Research Article

Polyethylene degradation by *Klebsiella pneumoniae* with thermostable lipase and lignolytic enzymes

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Abstract

Polyethylene is a synthetic plastic polymer that causes increasing terrestrial and aquatic pollution due to improper disposal. However, extracellular enzymes have been revealed to play key roles in polyethylene biodegradation. In this study, the growth of *Klebsiella pneumoniae in a* polyethylene-based medium (PBM) *was determined spectrophotometrically at 600 nm in a shaking incubator at pH 7.0, 37 ^{o}C, and 180 rpm. Production of li*pase, manganese peroxidase, and laccase by *K. pneumoniae* in PBM were screened and their physicochemical properties were evaluated. Furthermore, the biodegradation efficiency of *Klebsiella pneumoniae* on a PE sheet was determined using a Fourier transform infrared spectrophotometer (FTIR). The result indicated that the growth of *K. pneumoniae* was optimum on the fourth day with an optical density of 0.363. *K. pneumoniae* produced lipase and manganese peroxidase activities (2.64 U/mg and 6.89 U/mg respectively) optimally on the tenth day, while laccase activity (7.53 U/mg) was optimum on the eighth day. The physicochemical study showed lipase, manganese peroxidase, and laccase had optimum activity and were stable at pH 9, pH 7, and pH 5 with 84 %, 81 %, and 78% residual activities, respectively. Manganese peroxidase and laccase revealed optimum activity and stability at 70 ^{o}C, and 50 ^{o}C while lipase was optimally active at 60 ^{o}C and stable at 50 ^{o}C. Activities of MnP, Lac, and lipase were increased by Ca^{2+}, Mn^{2+} $Cu^{2+,}$ and Mg^{2+} and chelated by EDTA. The FTIR spectra of the biodegraded PE sheet revealed the presence of the aromatic OH group and carboxylic group. The result shows that *K. pneumoniae* can utilize PE as the source of carbon for growth and biodegrade PE sheets which was displayed by the production of thermostable lipase and lignolytic enzymes with new functional groups on the PE FTIR spectra. Hence, *K. pneumoniae* can be used for bio-remediating polyethylene and other environmental pollutants.

1. Introduction

Polyethylene (PE) is a synthetic plastic polymer synthesized by the polymerization reaction of ethene monomer [1]. The vast rising rates of PE production and utilization in industries and homes make polyethylene wastes enormously persistent in aquatic and terrestrial environments [2]. Landfilling, recycling, and incineration are some of the conventional ways of handling the PE menace but due to the cost of operations, alternate environmentally friendly methods are essential. Bioremediation techniques have evolved as a prominently effective method of ameliorating the impacts of water and land pollution caused by PE [3]. Biodegradation involves the application of biological agents that are readily available to remediate polyethylene wastes without the release of toxic end products into the environment [4]. Bacterial and fungal species have been unveiled to biodegrade polyethylene; these include the genera of

Bacillus [5], *Streptomyces* [3], *Pseudomonas* [6], *Aspergillus* [7] and *Penicillium* [3]. During PE biodegradation, abiotic factors act as biodeteriorating agents, altering the physical and chemical properties of polyethylene. This process is followed by the breaking of polyethylene polymer into smaller monomers by enzyme activities (bio-fragmentation) [8]. The short-chain monomers are then absorbed and metabolized into the microbial cells through the activities of intra- and extracellular enzymes. Finally, the monomers are mineralized into carbon dioxide, nitrogen gas, methane, and water with the release of adenosine triphosphate (ATP) [9].

Klebsiella pneumoniae is an opportunistic gram-negative bacterium of the Enterobacteriaceae family. It has been revealed to be effective in the treatment of polluted water [10], and the decomposition of citrate [11]. Moreover, *Klebsiella pneumoniae* has been reported to secrete extracellular enzymes such as lipase, tyrosinase, laccase, and peroxidase enzymes [12, 13], which are important for the biodegradation of PE [11].

