Annals of Science and Technology - A, Vol 10 (1): 24-37, 2025
Copyright: An Official *Journal* of the Nigerian Young Academy
ISSN: 2 544 6320

ARTICLE

Preliminary Assessment of Microbial Enzyme Potential for Plastic Degradation: An *In-Silico* to *In-Vitro* Investigation

Bilikis T. Folarin[1*], Blessing A. Oyedeji[2], Rosemary A. Adedokun[1], Odunayo A. Taiwo[3], Ruth N. Oloye[4], Oluwatoyin A. Babalola[5]

[1] *Department of Chemistry, Chrisland University, Ogun State, Nigeria.*
[2] *Department of Microbiology, Chrisland University, Ogun State, Nigeria*
[3] *Department of Biochemistry, Chrisland University, Ogun State, Nigeria*
[4] *Department of Microbiology, Federal University of Agriculture Abeokuta, Nigeria*
[5] *Department of Molecular Biology & Biotechnology, Chrisland University, Ogun State, Nigeria*
Received 17th February, 2025, Accepted 27th July, 2025
DOI: 10.2478/ast-2025-0003

[*]*Corresponding Author: bfolarin@chrislanduniversity.edu.ng:Tel - +2348130976147*

Abstract
Plastic pollution remains a global issue due to the increasing production of plastics, which has more than doubled in the last two decades, raising both environmental and health concerns. This preliminary study explored the potential of microbial enzymes in plastic degradation using both in-silico and in-vitro approaches. Molecular docking was employed to evaluate the binding affinities of three enzymes: esterase hydrolase (PDB ID: 3KVN), dehydrogenase oxidoreductase (PDB ID: 4JZ6), and laccase oxidoreductase (PDB ID: 5LM8) with 23 plastic compounds from four major plastic types: polyester, polyolefins, polystyrene, and polyvinyl chloride. Fourteen of the plastic compounds exhibited strong binding affinities (≤ −5.0 kcal/mol) with the three enzymes. Notably, 2,6-naphthalene dicarboxylic acid (PubChem ID: 14357), a polyester precursor, showed the highest binding affinity with all the enzymes (−7.1, −7.9, and −7.2 kcal/mol, respectively). Microbial isolates (bacteria and fungi) from a local multi-waste dumpsite were screened for their ability to produce laccase and lipase (enzymes relevant to plastic degradation) employing both qualitative and quantitative assays. Positive enzyme activity was indicated by distinct reddish-brown and yellow colorations. Isolates with the highest enzyme activity were identified through molecular characterization as *Bacillus pumilus*, *Staphylococcus cohnii*, and *Penicillium citrinum*. These initial findings suggest that soil microorganisms may harbor enzymes with potential plastic-degrading capabilities. However, further in-vitro studies are required to validate the actual degradation of plastic compounds by these enzymes. This work lays the foundation for future development of enzyme-based biotechnological solutions for plastic waste management.

Keywords: Biodegradation; Microbial enzymes; Molecular docking; Plastic compounds; Plastic pollution

1.0 Introduction

Synthetic plastics have become an essential part of modern society due to their versatility, particularly in packaging applications (Nikolaivits et al., 2020). Unlike conventional packaging materials, synthetic plastics have unique properties such as mechanical strength, affordability, durability, and superior barrier qualities (AlMaadeed et al., 2020; Desidery & Lanotte, 2022). An estimated 350-400 million metric tons of plastics are produced globally each year (Danso et al., 2019), with Polyethylene Terephthalate (PET), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP), polyamide (PA) and pjaolyurethane (PUR) among the most commercially significant types.

Despite their usefulness, plastics are notoriously resistant to degradation and can persist in the environment for several decades or centuries. This contributes to severe waste disposal challenges and associated health risks (Duddu et al., 2015). Equally worrisome is that treatment processes such as photolysis or natural weathering can degrade these plastics to potentially more hazardous secondary pollutants such as micro and nano- plastics which enter the food chain (Hantani et al., 2018). Plastics may also contain toxic additives such as plasticizers, flame retardants, xylenes, zinc acetate (Folarin et al., 2024; Lahimer et al., 2017) as well as solvents, initiators and catalysts employed during preparation (Mendiburu-Valor et al., 2021). These pollutants and their by-products are released during conventional waste management practices such as informal recycling, incineration, and landfilling, thus making these methods ineffective and potentially harmful (Rajmohan et al., 2019). Developing countries are the most affected, accounting for about 92% of reported deaths linked to plastic pollution (Bartrem et al., 2014; Landrigan et al., 2018). It is thus imperative to devise novel technologies and eco-friendly remediation strategies (Almeida et al., 2019; Ru et al., 2020).

Remediation techniques may be chemical, thermal, biological or even excavation and dredging of polluted sites, possibly achieved in situ or ex situ. However, biological remediation remains the most sustainable technique employed for degradation and detoxification of pollutants in plastic waste management (Kumar et al., 2018). A wide range of chemical pollutants like pesticides, nitroaromatic compounds, metals, polyaromatic hydrocarbons and halogenated petroleum hydrocarbons have been successfully biodegraded (Chen et al., 2015). Microorganisms used for bioremediation are eco-friendly and are promising agents in reducing environmental threats. The use of microorganisms in bioremediation is an attractive alternative because of its lower cost and high efficiency when compared with other physicochemical methodologies (Abatenh et al., 2017). Microbial enzymes are superior biological substances derived from various microorganisms (bacteria, mold and yeast), particularly for application in industries on commercial scales. Oxidoreductase and hydrolase are two major classes of enzymes used in plastic degradation (Bhandari et al., 2021). Hydrolase includes esterases, proteases, and cutinases while oxidoreductase includes dehydrogenases, laccases, and peroxidases. The degradation of plastics by hydrolases, specifically esterases, proceeds by ester bond cleavage and are active mainly on aliphatic polyesters while oxidoreductases catalyse bond cleavage in plastics via oxidation-reduction reactions (Chamas et al., 2020).

In silico methods have emerged as efficient, cost-effective tools for screening microorganisms capable of plastic degradation. Studies on plastic degradation using *in silico* techniques are limited. A study by Almeida and co-researchers is one of the few available reports. They reported an *in silico – in vitro* approach for screening bacterial PET hydrolase enzymes from *Streptomyces* for PET degradation (Almeida et al., 2019). Another study employed an *in vitro to in silico* approach to determine the biodegradation potentials of newly isolated *Pseudomonas* species from municipal landfill soils (Bhatia et al., 2014). These microorganisms were used for low density polyethylene biodegradation (LDPE). A pure *in silico* study assessed the binding affinity of some plastic compounds on *Ideonella sakaiensis,* a hydrolase enzyme (Duru et al., 2021).

In this preliminary study, we aimed to assess the degradation potential of 3 enzymes: esterase (PDB ID: 3KVN), laccase (PBD ID: 5LM8), and dehydrogenase (PBD ID: 4JZ6) on 23 plastic compounds of 4 plastic types (polyester, polyolefins, polystyrene and polyvinyl chloride) employing *in silico* methods. We also examined the capacity of two types of microorganisms isolated from a dumpsite, to produce two of these enzymes, specifically lipase (an esterase) and laccase in-vitro. This two-tiered approach offers preliminary insights into the feasibility of microbial enzyme application for plastic degradation and lays the groundwork for more robust experimental validation and optimization in future studies.

2.0 Experimental

2.1 Molecular docking

The crystal three-dimensional (3D) structures of three microbial enzymes: Esterase from *Pseudomonas aeruginosa* (3KVN), Salicylaldehyde dehydrogenase NahF, from *Pseudomonas putida* (4JZ6) and -laccase-like oxidoreductase from *Aspergillus niger* (5LM8) were selected as the protein target for molecular docking. These structures were retrieved from the Protein Data Bank (PDB) with resolutions of 2.50 Å, 2.42 Å and 1.70 Å respectively. Selected enzymes were screened based on absence of mutation, possessing resolution not lower than 1.60 Å, and belonging to the hydrolase or oxidoreductase family. The hydrolase and oxidoreductase enzymes have the potential to degrade plastics through reactions like oxidation, reduction, and ester bond cleavage. Enzyme preparation was performed using UCSF Chimera (Pettersen et al., 2004), during which non-amino acid residues and water molecules were removed, and polar hydrogens were added to the protein structures. This was necessary to enhance the intermolecular interactions between ligands and protein residues.

