan, K.P., *et al*. 2021. Biodegradation and biodetoxification of batik dye wastewater by laccase from *Trametes hirsuta* EDN 082 immobilised on light expanded clay aggregate. 3 Biotech. 11: 247, https://doi.org/10.1007/s13205-021-02806-8.

[23] Asif, M.B., Hai, F.I., Singh, L., Price, W.E., Nghiem, L.D. 2017. Degradation of pharmaceuticals and personal care products by white-rot fungi—a critical review. Current Pol Rep. 3: 88–103, https://doi.org/10.1007/s40726-017-0049-5.

[24] Malhotra, M., Suman, S.K. 2021. Laccase-mediated delignification and detoxification of lignocellulosic biomass: removing obstacles in energy generation. Environ. Sci. Pollut. Res. 28: 58929–58944, https://doi.org/10.1007/s11356-021-13283-0.

[25] Tavares, M.F., Avelino, K.V., Araújo, N.L., Marim, R.A., Linde, G.A., Colauto, N.B., *et al*. Decolorization of azo and anthraquinone dyes by crude laccase produced by *Lentinus crinitus* in solid state cultivation. Braz. J. Microbiol. 51: 99–106, https://doi.org/10.1007/s42770-019-00189-w.

[26] Vaishnavi, J., Arulprakash, A., Selvi, A., Rajasekar, A. 2020. Marine Biomass Toward Biofuel Production. In Kumar, R.P., Gnansounou, E., Raman, J.K., Baskar, G. (eds.), Refining Biomass Residues for Sustainable Energy and Bioproducts. Elsevier Inc. USA. pp. 451–462.

[27] Piontek, K., Antorini, M., Choinowski, T. 2002. Crystal structure of a laccase from the fungus *Trametes versicolor* at 1.90-Å resolution containing a full complement of coppers. J. Biol. Chem.

