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SCREENING OF LACCASE AND MANGANESE PEROXIDASE IN CYANOBACTERIA

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This study investigates the presence of laccase and manganese peroxidase enzymes in cyanobacterial strains isolated from polyethylene waste material at refuse disposal sites. Despite polyethylene representing over 36.3% of global synthetic plastic production, its biodegradation has been challenging due to its hydrophobic nature and surface properties. While laccase and manganese peroxidase are known to facilitate polyethylene biodegradation in certain fungi and bacteria, their occurrence in cyanobacteria remains largely unexplored. Seven cyanobacterial isolates (*Phormedium abronema*, *Phormedium faveolarum*, *Oscillatoria laete-virens*, *Oscillatoria ornata*, *Westeollopsis prolifica*, *Synecocystis pevallikii*, and *Synechococcus elongatus*) were screened for these enzymes. Six isolates demonstrated positive laccase activity with varying intensity, as evidenced by the bluish-green coloration resulting from ABTS oxidation. In contrast, no manganese peroxidase activity was detected in any of the isolates, indicated by the absence of oxidation zones in phenol red-supplemented media. These findings suggest that cyanobacteria colonizing polyethylene waste possess laccase but likely lack manganese peroxidase enzymes, providing new insights into the enzymatic mechanisms potentially involved in cyanobacterial interactions with synthetic polymers in waste environments.

Keywords: Biodegradation, Cyanobacteria, Laccase, Manganese peroxidase, and Polyethylene.

Introduction

The introduction of plastics in the early 1900s has significantly altered modern life, providing versatile, durable, and economical materials for a wide range of industries and consumers[1]. Plastic production worldwide has grown exponentially, surpassing 300 million tons annually by 2015, underscoring the crucial role plastics play in economic and social structures[2,3]. However, the environmental and health consequences of such widespread plastic use have become increasingly apparent[4]. A major ecological issue is the growing accumulation of plastic waste, which is now polluting land and water ecosystems globally. Polyethylene is the most commonly produced synthetic plastic worldwide, constituting over 36.3% of total synthetic plastic production[5]. Among polyethylene types, low-density polyethylene (LDPE) forms a substantial portion due to its beneficial properties for flexible packaging, shopping bags, clothing, and various disposable items[6]. LDPE shows remarkable environmental persistence, demonstrating high resistance to breakdown[7]. Considering that many global LDPE-based products have a short functional life before disposal, LDPE plastic waste streams continue to increase by 5% yearly[5].

Reducing the use of polyethylene (PE) and other synthetic polymers is

crucial, while also developing effective methods for their breakdown and reuse. Extensive research has been conducted on PE degradation, using physico-chemical techniques, microbial approaches, or a combination of both[8,9,10]. The biodegradation of PE involves microorganisms or microbial communities that modify and consume the polymer as an energy source, leading to changes in its physical and chemical properties, including mass reduction, structural breakdown, and carbon conversion into biomass[9,10].

However, the formation of biofilms by polymer-degrading microorganisms on PE has been challenging due to its highly hydrophobic nature, limited specific surface area, and smooth surface texture[11,9]. Additionally, the adsorption and catalytic activity of polymer-degrading enzymes have been found to be incompatible with hydrophobic polymer surfaces [12,13].

Polyethylene biodegradation is a natural process carried out by microorganisms such as bacteria, fungi, actinomycetes, or algae[14,15,16,17]. The rate of polyethylene degradation depends on factors like crystallinity, surface modification, additives, molecular weight, and surfactants. Both extracellular and intracellular enzymes are essential in the biological breakdown process [18].