Lipases occur widely in nature and have been produced from different microbial strains. Lipase has been used by several researchers for the hydrolysis of plastic polymers [14]. Likewise, lipase has also been revealed to catalyze, synthesize, and hydrolyze esters formed during polyethylene biocatalysis [14]. In addition, lignolytic enzymes are extracellular oxidative enzymes with diverse species-specific chemical compositions [15]. Examples include peroxidases (manganese peroxidase and lignin peroxidase) and oxidase (laccase) [16]. Studies have shown that these enzymes are not only biocatalysts responsible for the degradation of lignin and cellulosic materials but also environmental pollutants such as crude oil wastes, textile effluents, and synthetic plastic polymers [15]. Although lignolytic enzymes have been revealed to play an important role in polyethylene biodegradation, there are limited studies on the production and characterization of these enzymes from *Klebsiella pneumoniae* and the biodegradation potential of *Klebsiella pneumoniae* on untreated polyethylene sheets is scarce. Hence, this study aimed to produce and characterize lipase, manganese peroxidase (MnP), and laccase (Lac) from *K. pneumoniae* and biodegrade untreated polyethylene sheets.

2. Materials and methods

2.1. Materials
Media constituents and other chemicals used were of analytical grade and products of Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of polyethylene (PE) sheet
Polyethylene (PE) sheets were obtained from the water-producing company of the Federal University of Technology Akure, Ondo State, Nigeria. The PE sheets were confirmed as polyethylene by the Department of Chemistry, Elizade University, Ondo State, Nigeria. The PE sheets were manually minced and sieved through a 0.6 mm sieve to obtain uniform particles. The PE sheet and PE particles were decontaminated with distilled water and 80% ethanol for 40 min [17].

2.3. Microorganism
The microorganism used for this study was a bacterium isolated from a plastic-polluted dump site in Akure, Ondo State, Nigeria. The bacterium was molecularly identified as *Klebsiella pneumoniae* by the Bioscience Centre, International Institute of Tropical Agriculture (IITA) based on the 16S rRNA sequence. The bacterium was maintained on a nutrient agar slant and stored at $4°C$.

2.4. Production of lipase and lignolytic enzymes
Seed inoculum was prepared by growing a loopful of slant culture in sterile nutrient broth containing peptone (0.25 g/50 mL), NaCl (0.25 g/50 mL), beef extract (0.075 g/50 mL), and yeast extract (0.075 g/50 mL) incubated at pH 6.0, $37°C$ and 180 rpm for 24 h in a shaking incubator (Stuart, UK). Then, polyethylene-based medium (PBM) was prepared by introducing 1 % polyethylene powder as the sole carbon source into mineral salt medium containing NH_4NO_3 (0.4 g/200 mL), KH_2PO_4 (0.04 g/200 mL), $KH_2PO_4 \cdot 12H_2O$ (0.04 g/200 mL), NaCl (0.16 g/200 mL), KCl (0.16 g/200 mL), $CaCl_2 \cdot 2H_2O$ (0.02 g/200 mL), $MgSO_4$ (0.04 g/200 mL) and $FeSO_4 \cdot 7H_2O$ (0.0004 g/200 mL). Thereafter, five percent (5 % v/v) inoculum was taken from the seed culture and transferred to the PBM. The experiment set was replicated twice and incubated at 180 rpm, $37°C$, and pH 7.0 for 30 days to study the growth and enzyme production from *K. pneumoniae*.

Fresh cultures of *K. pneumoniae* were prepared for lipase, manganese peroxidase, and laccase production on PBM incubated at 37°C, pH 7.0, and 180 rpm. At the end of the cultivation period, the broths were centrifuged at 10,000 rpm for 20 min at 4°C. Clear supernatants were recovered and assayed for lipase, manganese peroxidase, and laccase activities.

2.5. Lipase assay

Lipase activity was quantified by measuring the release of p-NP palmitate (C16) in 50 mM Tris-HCl buffer at pH 8.0 [18]. The reaction mixture contained 50 mM Tris-HCl buffer (pH 8) with 0.1% Triton X-100 and 0.5 mM p-NP palmitate (C16) in methanol. Thereafter, 600 µl was withdrawn into a sterile test tube and 100 µl of the supernatant was added and incubated at 37 °C for 1 h. The release of 1 nM of p-NP in the reaction mixture was measured via a visible spectrophotometer at 405 nm (ε_{405}= 18.6 mM^{-1} cm^{-1}).