277(40): 37663–37669, https://doi.org/10.1074/jbc.M204571200.
[28] Nasution, M.A.F., Firmanti, M.I., Riyanto, H.G., Sanjaya, A.R., Saepudin, E., Ivandini, T.A. 2023. Electrochemical and computational studies of citrate-modified β-cyclodextrin@Fe_3O_4 Nanocomposite as a Nonenzymatic Sensor for Cholesterol. Sensor. Mater. 35(12): 4215–4234, https://doi.org/10.18494/SAM4698.
[29] Polyakov, K.M., Fedorova, T.V., Stepanova, E.V., Cherkashin, E.A., Kurzeev, S.A., Strokopytov, B.V., *et al*. 2009. Structure of native laccase from *Trametes hirsuta* at 1.8 Å resolution. Acta Cryst. D65: 611–617, https://doi.org/10.1107/S0907444909011950.
[30] Nasution, M.A.F., Riyanto, H.G., Saepudin, E., Ivandini, T.A. 2020. Molecular investigation of modified β-cyclodextrin and cholesterol inclusion complexes through molecular docking simulations. IOP Conf. Ser. Mater. Sci. Eng. 902: 012017, https://doi.org/10.1088/1757-899X/902/1/012017.
[31] Abraham, M.J., Murtola, T., Schulz, R., Páll, S., Smith, J.C., Hess, B., *et al*. 2015. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 1–2: 19–25, https://doi.org/10.1016/j.softx.2015.06.001.
[32] Lindahl, E., Bjelkmar, P., Larsson, P., Cuendet, M.A., Hess, B. 2010. Implementation of the CHARMM force field in GROMACS: Analysis of protein stability effects from correction maps, virtual interaction sites, and water models. J. Chem. Theory Comput. 6(2): 459–466, https://doi.org/10.1021/ct900549r.
[33] Oostenbrink, C., Villa, A., Mark, A.E., Gunsteren, W.F.V. 2004. A biomolecular force field based on the free enthalpy of hydration and solvation: The GROMOS force-field parameter sets 53A5 and 53A6. J. Comput. Chem. 25(13): 1656–1676, https://doi.org/10.1002/jcc.20090.
[34] Lindorff-Larsen, K., Piana, S., Palmo, K., Maragakis, P., Klepeis, J.L., Dror, R.O., *et al*. 2010. Improved side-chain torsion potentials for the Amber ff99SB protein force field. Proteins Structure Function Bioinformat. 78(8): 1950–1958, https://doi.org/10.1002/prot.22711.
[35] Huang, Z., Wong, C.F. 2009. Docking flexible peptide to flexible protein by molecular dynamics using two implicit-solvent models: An evaluation in protein kinase and phosphatase systems. J. Phys. Chem. B. 113(43): 14343–14354, https://doi.org/10.1021/jp907375b.
[36] Lexa, K.W., Carlson, H.A. 2012. Protein flexibility in docking and surface mapping. Q. Rev. Biophys. 45(3): 301–343, https://doi.org/10.1017/S0033583512000066.
[37] Terefe, E.M., Ghosh, A. 2022. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of phytochemicals isolated from *Croton dichogamus* against the HIV-1 reverse transcriptase. Bioinformatics Biol. Insigh. 16: 1–20, https://doi.org/10.1177/1177932222112 5605.
[38] Castro-Alvarez, A., Costa, A.M., Vilarrasa, J. 2017. The performance of several docking programs at reproducing protein-macrolide-like crystal structures. Molecules. 22(1): 136, https://doi.org/10.3390/molecules22010136.
[39] Yosberto, C.-M., Espinosa, L.A., Vieyto, J.C., González-Durruthy, M., del Monte-Martinez, A., Guerra-Rivera, G., *et al*. 2019. Theoretical study on binding interactions of laccase-enzyme from Ganoderma weberianum with multiples ligand substrates with environmental impact. Ann. Proteom. Bioinform. 3: 001–009, https://doi.org/10.29328/journal.apb.1001007.
[40] Cordova, M.R., Iskandar, M.R., Surinati, D., Kaisupy, M.T., Wibowo, S.P.A., Subandi, R., *et al*. 2024. Microplastic occurrence in sub-surface waters of the Indonesian archipelago. Front. Mar. Sci. 11: 1362414, https://doi.org/10.3389/fmars.2024.1362414.
[41] Lebreton, L., Andrady, A. 2019. Future scenarios of global plastic waste generation and disposal. Palgrave Communic. 5: 6, https://doi.org/10.1057/s41599-018-0212-7.
[42] Vishvakarma, V.K., Singh, M.B., Jain, P., Kumari, K., Singh, P. 2022. Hunting the main protease of SARS-CoV-2 by plitidepsin: Molecular docking and temperature-dependent molecular dynamics simulations. Amino Acids. 54: 205–213, https://doi.o rg/10.1007/s00726-021-03098-1.
[43] Verma, C., Mishra, G., Omkar. 2021. Bioinformatics. In Omkar. (eds.), Molecular Approaches for Sustainable Insect Pest Management. Springer. Singapore. pp. 343–376.
[44] Dror, R.O., Jensen, M., Borhani, D.W., Shaw, D.E. 2010. Exploring atomic resolution physiology on a femtosecond to millisecond timescale using molecular dynamics simulations. J. Gen. Physiol. 135(6): 555–562, https://doi.org/10.1085/jgp.200910373.
[45] Rai, S.K., Pathak, R.K., Singh, D.B., Bhatt, A., Baunthiyal, M. 2021. Chemo-informatics guided study of natural inhibitors targeting rho GTPase: A lead for treatment of glaucoma. In Silico Pharmacol. 9: 4, https://doi.org/10.1007/s40203-020-00061-y.
[46] Kumar, D., Kumari, K., Jayaraj, A., Kumar, V., Kumar, R.V., Dass, S.K., *et al*. 2021. Understanding the binding affinity of noscapines with protease of SARS-CoV-2 for COVID-19 using MD simulations at different temperatures. J. Biomol. Struct. Dyn. 39(7): 2659–2672, https://doi.org/10.1080/07391102.2020.1752310.
[47] Surabhi, S., Singh, B. 2018. Computer aided drug design: An overview. J. Drug Deliv. Ther. 8(5): 504–509, https://doi.org/10.22270/jddt.v8i5.1894.

[48] Yang, L.H., Qiao, B., Xu, Q.M., Liu, S., Yuan, Y., Cheng, J.S. 2021. Biodegradation of sulfonamide antibiotics through the heterologous expression of laccases from bacteria and investigation of their potential degradation pathways. J. Hazard. Mater. 416: 125815, https://doi.org/10.1016/j.jhazmat.2021.125815.

[49] Bhatt, K., Maheshwari, D.K. 2020. Zinc solubilizing bacteria (*Bacillus megaterium*) with multifarious plant growth promoting activities alleviates growth in *Capsicum annuum* L. 3 Biotech. 10: 36, https://doi.org/10.1007/s13205-019-2033-9.