While bacterial and fungal biodegradation of polyethylene has been widely studied, the potential of algae in this context has received less attention[19,20]. Algae can colonize polyethylene materials in wastewater through the secretion of extracellular polymeric substances (EPS)[19,21,22]. Environmental factors largely influence the colonization of microbial communities on polyethylene surfaces, potentially creating favorable conditions for colonization[23]. Polyethylene discarded in aquatic environments and exposed to sunlight breaks into smaller particles due to bacterial and algal adhesion. The literature has confirmed direct microbial degradation of PE, using the polymer as the sole carbon source[24]. Previous studies have examined the biodegradation capabilities of PE by species like *Anabaena spiroides*, *Scenedesmus dimorphus*, *Navicula pupula*, *Phormidium lucidum* and *Oscillatoria subbrevis*[20,25]. In one of the recent studies, ten microalgal strains (*Neglectella solitaria* viz. *Oocystis solitaria, Chlorella vulgaris*, *Scenedesmus opoliensis*, *Scenedesmus carinatus*, *Coelastrum cambricum*, *Microcystis aeruginosa*, *Chroococcus turgidus*, *Arthrospira platensis*, *Arthrospira subsalsa* viz. *Spirullina subsalsa*, *Oscillatoria curviceps,* and *Leptolyngbya tenuis* viz. *Phormidium tenue)* were isolated from colonized polythene and tested for their biodegradation potential[26]. The choice of microorganisms for polyethylene biodegradation is critical, with those capable of producing oxidative and lignolytic enzymes showing greater effectiveness [27].

Multiple studies have identified microbial strains that produce extracellular enzymes, including LDPE-degrading enzymes, lipases, peroxidases, cutinases, esterases, laccases, and peroxidases. These enzymes specifically target LDPE through cleavage, depolymerization, or other catabolic processes[28,6,7].

The enzymes laccase and manganese peroxidase are integral to the process of biodegradation of polythene, as they facilitate the disassembly of its intricate polymeric framework. These enzymes are synthesized by a diverse array of microorganisms that exploit polythene as a carbon substrate, thereby promoting its degradation. The principal organisms recognized for the biosynthesis of these enzymes encompass specific fungi and bacteria, which have been successfully isolated from environments that are abundant in plastic refuse. The laccase enzyme exhibits a notable capacity for the oxidation of both phenolic and non-phenolic lignin-related compounds, thereby contributing to the degradation of polythene through modifications to its chemical

constitution[29,30]. Manganese peroxidase catalyzes the oxidation of manganese ions, which subsequently oxidize a variety of organic substrates, inclusive of polythene, culminating in its degradation[29,31]. Numerous fungal species have been identified as significant producers of laccase and manganese peroxidase. Exemplary species include *Penicillium italicum, Penicillium simplicissimum,* and *Trichoderma harzianum*, which have exhibited considerable enzymatic activity and efficacy in the degradation of polythene[32,30,31]. Certain bacterial strains, such as *Escherichia coli* and *Pseudomonas aeruginosa*, have also been documented to generate these enzymes, albeit with varying degrees of enzymatic activity when contrasted with fungi[33].

Numerous researches have documented the occurrence of the enzymes laccase and manganese peroxidase in various bacterial and fungal species; however, the volume of investigations pertaining to the occurrence of these enzymes in algal and cyanobacterial populations remains markedly limited. In the current study, a preliminary screening of the aforementioned enzymes was conducted in cyanobacterial strains that were isolated from polyethylene waste material sourced from refuse disposal sites.

Material and Methods

Algal-colonized submerged polyethylene substrates were systematically collected from the sewage effluent of Ajmer town, located in the state of Rajasthan, India (Fig. 1and 2). The samples that had developed on polyethylene carry bags immersed in wastewater were examined microscopically, leading to the identification of seven predominant cyanobacterial species suitable for monoculture cultivation. The collected cyanobacterial samples underwent centrifugation, following which the resultant pellets were homogenized in sterilized BG-11(N+) medium, and the resulting suspension was subsequently inoculated onto agar petri plates utilizing the pour plate technique. The plates were subjected to a 15-day incubation period under constant illumination (2000 lx) at a temperature of 24 ± 1 °C, in accordance with the methodology delineated by Rippka et al. (1979)[34]. The colonies proliferating in the agar medium have been preserved in liquid BG-11(N+) medium, as detailed in Table 1. The algal samples were scrutinized microscopically and identified based on their distinctive morphological characteristics, as established by Desikachary (1959)[35].

(a) (b)

Figure 1.: (a) Location information and a topographical image of the Ajmer. (b) Rajasthan is depicted in topographical picture of the North Indian subcontinent.

(a) (b)

Figure 2.:Topographical view (a) and location details (b) of the sampling sites.