A total of twenty-three (23) chemical constituents of different plastic types were used as ligands for docking in this study. The 3D structures of these compounds were retrieved from the PubChem database and energy-minimized using the PyRx software package (Dallakyan & Olson, 2015). Molecular docking was done using AutoDock Vina (Trott & Olson, 2009) in order to understand the mode of binding interaction between target plastic compounds and enzymes (Rakesh et al., 2019). As a predictive tool, it allows for the calculation of the binding energies of compounds when they interact with enzymes. A lower binding energy indicates a better ability of the compound to bind within the active site of the enzyme or protein target. To ensure accurate docking, grid boxes were set to cover the active site of each enzyme. The grid box centers and dimensions were set as follows:

- Esterase (3KVN):
  Center: x = -4.45, y = 40.89, z = 48.34
  Size: x = 57, y = 51, z = 93
- Salicylaldehyde dehydrogenase (4JZ6):
  Center: x = 63.57, y = -8.75, z = 14.34
  Size: x = 53, y = 59, z = 50
- Laccase-like oxidoreductase (5LM8):
  Center: x = -23.57, y = 0.71, z = -26.47
  Size: x = 47, y = 56, z = 44

These grid parameters allowed for accurate targeting of the active sites, enabling reliable estimation of binding energies and molecular interactions between the enzymes and the plastic compounds.

2.2 Isolation and characterisation of microorganisms

Soil was collected from a compost pile at a depth of 6 inches from a multi-waste dumpsite in Abeokuta, Ogun State, Nigeria. For bacteria and mold isolation, the sample was serially diluted and plated at 10^{-3} and 10^{-5} dilutions on Nutrient Agar (NA) and Potato Dextrose Agar (PDA), respectively. The NA plates were incubated at 37 °C for twenty-four hours while the PDA plates were incubated at 25 °C for five days (Thabet et al., 2023). After incubation, bacterial colonies were identified based on their morphological and biochemical properties, while molds were identified based on their morphological appearance on PDA plates and micro-morphological features using x10 and x40 objective lenses (Akinyemi et al., 2022). Pure cultures obtained by subculturing on fresh NA and PDA using the same incubation conditions were preserved on agar slants for further identification and characterisation.

2.2.1 Qualitative screening of mold and bacteria for laccase and lipase production

Qualitative screening was carried out to assess the ability of bacterial and mold isolates to produce laccase and lipase enzymes. For laccase screening, pure cultures were inculated on NA and PDA plates containing 0.04% Guaiacol as an indicator. The plates were incubated at 37 ℃ for 5 days (bacteria) and 25 ℃ for 6 days (mold) as described by Salami et al. (2022). The development of a reddish-brown halo in the Guaiacol supplemented plates indicated a positive laccase secretion.

Lipase production was assessed using phenol red-based NA and PDA media. The media were prepared by dissolving PDA (39.5 g) or NA (14 g), peptone (6.67 g), phenol red dye (0.5 g) and substrate olive oil (5 mL) in 500 mL of distilled water. The mixtures were heated, agitated, and left to cool before adjusting the pH to 7.0 (PDA) and 6.7 (NA). Plates were autoclaved, inoculated with mold or bacterial cultures, and incubated at 25 ℃ for 3 days (molds) and 37 ℃ for 2 days (bacteria) (Ilesanmi et al., 2020; Tsui et al., 2019). A colour change from red to yellow indicated the release of fatty acids as a result of lipolysis.

2.2.2 Quantitative screening of mold and bacteria for laccase and lipase production

Isolates that tested positive for laccase and lipase production in qualitative screening were subjected to quantitative screening adapting the methods of Ilesanmi et al. (2020) and Tsui et al.

(2019). For laccase, 500 mL Erlenmeyer flask was filled with a nutrient medium containing 3.0 g peptone, 0.6 g KH_2PO_4, 0.4 g K_2HPO_4, 0.001 g $ZnSO_4$, 0.05 g $MnSO_4$, 0.0005 g $FeSO_4$, 0.5 g $MgSO_4$, 0.01 g $CuSO_4$, and 2 g rice bran was added as substrate for molds. The pH was adjusted to 6.7 for bacterial cultures and 7.0 for molds. Flasks were autoclaved and inoculated after cooling and incubated in triplicates at 25 ℃ under static conditions for 15 days (molds), and at 37 °C on a gyratory shaker (125 rpm) for 6 days (bacteria).

For lipase, the isolates were inoculated on a medium containing 10 g peptone, 5 g yeast extract, 5 g NaCl, 2 g $CaCl_2$, 0.3 g K_2HPO_4, 1 g $KHPO_4$, 2 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4$. $7H_2O$, and 2% (v/v) olive oil, while rice bran 2 g was added as a substrate for mold. The media were incubated at 37º C for 3 days at pH 6.7 on a gyratory shaker (125 rpm) (bacteria), and at 25 ℃ for 7 days at pH 7 under static conditions (molds). After incubation, mold cultures were harvested by adding 50 mL of sterillized distilled water and shaking for one hour. The content was then filtered using 125 mm Whatman filter paper. Both bacterial and mold cultures were centrifuged at 10,000 rpm for 10 minutes at 4 °C using a high-speed tabletop centrifuge (HI850R, Clifton, Nikel, England). The supernatants were collected and labeled as crude enzyme extracts (Senthivelan et al., 2019).

2.2.3 Laccase enzyme assay

Laccase activity was determined by mixing 1 mL of 0.02% guaiacol, 3 mL of 10 mM sodium acetate buffer and 1 mL of the crude enzyme extract in test tubes. A blank solution consisted of 1 mL each of substrate, buffer distilled water. Reaction mixtures were incunbated at 30 °C for 15 minutes in a water bath, and absorbance was measured at 470 nm using a UV spectrophotometer (Biobase 752N). One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1µmol of guaiacol per minute (Solanki et al., 2022). Laccase enzyme activity was calculated using the equation below.

$$E.A_{laccase} = \frac{A \times V}{t \times e \times v}$$

where $E.A_{laccase}$ is the laccase enzyme activity, A is absorbance, V is the total reaction mixture volume (mL), t is incubation time, e is extinction coefficient for guaiacol (0.6740 µM/ cm), and v is enzyme volume (mL).

2.2.4 Lipase enzyme assay

Lipase activity was measured using the titrimetric technique described by Edupuganti et al. (2017). Crude enzyme extract (2 mL) was added to 18 mL of a 100 mM phosphate buffer (pH 7.0) emulsion containing 5% acacia Arabic gum and 10% olive oil, The mixture was incubated at 37 °C for 15 minutes on a thermostatic orbital shaker. To terminate the reaction and extract fatty acids, 1 mL of a 1:1 (v/v) acetone and ethanol mixture were added. Using phenolphthalein indicator, the fatty acids released were titrated with 0.05M NaOH. One unit of lipase activity was defined as the amount of enzyme required to liberate 1 µmol of fatty acid per minute under the specified standard conditions. Experimentation was carried out in triplicates and lipase activity was calculated employing the equation below.

$$E.A_{lipase} = \frac{\Delta V \times C \times 1000}{v \times t}$$

Where $E.A_{lipase}$ is the lipase enzyme activity ΔV represents NaOH consumption expressed in ml/min, C is the concentration of NaOH in mM, 1000 is a coefficient that converts the concentration into g/mL, v is the volume (ml) of enzyme used, and t is the incubation time.