The isolated culture was cultivated by inoculating a loopful of each strain individually into a 250 ml flask containing 100 ml of BG11 and was subsequently induced with guaiacol for the biosynthesis of laccase (Fig. 3). The cultures without the induction of guaiacol were considered as control. The cultures were incubated at 25°C with a light/dark cycle of 16/8 hours. The concentration of guaiacol added was 100 µM, which was prepared by dissolving it in 50% ethanol and subjected to filter sterilization prior to application. A 1 ml sample of culture was extracted every 24 hours of growth, followed by centrifugation for 20 minutes at 8000 g, with the supernatant being collected and utilized as a crude enzyme extract [3].

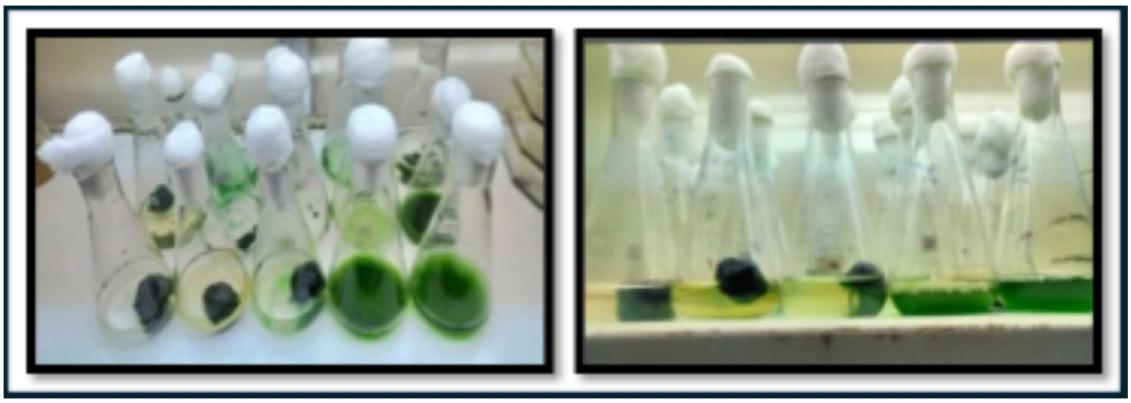

Figure 3.: Cultures of isolated cyanobacterial strain maintained in BG11 medium.

Table 1.: Composition of BG11 medium (1000ml) (Final pH after sterilization:7.1 at 25°C).

$NaNO_3$	**1.5 g**
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
Na_2CO_3	0.02 g
Trace metal mix A5	1.0 ml
Agar (if needed)	10.0 g
Distilled water	1.0 L

Screening of the aforementioned extract was conducted utilizing ABTS (2-2 Azinobis (3-ethylbenzthiazoline- 6-sulfonic acid) as a substrate, which undergoes oxidation by the laccase enzyme, resulting in the formation of a more stable cation radical state and imparting a blue-green coloration to the mixture. The reaction mixture was formulated by combining 200 µl of culture supernatant with 800 µl of a 2 mM ABTS solution (which was prepared by dissolving in sodium acetate buffer at pH 4.5 at 30°C). The manifestation of blue-green coloration was monitored and can be correlated with enzyme activity[36].

The assessment of cyanobacterial strains for the production of manganese peroxidase was conducted employing phenol red as an indicator. The phenol red indicator is oxidized by the enzyme, resulting in a colorimetric transition from red to yellow[37,38]. The cyanobacterial cultures were streaked onto freshly prepared sterile BG11 agar plates containing 0.01 g per 100 ml of phenol red. Incubation was performed at 25°C with a light/dark cycle of 16/8 hours. A colorimetric shift was interpreted as a positive indication of the enzyme's presence.

Results and Discussion

A significant accumulation of plastic bags, along with various other waste materials, was observed at multiple sample collection locations adjacent to Anasagar Lake in Ajmer, and these bags exhibited a pronounced colonization by algae. The cyanobacterial strains that were found colonizing the collected waste were isolated and subsequently examined under a light microscope utilizing various levels of magnification and were categorized according to their morphological characteristics including cell shape, size, and arrangement. The identification of these strains was achieved through the utilization of monographs and keys as delineated by Desikachary (1959). The investigation incorporated seven cyanobacterial isolates, specifically *Phormedium abronema*, *Phormedium faveolarum*, *Oscillatoria laete-virens*, *Oscillatoria ornata*, *Westeollopsis prolifica*, *Synecocystis pevallikii*, and *Synechococcus elongatus*. Digital photomicrographs of the specimens are illustrated in Fig. 4(a)-(g). Numerous prior investigations have documented that algal and cyanobacterial strains were observed to colonize the surfaces of waste polythene, and this study corroborates such findings. According to the literature, cyanobacterial strains possess the capability to colonize polyethylene materials present in wastewater through the secretion of extracellular polymeric substances (EPS)[19,21,22].

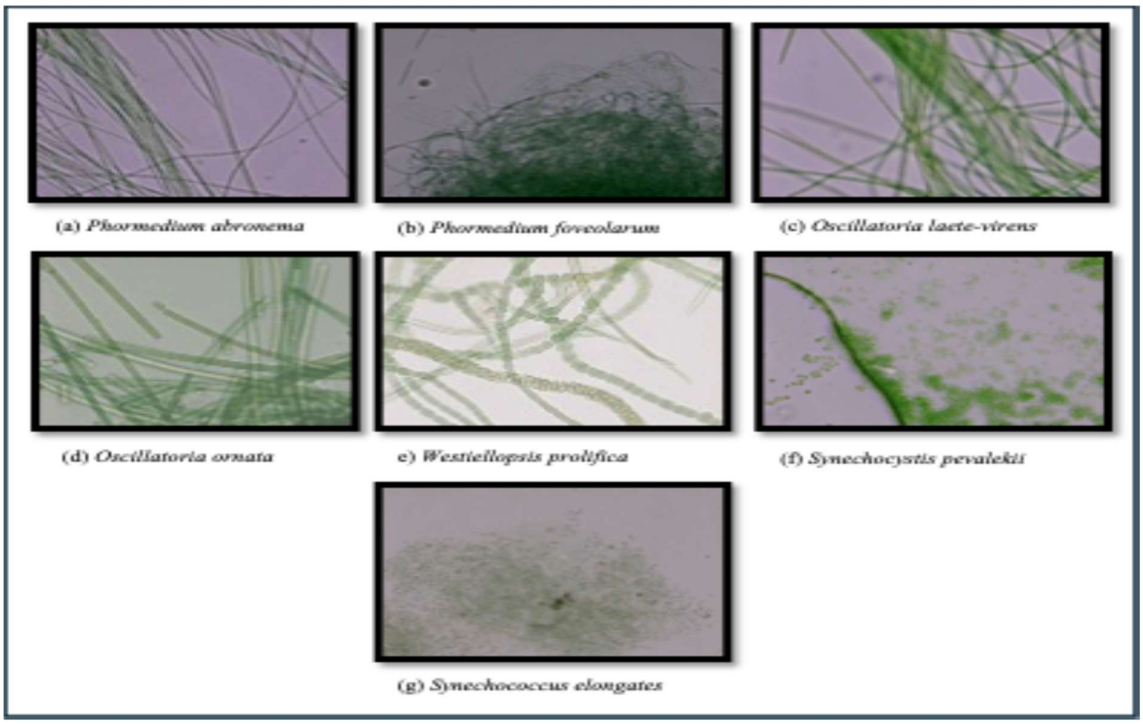

Figure 4.: Digital photomicrographs of the isolated cyanobacteria at 400x

The cyanobacterial isolates, *Phormedium abronema*, *Phormedium faveolarum*, *Oscillatoria laete-virens*, *Oscillatoria ornata*, *Westeollopsis prolifica*, *Synecocystis pevallikii* and *Synechococcus elongatus*, were evaluated for laccase presence on the second day of cultivation subsequent to induction by guaiacol. The bluish-green hue of the resultant mixture was attributed to the enzymatic oxidation of the substrate ABTS which was absent in control of each isolate (shown in Fig. 5)[38]. Among the seven isolates, six exhibited positive laccase activity; however, the intensity of the coloration varied, and one isolate did not show the presence of the enzyme, as indicated in Table 2. The color mixture containing extract of *Phormedium abronema* and *Oscillatoria ornate* was very intensed as compared to that of others. In a preceding study, thirty-five cyanobacterial isolates were assessed for laccase presence, with twenty-nine isolates yielding positive results. Given the advantageous properties of cyanobacteria, including their capacity to enhance environmental oxygen levels, rapid generation time, and ease of mass cultivation, they may serve as a valuable resource for laccase production[36].