2.3 Molecular identification of isolates

The identities of the two bacterial isolates and one mold isolate with the best laccase and lipase activity were confirmed using molecular techniques. Genomic DNA was extracted using. the Quick-DNA™ Fungal/Bacteria Miniprep Kit (Zymo Research, Cat. No. D6005). Evaluation of the DNA concentration and purity was done with the use of Nanodrop Spectrophotometer (ND-1000, Themo Scientific, USA). PCR amplification was carried out using the following primers: 16S-27F (5'-AGAGTTTGATCMTGGCTCAG) and 16S-1492R (CGGTTACCTTGTTACGACTT) for bacteria, and ITS-1 (5'-TCCGTAGGTGAACCTGCGG) and ITS-4 (5' TCCTCCGCTTATTGATATGC) for mold. Amplification was done using OneTaq® Quick-Load® 2X Master Mix (NEB, Cat. No. M0486). PCR products were subjected to gel electrophoresis and purified using the EXOSAP method. Sequencing and alignment were followed by BLAST analysis (NCBI) for species identification (Tamura et al., 2011).

2.4 Statistical Analysis

All enzyme data were analyzed using SPSS version 25 for Windows (SPSS, Chicago II., U.S.A.). Results were obtained from triplicate measurements, and significant differences between means were separated using Duncan's Multiple Range Test (DMRT) at a significance level of $\alpha \leq 0.05$ (Adelabu & Kareem, 2022).

3.0 Results and discussion

3.1 Binding affinities and molecular interactions of target enzymes with investigated plastic compounds (In Silico Analysis)

Microbial enzymes represent one of the most sustainable approaches to plastic degradation (Mohanan et al., 2020). In this study, twenty-three chemical constituents of different types of plastics were used as ligands for docking against three microbial enzymes: esterase (3KVN), a dehydrogenas e (4JZ6), and a laccase (5LM8). The results of these docking simulations are summarized in Table 1, with selected 2D and 3D interaction visualizations for the two compounds with the highest binding affinity shown in Figs. 1 and 2 respectively.

Binding energies of the plastic ligands with 3KVN (esterase), 4JZ6 (dehydrogenase), and 5LM8 (laccase) ranged from -2 to -7.1 kcal/mol, -2 to -7.9 kcal/mol and -2.4 to -7.2 kcal/mol respectively (Table 1). The ligand 2,6-naphthalene dicarboxylic acid (NDA) showed the lowest binding energy across all the three enzymes (-7.1, -7 and -7.2 kcal/mol respectively), indicating the strongest binding affinity. In contrast, ethylene showed the highest binding energy (-2, -2, -2.4 kcal/mol respectively, suggesting the weakest interaction. Overall, fourteen of the ligands showed strong binding affinity, with energies ≤ -5 kcal/mol across the three enzymes (Table 1).

Table 1: Studied plastic chemical constituents and their binding energies (kcal/mol) with three target microbial enzymes

s/n	Compounds	PubChem ID	Canonical Smiles	3KVN	4JZ6	5LM8
1	2,6-naphthalene dicarboxylic acid	14357	C1=CC2=C(C=CC(=C2)C(=O)O)C=C1C(=O)O	-7.1	-7.9	-7.2
2	Diisononylphthalate	590836	CC(C)CCCCCCOC(=O)C1=CC=CC=C1C(=O)OCCCCCCC(C)C	-5	-7.5	-6.7
3	Bis(2-ethylhexyl) terephthalate	22932	CCCCC(CC)COC(=O)C1=CC=C(C=C1)C(=O)OCC(CC)CCCC	-5.1	-7.3	-6.5
4	Bis (2-hydroxyethyl) terephthalate	13739	C1=CC(=CC=C1C(=O)OCCO)C(=O)OCCO	-6.6	-6.4	-5.7
5	Mono-Methyl Terephthalate	15513	COC(=O)C1=CC=C(C=C1)C(=O)O	-6.1	-6.3	-6
6	Dimethyl Terephthalate	8441	COC(=O)C1=CC=C(C=C1)C(=O)OC	-6.5	-6.3	-5.6
7	Diethyl phthalate	6781	CCOC(=O)C1=CC=CC=C1C(=O)OCC	-5.1	-6.2	-5.6
8	Dimethyl phthalate	8554	COC(=O)C1=CC=CC=C1C(=O)OC	-5	-6.2	-6.3
9	Terephthalic acid	7489	C1=CC(=CC=C1C(=O)O)C(=O)O	-6.4	-6.1	-6
10	phenyl acetic acid	999	C1=CC=C(C=C1)CC(=O)O	-6.2	-5.8	-6
11	Phenylacetaldehyde	998	C1=CC=C(C=C1)CC=O	-5.6	-5.6	-5.3
12	Styrene maleic anhydride	62707	C=CC1=CC=CC=C1.C1=CC(=O)OC1=O	-5.8	-5.4	-5.4
13	styrene oxide	7276	C1C(O1)C2=CC=CC=C2	-5.6	-5.4	-5.4
14	Styrene	7501	C=CC1=CC=CC=C1	-5.7	-5.4	-5.4
15	1,3 propanediol	10442	C(CO)CO	-3.7	-3.9	-3.9
16	Ethylene glycol	174	C(CO)O	-3.7	-3.1	-3.4
17	Styrene butadiene	62697	C=CC=C.C=CC1=CC=CC=C1	-3.7	-3.1	-3.6
18	Butadiene	7845	C=CC=C	-3.7	-3.1	-3.7
19	Isobutylene	8255	CC(=C)C	-3.6	-3.1	-3.5
20	Acrylonitrile	7855	C=CC#N	-3.7	-3	-3.6
21	Propene	8252	CC=C	-2.9	-2.5	-3.2
22	Vinyl chloride	6338	C=CCl	-2.6	-2.2	-2.8
23	Ethylene	6325	C=C	-2	-2	-2.4

The binding energy (B.E) values were categorized using a 3-level colour scale and icon sets, *viz.,* (I) B.E ≥ -4 (red diamond, low affinity/high energy); (II) -4 > B.E > -6 (brown triangle, intermediate affinity); (III) BE ≤ -6 (green circle, high affinity/ low energy)

The order of ligand affinity for esterase was: 2,6-naphthalene dicarboxylic acid > terephthalic acid > styrene > bis(2-ethylhexyl) terephthalate > diisononyl phthalate. For dehydrogenase, the order was: 2,6 naphthalene dicarboxylic acid > diisononyl phthalate > bis(2-ethylhexyl) terephthalate > terephthalic acid > styrene. The same pattern as that in dehydrogenase was observed for laccase, although overall interactions were weaker, as reflected in generally higher binding energies. The exceptional binding affinity of NDA for all the three enzymes may be due to its structural complexity and polyaromatic nature which allows for multiple types of interactions- especially hydrogen bonding and pi interactions with enzyme active sites (El-Haj & Ahmed, 2020; Lu et al., 2020). NDA contains functional groups such as hydroxyl and carboxyl groups, which increase reactivity, and two benzene rings that enhance hydrophobic interactions with enzyme residues (Shi et al., 2023). This plastic compound is a precursor to the high-performance polyethylene naphthalate (PEN), a polyester introduced more recently to replace polyethylene terephthalate (PET) where a high tensile strength and higher modulus are of utmost importance (Menczel, 2020). Similarly, diisononyl phthalate (DINP) exhibited high binding affinity with the three enzymes. DINP is a widely used plasticizer in polyvinyl chloride (PVC) to soften the plastic (Folarin et al., 2024; Wang & Qian, 2021) and is known for its potential health hazards, including endocrine disruptions causing irreversible reproductive dysfunctions and other chronic health effects in humans and other animals (Verma et al., 2016). Another plastic compound with notable interactions with the enzymes was bis (2-hydroxyethyl) terephthalate (BHET), a PET hydrolysis product used in PET recycling. PET is one of the commonly used compounds in the packaging and textile industries- ranking fifth in the list of major polymers produced globally, and contributing to the enormous amount of wastes generated annually (Westover & Long, 2023). The ability of these enzymes to bind BHET suggests potential application in downstream plastic degradation.