Table 2.: Laccase detection in isolated cyanobacterial strain

S. No.	Cyanobacteria	Laccase detection
1	*Phormedium abronema*	+ve
2	*Phormedium faveolarum*	+ve
3	*Oscillatoria laete-virens*	+ve
4	*Oscillatoria ornata*,	+ve
5	*Westeollopsis prolific*	+ve
6	*Synecocystis pevallikii*	+ve
7	*Synechococcus elongates*	-ve

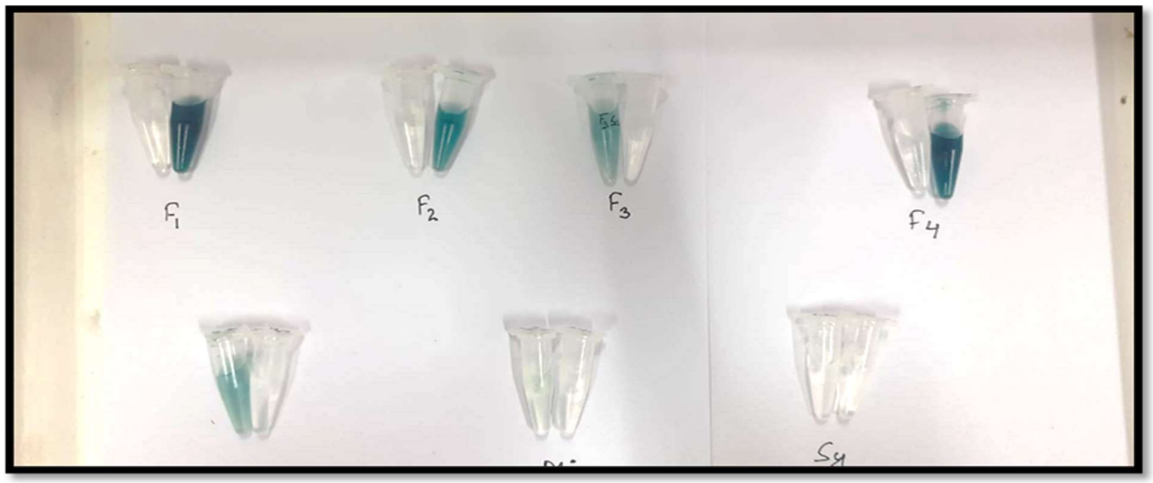

Figure 5.: Laccase detection in isolated stains

The isolated cyanobacterial strains were screened for their capacity to produce manganese peroxidase through the utilization of phenol red as a chromogenic indicator. The phenol red indicator undergoes oxidation catalyzed by the enzyme, leading to a discernible colorimetric shift from red to yellow[38,37]. The control sample devoid of cyanobacterial inoculation exhibited an orange hue. There was an absence of the transition from orange to yellow

subsequent to the cultivation of cyanobacteria on BG11 agar plates supplemented with phenol red. The color of the medium transitions to dark pink immediately following the streaking procedure and maintains this coloration post-cyanobacterial growth due to the alkaline nature of the culture[39]. No oxidation zones were observed in any of the cultures of the isolated cyanobacteria, as illustrated in Fig 6. Thus, this observation may suggest that the investigated cyanobacteria potentially lack the enzyme manganese peroxidase.

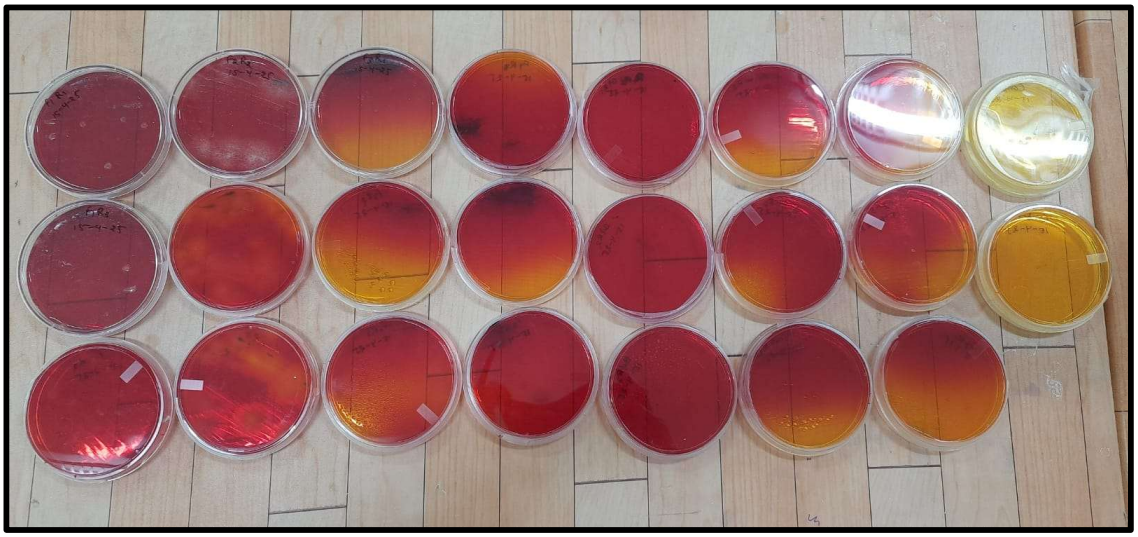

Figure 6.: BG11 agar plates containing phenol red after the growth of cyanobacteria

Conclusion

The examination of laccase and manganese peroxidase enzymes in cyanobacterial strains procured from polyethylene waste material has produced several important conclusions. Six of the seven cyanobacterial isolates exhibited positive laccase activity, though the intensities varied, thereby underscoring the extensive yet heterogeneous distribution of this enzyme among cyanobacterial species inhabiting plastic waste. This revelation implies that laccase enzymes may serve a pivotal function in the interaction dynamics between cyanobacteria and polyethylene substrates within waste environments.

In contrast, the lack of oxidation zones in the culture medium signifies that manganese peroxidase activity was undetected in any of the seven isolates. This observation highlights a potential enzymatic deficiency in these cyanobacterial species, thereby differentiating their metabolic capabilities from those of various fungi and bacteria that are known to possess both enzymes.

The assessed presence of laccase in these cyanobacterial strains, alongside their intrinsic ecological benefits—including oxygen production, accelerated growth rates, and suitability for mass cultivation—offers promising prospects for biotechnological applications. These organisms have the potential to act as sustainable biocatalysts for laccase production, with implications for the bioremediation of polyethylene waste as well as other environmental pollutants.

Further investigation is essential to delineate the specific laccase enzymes produced, optimize their expression, and examine potential genetic or environmental modifications that may elicit manganese peroxidase activity in these strains. Additionally, probing into the exact mechanisms through which these cyanobacteria colonize polyethylene surfaces via extracellular polymeric substances (EPS) secretion could yield

valuable insights for the development of improved bioremediation strategies targeting plastic pollution.