On the other hand, ethylene and vinyl chloride, important monomers in the production of polythene and PVC- two of the most widely used plastics, showed the lowest affinities, indicating that these enzymes may not target monomers effectively. However, high binding affinities with complex compounds, such as polyester and plasticizers suggest that these enzymes may find application in post-consumer plastic degradation. Notably, interactions with TRP148 and TRP403 amino acid residues in laccase and dehydrogenase were observed to be consistently associated with higher binding affinity. These residues may play a key role in forming stable enzyme-substrate complexes with plastic compounds.

3.2 Isolation, characterization and assay of potential plastic degrading microorganisms

A total of eighteen (18) mold and twenty-one (21) bacterial isolates were obtained using standard microbiological techniques. Qualitative screening for laccase production carried out on bacterial isolates revealed varying intensities of dark brown colouration on guaiacol supplemented agar plates. Eight (8) of the twenty-one (21) bacterial isolates produced reddish-brown coloration, with three (3) isolates showing most reddish-brown coloration (Plate 1). *B. cereus* showed the deepest colouration followed by *Staphylococcus sp* while *E. aerogenes* had the faintest colouration. Lipase production was screened using phenol-red olive oil agar. A red-to-yellow color shift indicated the secretion of extracellular lipase (Plate 2). Five (5) bacterial isolates were confirmed as lipase producing organisms (Table 2), with *Bacillus cereus* showing the deepest yellow colouration, followed by *Staphylococcus sp* while *E. coli* showed the least colouration. Among the mold isolates, *Penicillium sp* produced the most intense reddish-brown (laccase) and yellow (lipase) colorations, followed by *Aspergillus sp* while *Mucor sp*. showed the faintest color development in both assays.

3.2.1 Quantitative assay of enzyme activity in Bacterial isolates.

To confirm enzyme production, laccase and lipase activities in bacterial isolates were quantified using standard enzyme assays (Table 2). *Bacillus cereus* had the highest laccase activity (1.122±0.01 U/mL), followed by *Staphylococcus* sp (0.983±0.09 U/mL) while *Enterobacter aerogenes* and *E. coli* had the lowest laccase activity (0.124±0.00 U/mL). Similarly, Bacillus sp exhibited the highest lipase activity (1.49±0.01 U/mL) followed by *Staphylococcus* sp (1.26±0.10 U/mL) while the least lipase activity was observed in *E. coli* (0.07±0.11 U/mL) (Table 2). Duncan's Multiple Range Test revealed that laccase by *Bacillus cereus* showed statistically distinguishable activity at $\alpha \leq 0.05$, implying that this bacterium is a potential laccase producing microorganism.

Our study agrees with the report of Narayanan et al. (2015) where *Bacillus subtilis*, *Enterobacter aerogenes* and *E. coli* were isolated from agricultural residue dumpsite. Comparably, Jyothi et al. (2021) reported *Bacillus* and *Enterobacter* sp isolated from contaminated sites as laccase producing bacteria. Another study

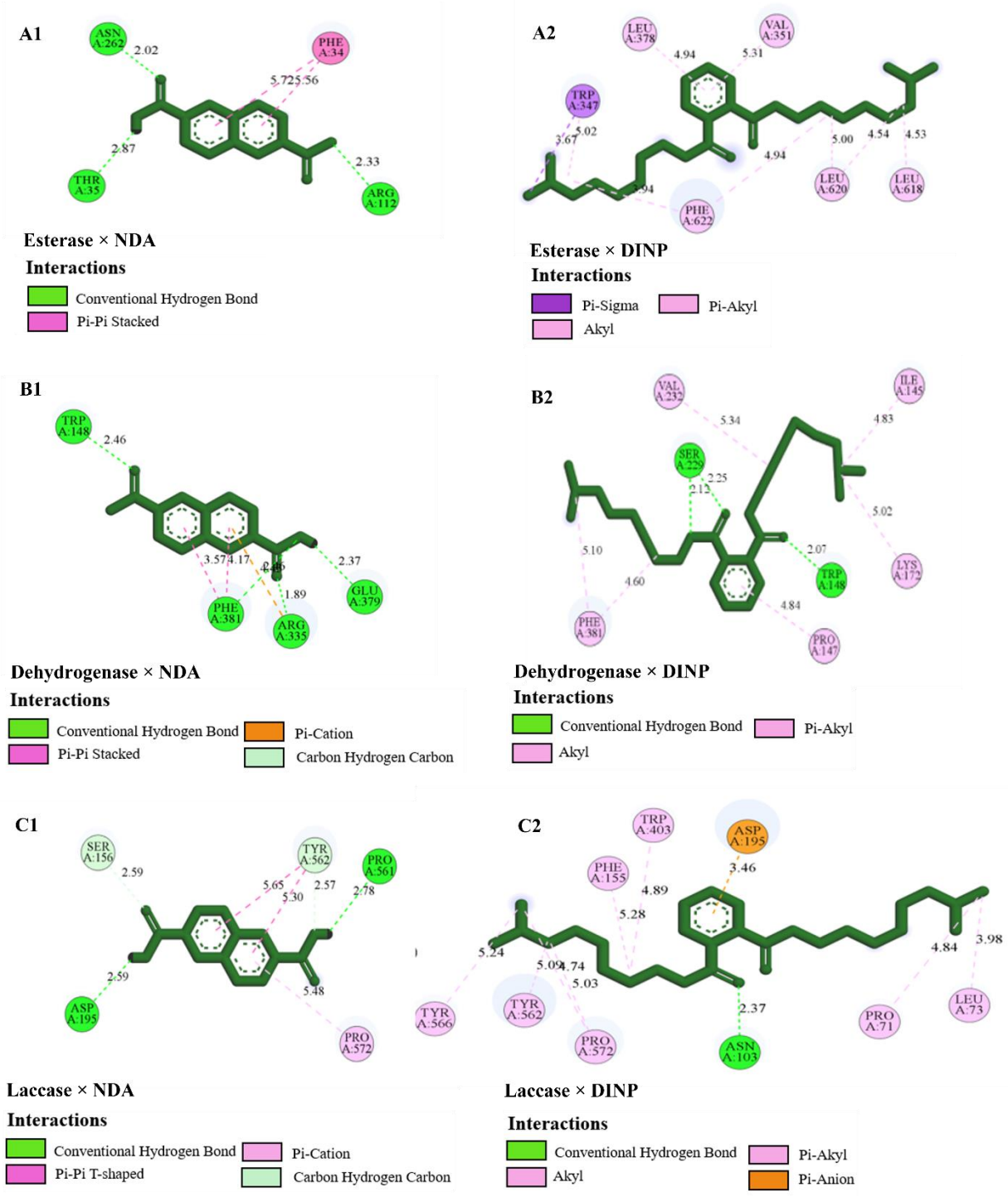

Fig. 1: 2D interactions of 2,6-naphthalene dicarboxylic acid (NDA) and Diisononylphthalate (DINP) with esterase (3KVN), dehydrogenase (4JZ6) and laccase (5LM8). The interactions of the plastic compound with the highest binding affinity (NDA) with target enzymes are on the left and labelled A1, B1, and C1 for 3KVN, 4JZ6, and 5LM8 respectively. Likewise, the interactions of the plastic compound with the second highest binding affinity with target enzymes are on the right, and labelled as A2, B2, and C2 respectively.