References

1. Ali SS, Elsamahy T, Al-Tohamy R and Sun J 2024, A critical review of microplastics in aquatic ecosystems: Degradation mechanisms and removing strategies. *Environ Sci Ecotechnology* **21**(5) 100427. https://doi.org/10.1016/j.ese.2024.100427
2. Geyer R, Jambeck JR, Law KL 2017, Production , use , and fate of all plastics ever made. *Science Advances* **3**(**7**) 25–29.
3. Ali SS, Elsamahy T, Al-Tohamy R, Zhu D, Mahmoud YAG and Koutra E 2021, Plastic wastes biodegradation: Mechanisms, challenges and future prospects. *Sci Total Environ.* **780**(1) 146590. https://doi.org/10.1016/j.scitotenv.2021.146590
4. Al-Tohamy R, Ali SS, Zhang M, Sameh M, Zahoor and Mahmoud YAG 2023, Can wood-feeding termites solve the environmental bottleneck caused by plastics? A critical state-of-the-art review. *J Environ Manage*. **326** (PtA) 116606. https://www.sciencedirect.com/science/article/pii/S030147972202179X
5. Ali SS, Elsamahy T, Koutra E, Kornaros M, El-Sheekh M and Abdelkarim EA 2021, Degradation of conventional plastic wastes in the environment: A review on current status of knowledge and future perspectives of disposal. *Sci Total Environ*. **771** 144719. https://doi.org/10.1016/j.scitotenv.2020.144719
6. Ali SS, Elsamahy T, Zhu D and Sun J 2023, Biodegradability of polyethylene by efficient bacteria from the guts of plastic-eating waxworms and investigation of its degradation mechanism. *J Hazard Mater*. **443** 130287. https://www.sciencedirect.com/science/article/pii/S0304389422020817
7. Elsamahy T, Sun J, Elsilk SE and Ali SS 2023, Biodegradation of low-density polyethylene plastic waste by a constructed tri-culture yeast consortium from wood-feeding termite: Degradation mechanism and pathway. *J Hazard Mater*. **448** 130944. https://www.sciencedirect.com/science/article/pii/S0304389423002261
8. Bonhomme S, Cuer A, Delort AM, Lemaire J, Sancelme M and Scott G 2003, Environmental biodegradation of polyethylene. *Polym Degrad Stab.* **81**(3) 441–52.
9. Restrepo-Flórez JM, Bassi A and Thompson MR 2014, Microbial degradation and deterioration of polyethylene - A review. *Int Biodeterior Biodegradation.***88** 83–90.
10. Kumar Sen S and Raut S 2015, Microbial degradation of low density polyethylene (LDPE): A review. *J Environ Chem Eng* **3**(1) 462–73. http://dx.doi.org/10.1016/j.jece.2015.01.003
11. Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F and Nava-Saucedo JE 2008, Polymer biodegradation: Mechanisms and estimation techniques - A review. *Chemosphere.* **73**(4) 429–42.
12. Espino-Rammer L, Ribitsch D, Przylucka A, Marold A, Greimel KJ and Acero EH 2013, Two novel class ii hydrophobins from Trichoderma spp. Stimulate enzymatic hydrolysis of poly (ethylene terephthalate) when expressed as fusion proteins. *Appl Environ Microbiol*. **79**(14) 4230–8.
13. Sammond DW, Yarbrough JM, Mansfield E, Bomble YJ, Hobdey SE and Decker SR 2014, Predicting enzyme adsorption to lignin films by calculating enzyme surface hydrophobicity. *J Biol Chem* **289**(30) 20960–9. http://dx.doi.org/10.1074/jbc.M114.57