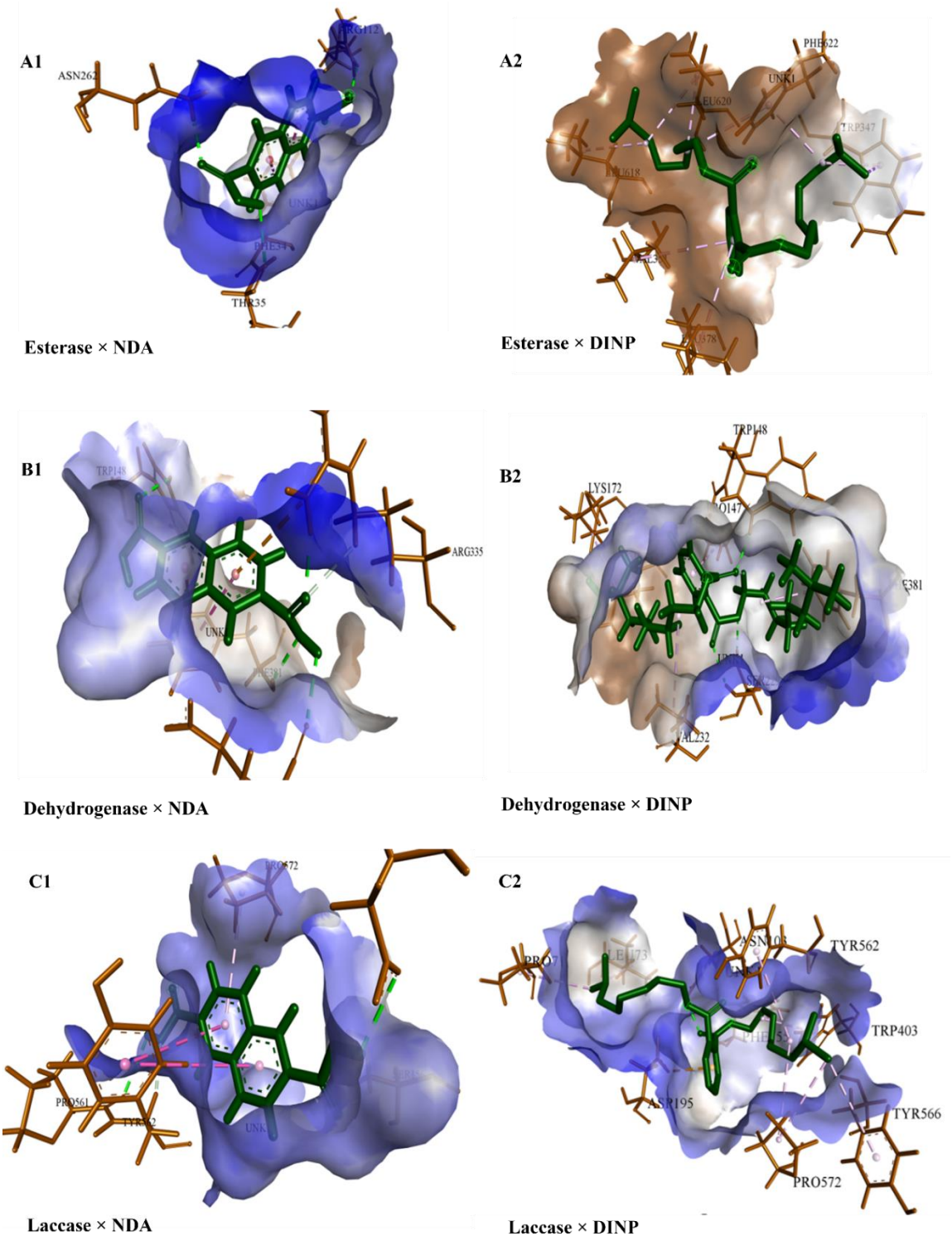

Fig. 2: 3D interactions of 2,6-naphthalene dicarboxylic acid (NDA) and Diisononylphthalate (DINP) with esterase (3KVN), dehydrogenase (4JZ6) and laccase (5LM8). The interactions of the plastic compound with the highest binding affinity (NDA) with target enzymes are on the left and labelled A1, B1, and C1 for 3KVN, 4JZ6, and 5LM8 respectively. Likewise, the interactions of the plastic compound with the second highest binding affinity with target enzymes are on the right, and labelled as A2, B2, and C2 respectively.

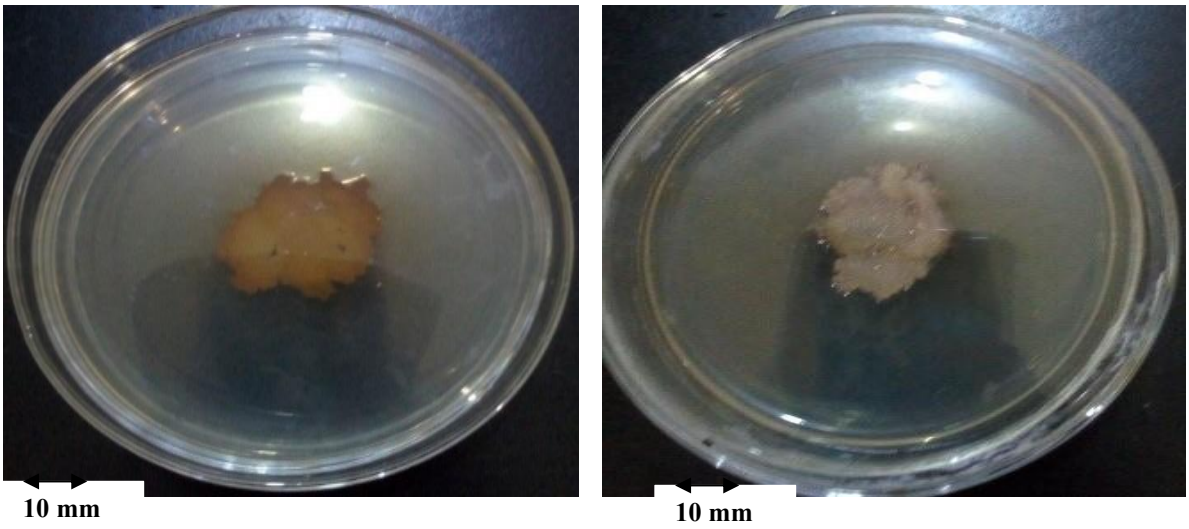

Plate 1: Plate assay for laccase activity. The **left plate**, supplemented with guaiacol shows reddish **brown coloration** indicating **positive guaiacol oxidation** by the isolate. The **right plate** serves as a control with no visible color change. **Scale bar = 10 mm.**

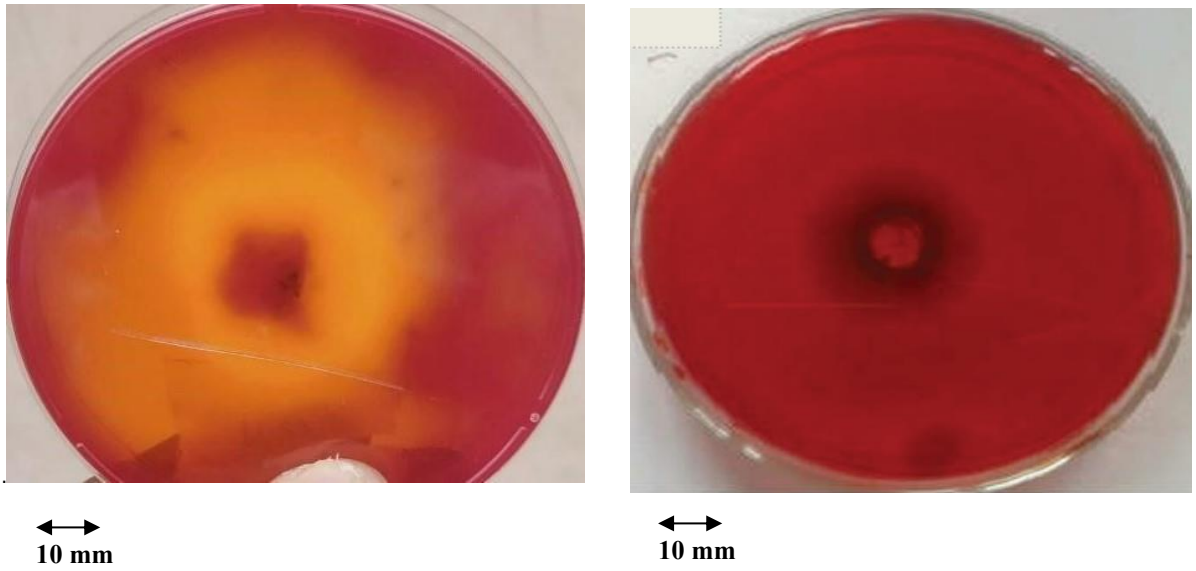

Plate 2: Plate assay for lipase activity. The phenol red-olive oil agar plate (left) shows yellow **coloration** indicating **positive** secretion of extracellular lipase by the isolate. The **right plate** serves as a control with no visible color change. **Scale bar = 10 mm.**

also produced and purified laccase enzyme secreted by *Bacillus* spp (Srinivasan et al., 2019). The ability of these organisms to oxidize guaiacol faster than others could be that they possess genes that code for rapid laccase production (Janusz et al., 2020).

Bacillus sp has been reported to secret lipase enzymes for growth on different substrates. This is attributed to its high reaction specificity, stereo specificity and less energy consumption (Lee et al., 2015). The presence of lipids at the dumpsite may have enhanced lipase gene expression in *B. pumilus* and the other bacteria, consistent with earlier observations (Demirkan et al., 2021).

Table 2: Screening for laccase and lipase production in bacterial and mold isolates

Bacterial isolates	Enzyme activity (U/mL)		Mold isolates	Enzyme activity (U/mL)	
	Laccase	Lipase		Laccase	Lipase
Bacillus cereus	1.12±0.01^{f}	1.49±0.01^{e}	*Aspergillus* sp	0.17±0.00^{d}	0.08±0.00^{d}
Staphylococcus sp	0.98±0.09^{e}	1.26±0.10^{d}	*Penicillum* sp	0.19±0.11^{e}	0.09±0.00^{e}
Burkholderia cepacian	0.71±0.11^{d}	0.15±0.00^{b}	*Mucor* sp	0.16±0.00^{bc}	0.07±0.00^{c}
E.coli	0.12±0.00^{a}	0.07±0.11^{a}	*Aspergillus* sp	0.11±0.11^{b}	0.05±0.00^{b}
B. subtilis	0.39±0.01^{b}	0.16±0.10^{c}	*Trichoderma* sp	0.09±0.10^{a}	0.04±0.00^{a}
E. aerogenes	0.12±0.10^{a}	Nil	*Fusarium* sp	0.15±0.01^{c}	0.05±0.00^{b}
K. pneumonia	0.41±0.12^{b}	Nil	*Aspergillus* sp	0.16±0.01^{c}	0.06±0.00^{bc}
Seratia. Marcescens	0.52±0.00^{c}	Nil	-	-	-

Results are values of mean ± standard deviation for three replicate determinations.
Values in a column that are followed by various letters (superscripts) indicate significant differences in enzyme activity of organisms according to Duncan's Multiple Range Test at $\alpha \leq 0.05$.

3.2.2 Screening for enzyme production in mold isolates

Five mold genera were identified: *Aspergillus, Penicillium, Mucor, Trichoderma* and *Fusarium.* Of these, *Aspergillus* and *Penicillium* were the most predominant. They are widely distributed and have the capacity to thrive on a wide variety of substrates (Kareem et al., 2017). Enzyme assay results showed *Penicillum* sp to have the highest laccase and lipase activity (Table 2). This is supported by Khan et al. (2023) who reported *Penicillium* sp from plastic-polluted dumpsite soils in India as a potent laccase and lipase producing mold. *Mucor* sp and *Aspergillus* sp also showed moderate enzyme activities, consistent with previous findings (Abdel-Razek et al., 2020; Alabdalall et al., 2020; Kareem et al., 2017). *Aspergillus* sp are known to withstand harsh environmental conditions, hence can secrete different enzymes for survival including lipase (Abdel-Razek et al., 2020).

3.2.3 Molecular Identification of high enzyme-producing isolates

The three isolates with the highest enzyme activity- two bacteria and one mold, were subjected to molecular identification via 16S rRNA and ITS sequencing. Biochemical characterization identified the predominant bacterium as *Bacillus cereus.* However, molecular characterization confirmed it as *Bacillus pumillus* (NCBI Accession No. PP140677.1, 99.47% identity). *Bacillus pumilus* and *B. cereus* are both members of the Bacillus genus, and biochemical tests may not be able to distinguish between certain species. Hence, molecular confirmation is usually required (Parvathi et al., 2009). The second bacterium was identified as *Staphylococcus cohnii* (CP054807.1, 99.93%) while the mold isolate was confirmed as *Penicillium citrinum* (OP040154.1, 99.45%).

B. pumilus, like most other Bacillus species, can survive in extremely harsh environments, and also exhibits high protease and lipase activity (Parvathi et al., 2009); hence their ability to grow in multi-waste dumpsite. *B. pumilus* isolated from a municipal dumpsite in India showed in as little as 60 days, a significant biodegradative capacity of plastic beads containing low density polyetyhlene (LDPE) (Banerjee et al., 2024). A study in Owerri, Nigeria also confirms the biodegradative capacity of *B. pumilus* on LDPE using microbial growth in liquid medium (Uwakwe et al., 2025). *Staphylococcus cohnii* has been shown to enhance the biodegradability of LDPE/starch blended films (Samanta et al., 2024). *P. citrinum* has also been shown to have high capacity in the biodegradation of LDPE (Khan et al., 2023).

3.3 Limitations

Despite the promising findings from this preliminary study, a few limitations are hereby acknowledged. Firstly, while molecular docking provides predictive insights into enzyme-substrate interactions, it does not account for complex environmental factors that affect actual degradation, such as pH, temperature, and enzyme kinetics. Further in vitro and in vivo

tests are required to validate these binding interactions under simulated environmental conditions. In addition, this study did not directly measure the breakdown of plastic polymers into intermediate or final degradation products. Future studies should integrate analyses to confirm the actual degradation of plastics and characterize the resulting by-products. Notwithstanding, findings from this study provide a strong basis for further exploration into enzyme-based plastic bioremediation.

4.0 Conclusion

To the best of our knowledge, this is one of the first studies to investigate the degradation potentials of multiple enzymes on multiple plastic compounds. Our *in-silico* study revealed strong binding interactions between plastic compounds, most especially the polyester plastic compounds, with all the three enzymes tested. Additionally, our results from *in vitro* investigations validated high production of laccase and lipase enzymes in two bacteria and one mold (*Bacillus pumilus, Staphylococcus cohnii* and *Penicillium citrinium*) isolated from a multi-waste dumpsite soil sample. These findings suggest that these microorganisms and their enzymes hold significant promise for the biodegradation of plastic pollutants. Further studies are needed to validate their performance under real world environmental conditions and to scale up enzyme production for applied plastic waste bioremediation.

Acknowledgement

The authors express immense appreciation to Mr Abayomi Shofunde of the Department of Microbiology, Chrisland University for his technical support.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Conception: BTF

Design: BTF, BAO, RAA & OAT

Execution: BTF, BAO, RAA, OAT, RNO & OAB

Interpretation: BTF, BAO, RAA, OAT, RNO & OAB

Writing the paper: BTF, BAO, RAA, OAT & OAB

Funding

The authors declare no access to external fundings for this study.

References

Abatenh, E., Gizaw, B., & Wassie, Z. T. and M. (2017). The Role of Microorganisms in Bioremediation- A Review. *Open Journal of Environmental Biology*, *1*(1), 38–46. http://dx.doi.org/10.17352/ojeb.000007

Abdel-Razek, A. S., El-Naggar, M. E., Allam, A., Morsy, O. M., & Othman, S. I. (2020). Microbial natural products in drug discovery. *Processes*, *8*(4), 470. https://doi.org/10.3390/pr8040470

Adelabu, B. A., & Kareem, S. O. (2022). Cellulase Production By Immobilized Cells of Candida Tropicalis Isolated From Grasshopper Zonocerus Variegatus in Saw Dust and Rice Husk Medium. *Polish Journal of Natural Sciences*, *37*(2), 187–204. https://doi.org/10.31648/pjns.7538

Akinyemi, O. D., Ahuekwe, E. F., Akinduti, P. A., Oziegbe, O., Oniha, M. I., Ichor, T., Alade, B. M., Ezekiel, O. M., Adekeye, B. T., & Kuye, A. O. (2022). Isolation and Screening of Laccase-producing Fungi from Sawdust-contaminated Sites in Ado-Odo Ota, Ogun State, Nigeria. *IOP Conference Series: Earth and Environmental Science*, *1054*(1), 12006. https://doi.org/10.1088/1755-1315/1054/1/012006