3642
14. American Society for Testing and Materials. *ASTM standards on environmentally degradable plastics*. Philadelphia (PA): American Society for Testing and Materials; 1993. iv + 64 p. ISBN: 0-8031-1779-5.
15. Ghosh SK and Pal S, Ray S 2013, Study of microbes having potentiality for biodegradation of plastics. *Environ Sci Pollut Res Int*. **20**(7) 4339–55.
16. Qi X, Ren Y and Wang X 2017, New advances in the biodegradation of Poly (lactic) acid. *Int Biodeterior Biodegradation*. **117** 215–23. https://www.sciencedirect.com/science/article/pii/S0964830517300100
17. Ahmed T, Shahid M, Azeem F, Rasul I, Shah AA and Noman M 2018, Biodegradation of plastics: current scenario and future prospects for environmental safety. *Environ Sci Pollut Res.* **25**(8) 7287–98.
18. Gu JD 2003, Microbiological deterioration and degradation of synthetic polymeric materials: Recent research advances. *Int Biodeterior Biodegrad*. **52**(2) 69–91.
19. Suseela MR, Toppo K 2007, Algal biofilms on polythene and its possible degradation. *Curr Sci*. **92**(3) 285–7.
20. Vimal Kumar R, Kanna GR and Elumalai S 2017, Biodegradation of Polyethylene by Green Photosynthetic Microalgae. *J Bioremediation Biodegrad.* **8**(1).
21. Sharma M, Dubey A and Pareek A 2014, Algal Flora on Degrading Polythene Waste. *CIBTech Journal of Microbiology*. **3**(2) 43–7. http://www.cibtech.org/cjm.htm
22. Sarmah P and Rout J 2017 Colonisation of Oscillatoria on submerged polythenes in domestic sewage water of Silchar town, Assam (India) *J. Algal Biomass Utln.* **8**(4) 135-144. https://www.researchgate.net/publication/320407279
23. Pathak VM, Navneet 2017, Review on the current status of polymer degradation: a microbial approach. *Bioresour Bioprocess*. **4**(1) 15.
24. Roy PK, Titus S, Surekha P, Tulsi E, Deshmukh C and Rajagopal C 2008, Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. *Polym Degrad Stab*. **93**(10) 1917–22.
25. Sarmah P and Rout J 2018, Efficient biodegradation of low-density polyethylene by cyanobacteria isolated from submerged polyethylene surface in domestic sewage water. *Environ Sci Pollut Res*. 25(33) 33508–20.
26. Sharma M, Sharma A, Batra N and Pareek A 2025, Biodegradation assessment of polythene by microalgae growing on polythene debris from fresh water bodies. *Iran Polym J* . https://doi.org/10.1007/s13726-025-01475-1.
27. Nayak P, Tiwari A 2011, Biodegradability of Polythene and Plastic By The Help of Microorganism: A Way for Brighter Future. *J Environ Anal Toxicol.* **1**(2) 1–4.
28. Khruengsai S, Sripahco T and Pripdeevech P 2021, Low-density polyethylene film biodegradation potential by fungal species from thailand. *J Fungi*. *7*(8) 594.
29. Shahi MP and Shahi SK 2022, Enzyme profiling of microbes for biodegradation of polythene (LDPE) isolated from Chhattisgarh, India. *Research Square* 2056903/v1%0 https://www.researchsquare.com/article/rs-2056903/v1
30. Sowmya H V, Ramalingappa, Krishnappa M and Thippeswamy B 2015, Degradation of polyethylene by Penicillium simplicissimum isolated from local dumpsite of Shivamogga district. *Environ Dev Sustain.* **17**(4) 731–45.
31. Zige TD and O E O 2024, Screening of Laccase and Manganese Peroxidase Activities Produced By Escherichia

Coli and Penicillium Italicum Isolated From Plastic Waste Site in Akure, Nigeria. *Int J Chem Res*. **8**(1) 10–4.

32. Sowmya H V, Ramalingappa, Krishnappa M and Thippeswamy B 2014, Degradation of polyethylene by *Trichoderma harzianum*—SEM, FTIR, and NMR analyses. *Environ Monit Assess*. **186**(10) 6577–86.
33. Ganesh P, Dineshraj D, Yoganathan K 2017, Production and screening of depolymerising enzymes by potential bacteria and fungi isolated from plastic waste dump yard sites. *Int J Appl Res*. **3**(3) 693–5.
34. Rippka R, Deruelles J, Waterbury JB 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol*. **111**(1) 1–61.
35. Afreen S, Bano F, Ahmad N, Fatma T 2017, Screening and optimization of laccase from cyanobacteria with its potential in decolorization of anthraquinonic dye Remazol Brilliant Blue R. *Biocatal Agric Biotechnol* . **10** 403–10. http://dx.doi.org/10.1016/j.bcab.2017.05.004
36. Desikachary TV 1959, *Cyanophyta*. I.C.A.R. New Delhi. India.
37. Kheti NK, Rath S and Thatoi H 2023, Screening and optimization of Manganese peroxidase (MnP) production by Pseudoduganella violacea (SMB4), a bacterial isolate from Similipal Biosphere Reserve, Odisha and evaluation of Maillard reaction products degradation. *Sustain Chem Environ*. **2** 100009. https://doi.org/10.1016/j.scenv.2023.100009
38. Kumar V and Chandra R. 2018, Characterisation of manganese peroxidase and laccase producing bacteria capable for degradation of sucrose glutamic acid-Maillard reaction products at different nutritional and environmental conditions. *World J Microbiol Biotechnol*. **34**(2) 1–18. http://dx.doi.org/10.1007/s11274-018-2416-9
39. El-Nahhal IM, Livage J, Zourab SM, Kodeh FS and Al swearky A 2015, Entrapment of phenol red (PR) pH indicator into sol–gel matrix in presence of some surfactants. *J Sol-Gel Sci Technol*. **75**(2) 313–22. http://dx.doi.org/10.1007/s10971-015-3702-8