Alabdalall, A. H., ALanazi, N. A., Aldakeel, S. A., AbdulAzeez, S., & Borgio, J. F. (2020). Molecular, physiological, and biochemical characterization of extracellular lipase production by Aspergillus niger using submerged fermentation. *PeerJ*, *8*, e9425. https://doi.org/10.7717/peerj.9425

AlMaadeed, M. A. A., Ponnamma, D., & El-Samak, A. A. (2020). *Chapter 1 - Polymers to improve the world and lifestyle: physical, mechanical, and chemical needs* (M. A. A. AlMaadeed, D. Ponnamma, & M. A. B. T.-P. S. and I. A. Carignano (eds.); pp. 1–19). Elsevier. https://doi.org/10.1016/B978-0-12-816808-0.00001-9

Almeida, E. L., Carrillo Rincón, A. F., Jackson, S. A., & Dobson, A. D. W. (2019). In silico screening and heterologous expression of a polyethylene terephthalate hydrolase (PETase)-like enzyme (SM14est) with polycaprolactone (PCL)-degrading activity, from the marine sponge-derived strain Streptomyces sp. SM14. *Frontiers in Microbiology*, *10*, 2187. https://doi.org/10.3389/fmicb.2019.02187

Banerjee, D., Patel, C., & Patel, K. (2024). Degradation of Plastic Beads Containing Low Density Polyethylene (LDPE) by Sequential Photolysis, Hydrolysis and Bacterial Isolates. *Bulletin of Environmental Contamination and Toxicology*, *112*(3), 41. https://doi.org/10.1007/s00128-024-03853-6

Bartrem, C., Tirima, S., von Lindern, I., von Braun, M., Worrell, M. C., Mohammad Anka, S., Abdullahi, A., & Moller, G. (2014). Unknown risk: co-exposure to lead and other heavy metals among children living in small-scale mining communities in Zamfara State, Nigeria. *International Journal of Environmental Health Research*, *24*(4), 304–319. https://doi.org/10.1080/09603123.2013.835028

Bhandari, S., Poudel, D. K., Marahatha, R., Dawadi, S., Khadayat, K., Phuyal, S., Shrestha, S., Gaire, S., Basnet, K., & Khadka, U. (2021). Microbial enzymes used in bioremediation.

Journal of Chemistry, *2021*(1), 8849512. https://doi.org/10.1155/2021/8849512

Bhatia, M., Girdhar, A., Tiwari, A., & Nayarisseri, A. (2014). Implications of a novel Pseudomonas species on low density polyethylene biodegradation: an in vitro to in silico approach. *SpringerPlus*, *3*, 1–10. https://doi.org/10.1186/2193-1801-3-49

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., Abu-Omar, M., Scott, S. L., & Suh, S. (2020). Degradation rates of plastics in the environment. *ACS Sustainable Chemistry & Engineering*, *8*(9), 3494–3511. https://doi.org/10.1021/acssuschemeng.9b06635

Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D., & Zhang, J. (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. *Biotechnology Advances*, *33*(6, Part 1), 745–755. https://doi.org/https://doi.org/10.1016/j.biotechadv.2015.05.003

Dallakyan, S., & Olson, A. J. (2015). Small Molecule Library Screening by Docking with PyRx. *Methods in Molecular Biology (Clifton, N.J.)*, *1263*(January), 1–11. https://doi.org/10.1007/978-1-4939-2269-7

Danso, D., Chow, J., & Streit, W. R. (2019). Plastics: environmental and biotechnological perspectives on microbial degradation. *Applied and Environmental Microbiology*, *85*(19), e01095-19. https://doi.org/10.1128/AEM.01095-19

Demirkan, E., Çetinkaya, A. A., & Abdou, M. (2021). Lipase from new isolate Bacillus cereus ATA179: optimization of production conditions, partial purification, characterization and its potential in the detergent industry. *Turkish Journal of Biology*, *45*(3), 287–300. https://doi.org/10.3906/biy-2101-22

Desidery, L., & Lanotte, M. (2022). 1 - Polymers and plastics: Types, properties, and manufacturing. In F. Giustozzi & S. B. T.-P. W. for S. A. R. Nizamuddin (Eds.), *Woodhead Publishing Series in Civil and Structural Engineering* (pp. 3–28). Woodhead Publishing. https://doi.org/10.3906/biy-2101-22

Duddu, M. K., Tripura, K. L., Guntuku, G., & Divya, D. S. (2015). Biodegradation of low density polyethylene (LDPE) by a new biosurfactant-producing thermophilic Streptomyces coelicoflavus NBRC 15399T. *African Journal of Biotechnology*, *14*(4), 327–340. https://doi.org/10.5897/AJB2014.14224

Duru, C. E., Duru, I. A., & Enyoh, C. E. (2021). In silico binding affinity analysis of microplastic compounds on PET hydrolase enzyme target of Ideonella sakaiensis. *Bulletin of the National Research Centre*, *45*(1), 104. https://doi.org/10.1186/s42269-021-00563-5

Edupuganti, S., Parcha, L., & Mangamoori, L. N. (2017). Purification and characterization of extracellular lipase from staphylococcus epidermidis (MTCC 10656). *Journal of Applied Pharmaceutical Science*, *7*(1), 57–63. https://doi.org/10.7324/JAPS.2017.70108

El-Haj, B. M., & Ahmed, S. B. M. (2020). Metabolic-hydroxy and carboxy functionalization of alkyl moieties in drug molecules: prediction of structure influence and pharmacologic activity. *Molecules*, *25*(8), 1937. https://doi.org/10.3390/molecules25081937

Folarin, B. T., Poma, G., Yin, S., Altamirano, J. C., Cleys, P., Oluseyi, T., & Covaci, A. (2024). Source identification and human exposure assessment of organophosphate flame retardants and plasticisers in soil and outdoor dust from Nigerian e-waste dismantling and dumpsites. *Environmental Pollution*, *362*, 124998. https://doi.org/10.1016/j.envpol.2024.124998

Hantani, Y., Imamura, H., Yamamoto, T., Senga, A., Yamagami, Y., Kato, M., Kawai, F., & Oda, M. (2018). Functional characterizations of polyethylene terephthalate-degrading cutinase-like enzyme Cut190 mutants using bis (2-hydroxyethyl) terephthalate as the model substrate. *AIMS Biophysics*, *5*(4), 290–302. https://doi.org/10.3934/biophy.2018.4.290

Ilesanmi, O. I., Adekunle, A. E., Omolaiye, J. A., Olorode, E. M., & Ogunkanmi, A. L. (2020). Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. *Scientific African*, *8*, e00279. https://doi.org/10.1016/j.sciaf.2020.e00279

Janusz, G., Pawlik, A., Świderska-Burek, U., Polak, J., Sulej, J., Jarosz-Wilkołazka, A., & Paszczyński, A. (2020). Laccase properties, physiological functions, and evolution. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21030966

Jyothi, S.; Kumar, K.; Latha, E. (2021). Screening, Isolation and Biochemical Characterization of Laccase Producing Bacteria from Contaminated Sites. *International Journal of Pharmaceutical Sciences Review and Research*, *71*(2), 117–121. https://doi.org/10.47583/ijpsrr.2021.v71i02.018

Kareem, S. O., Adegoke, O. O., Balogun, S. A., Afolabi, A. T., & Akinde, S. B. (2017). Biodegradation of premium motor spirit (PMS) by lipase from Bacillus thuringiensis and Lysinibacillus sphaericus. *Nigerian Journal of Biotechnology*, *33*, 34–40. https://doi.org/10.4314/njb.v33i1.5

Khan, S., Ali, S. A., & Ali, A. S. (2023). Biodegradation of low density polyethylene (LDPE) by mesophilic fungus 'Penicillium citrinum'isolated from soils of plastic waste dump yard, Bhopal, India. *Environmental Technology*, *44*(15), 2300–2314. https://doi.org/10.1080/09593330.2022.2027025

Kumar, V., Shahi, S. K., & Singh, S. (2018). Bioremediation: an eco-sustainable approach for restoration of contaminated sites. *Microbial Bioprospecting for Sustainable Development*, 115–136. https://doi.org/10.1007/978-981-13-0053-0_6

Lahimer, M. C., Ayed, N., Horriche, J., & Belgaied, S. (2017). Characterization of plastic packaging additives: food contact, stability and toxicity. *Arabian Journal of Chemistry*, *10*, S1938–S1954. https://doi.org/10.1016/j.arabjc.2013.07.022

Landrigan, P. J., Fuller, R., Acosta, N. J. R., Adeyi, O., Arnold, R., Baldé, A. B., Bertollini, R., Bose-O'Reilly, S., Boufford, J. I.,

& Breysse, P. N. (2018). The Lancet Commission on pollution and health. *The Lancet*, *391*(10119), 462–512. https://doi.org/10.1016/S0140-6736(17)32345-0

Lee, L. P., Karbul, H. M., Citartan, M., Gopinath, S. C. B., Lakshmipriya, T., & Tang, T.-H. (2015). Lipase-Secreting Bacillus Species in an Oil-Contaminated Habitat: Promising Strains to Alleviate Oil Pollution. *BioMed Research International*, *2015*(1), 820575. https://doi.org/10.1155/2015/820575

Lu, H., Qi, Y., Zhao, Y., & Jin, N. (2020). Effects of hydroxyl group on the interaction of carboxylated flavonoid derivatives with S. Cerevisiae α-glucosidase. *Current Computer-Aided Drug Design*, *16*(1), 31–44. https://doi.org/10.2174/1573409914666181022142553

Menczel, J. D. (2020). Poly (ethylene naphthalate)[poly (ethylene-2, 6-naphthalene dicarboxylate)]. In *Thermal Analysis of Textiles and Fibers* (pp. 191–196). Elsevier. https://doi.org/10.1016/B978-0-08-100572-9.00010-0

Mendiburu-Valor, E., Mondragon, G., González, N., Kortaberria, G., Eceiza, A., & Peña-Rodriguez, C. (2021). Improving the efficiency for the production of bis-(2-hydroxyethyl) terephtalate (BHET) from the glycolysis reaction of poly (ethylene terephtalate)(PET) in a pressure reactor. *Polymers*, *13*(9), 1461. https://doi.org/10.3390/polym13091461

Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in Microbiology*, *11*, 580709. https://doi.org/10.3389/fmicb.2020.580709

Narayanan, M. P., Murugan, S., Eva, A. S., Devina, S. U., & Kalidass, S. (2015). Application of immobilized laccase from Bacillus subtilis MTCC 2414 on decolourization of synthetic dyes. *Res J Microbiol*, *10*(9), 421–432. https://doi.org/10.3923/jm.2015

Nikolaivits, E., Dimopoulou, P., Maslak, V., Nikodinovic-Runic, J., & Topakas, E. (2020). Discovery and biochemical characterization of a novel polyesterase for the degradation of synthetic plastics. *Chemistry Proceedings*, *2*(1), 33. https://doi.org/10.3390/ECCS2020-07572

Parvathi, A., Krishna, K., Jose, J., Joseph, N., & Nair, S. (2009). Biochemical and molecular characterization of Bacillus pumilus isolated from coastal environment in Cochin, India. *Brazilian Journal of Microbiology*, *40*, 269–275. https://doi.org/10.1590/S1517-83822009000200012

Rajmohan, K. V. S., Ramya, C., Viswanathan, M. R., & Varjani, S. (2019). Plastic pollutants: effective waste management for pollution control and abatement. *Current Opinion in Environmental Science & Health*, *12*, 72–84. https://doi.org/10.1016/j.coesh.2019.08.006

Ru, J., Huo, Y., & Yang, Y. (2020). Microbial degradation and valorization of plastic wastes. *Frontiers in Microbiology*, *11*, 442. https://doi.org/10.3389/fmicb.2020.00442

Salami, H. T. A., Hamza, T. A., Saleem, H. D., Fadhil, A. A., Abdulhasan, M. J., Adhab, A. H., Izzat, S. E., & Hamad, D. A. (2022). Efficiency of Purified Laccase from Pseudomonas Spp. as Bioremediator Agent in Gasoline Contaminated Soils. *Journal of Pharmaceutical Quality Assurance*, *13*(2), 141–144. https://doi.org/10.25258/ijpqa.13.2.8

Samanta, S., Datta, D., Tiwari, O. N., & Halder, G. (2024). Microbial enhancement of biodegradability inoculating Bacillus tropicus and Staphylococcus cohnii onto LDPE/starch blended films. *Biomass Conversion and Biorefinery*, *14*(13), 14003–14016. https://doi.org/10.1007/s13399-022-03478-x

Senthivelan, T., Kanagaraj, J., Panda, R. C., & Narayani, T. (2019). Screening and production of a potential extracellular fungal laccase from Penicillium chrysogenum: Media optimization by response surface methodology (RSM) and central composite rotatable design (CCRD). *Biotechnology Reports*, *23*, e00344. https://doi.org/10.1016/j.btre.2019.e00344

Shi, X.-Y., Liu, S.-J., Yu, L.-Q., Li, L.-F., & Lv, Y.-K. (2023). MOFs with 2, 6-naphthalene dicarboxylic acid as organic ligand for solid phase microextraction of aldehyde biomarkers in the exhalation of lung cancer patients. *Talanta Open*, 100286. https://doi.org/10.1016/j.talo.2023.100286

Solanki, J., More, S., Jodhani, K., Patel, D., & Nataraj, M. (2022). Efficacy of biosurfactant as a coating material for post-harvest preservation of jamun fruit (Syzygium cumini). *Environmental and Experimental Biology*, *20*(1), 11–23. https://orcid.org/0000-0001-8311-4270

Srinivasan, P., Selvankumar, T., Kamala-Kannan, S., Mythili, R., Sengottaiyan, A., Govarthanan, M., Senthilkumar, B., & Selvam, K. (2019). Production and purification of laccase by Bacillus sp. using millet husks and its pesticide degradation application. *3 Biotech*, *9*(11), 396. https://doi.org/10.1007/s13205-019-1900-8

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*(10), 2731–2739. https://doi.org/10.1093/molbev/msr121

Thabet, H. S., Seddik, I., Mohamed, W. A., Sayed, A. M., & Yusuf, S. (2023). Isolation, Screening, and Molecular Identification of Lipase-Producing Bacteria from Different Bacterial Sources. *Assiut Veterinary Medical Journal*, *69*(178), 134–144. https://dx.doi.org/10.21608/avmj.2023.213953.1147

Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc

Tsui, E., Luong, P. M., Fogel, J., Fogel, E. S., & Zegans, M. E. (2019). Microbial analysis of donor corneoscleral rims and storage media. *Ocular Immunology and Inflammation*, *27*(5), 817–820. https://doi.org/10.1080/09273948.2018.1459734

Uwakwe, F. E., Ezejiofor, T. I. N., Anyalogbu, E. A. A., & Ogbulie, T. E. (2025). The biodegradation of low-density

polyethylene by Bacillus species. *EQA-International Journal of Environmental Quality*, *67*, 26–35. https://doi.org/10.6092/issn.2281-4485/20474

Verma, R., Vinoda, K. S., Papireddy, M., & Gowda, A. N. S. (2016). Toxic pollutants from plastic waste-a review. *Procedia Environmental Sciences*, *35*, 701–708. https://doi.org/10.1016/j.proenv.2016.07.069

Wang, Y., & Qian, H. (2021). Phthalates and their impacts on human health. *Healthcare*, *9*(5), 603. https://doi.org/10.3390/healthcare9050603

Westover, C. C., & Long, T. E. (2023). Envisioning a BHET Economy: Adding Value to PET Waste. *Sustainable Chemistry*, *4*(4), 363–393. https://doi.org/10.3390/suschem4040025