2.6. Manganese peroxidase assay

Manganese peroxidase (MnP) activity was determined based on the oxidation of phenol red in the demethylation reaction of MnP and phenol red in the presence of H_2O_2. The reaction mixture contained 50 mM sodium succinate buffer (2 mL), 50 mM sodium lactate (2 mL), 0.1 mM manganese sulfate (0.8 mL), 0.1 mM phenol red (1.4 mL), gelatine 2 mg/mL, and 1 mL of the supernatant. The reaction mixture (1 mL) was then introduced into 40 µL of 5 N NaOH and 50 µM H_2O_2 (0.4 mL) was added to initiate the reaction at 30 °C. The absorbance was measured through a visible spectrophotometer at 610 nm (ε_{610} = 22 mM^{-1} cm^{-1}) [19].

2.7. Laccase assay

The activity of laccase (Lac) was measured through the oxidation of 2 mM ABTS (2, 2' azino-di-[3-ethylbenzothiazoline-6-sulphonic acid) in 100 mM phosphate-citrate buffer at pH 4.0. The reaction mixture incorporates 2 mL ABTS and 2 mL of the supernatant. The formation of the ABTS cation was monitored by reading the absorbance of 2 mL of the reaction mixture using a visible spectrophotometer at 420 nm (ε_{420} = 36 mM^{-1} cm^{-1}) [20].

2.8. Protein content determination

The concentration of protein in the supernatant was evaluated using bovine serum albumin (BSA) as a standard [20]. The reaction mixture comprises a diluted dye reagent pipetted into a test tube containing the supernatant, which was then incubated for color development at 27°C for 15 mins. The absorbance of the reaction mixture was measured at 595 nm against the blank. The result was used to calculate the specific activities of the enzymes.

2.9. Characterization of lipase, MnP and Lac from K. pneumoniae

The effect of pH on the activities of MnP, Lac, and lipase was studied using the following buffers: glycine-HCl (pH 3.0); sodium acetate (pH 5.0); Tris-HCl (pH 7.0), and glycine-NaOH (pH 9.0 and pH 11.0). The stability of the enzymes in the buffers was investigated by incubating them at 27 °C for 180 mins. In addition, the effect of temperature on the enzymes was determined in a range from 30 °C to 80 °C. The stability of the enzymes at the temperatures was obtained by incubating the enzymes for 180 mins [8]. Furthermore, the effect of metallic chlorides (Ca^{2+}, Cu^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}) of varying concentrations (1, 5, and 10 mM), and the metal chelating effect of ethylene diamine tetraacetic acid (EDTA) on the activities of MnP, lipase, and Lac was analyzed. The activities and stabilities of the enzymes were calculated using their respective protocols described above.

2.10. Biodegradation studies of polyethylene sheet

A polyethylene (PE) sheet that was not pre-treated with either UV radiation or heating was used for this study. Pre-weighed 0.2 g of polyethylene (PE) sheets (3 X 2 cm) were sterilized by soaking in 80% (v/v) ethanol and rinsed in a conical flask containing sterile distilled water. The pre-weighed PE sheet was then introduced into a conical flask containing a sterile nutrient medium with a loop full of *S. marcescens*. The conical flask containing only sterile nutrient medium and pre-weighed PE sheets served as a control. The flasks were firmly corked with aseptic cotton wool covered with aluminum foil and incubated at pH 7.0 and 50 °C in a shaking incubator at 180 rpm for 30 days. All experimental setups were in triplicate. At the end of the experimental period, the degree of biodegradation of the PE sheet was assessed with a Fourier transform infrared (FTIR) spectrophotometer to determine bond variations in the structure of the biodegraded PE sheet compared to the control PE sheet [11].

2.11. Statistical analysis

The data were analyzed using a one-way analysis of variance and presented as mean ± standard deviation. The mean values were correlated with the Duncan test and version 16 of the statistical package for social sciences (SPSS) by IBM.

3. Results and discussion

3.1. Growth of K. pneumoniae and screening of enzyme production

The growth of *K. pneumoniae* under polyethylene-based media (PBM) was progressive. At twenty-four hours (24 h) of exposure, the absorbance was 0.144 and increased to 0.189 after seventy-two hours (72 h). An optimum growth absorbance of 0.363 was obtained at ninety-eight (98) hours of exposure to PBM and *K. pneumoniae* entered the death phase after six days (Fig. 1a). The growth recorded by *K. pneumoniae* showed its ability to utilize polyethylene as a source of carbon needed for growth and other metabolic activities under mesophilic conditions. According to [21] bacterial strains isolated from dump sites are mesophilic because temperatures at dump sites in temperate regions are generally ambient. Previous studies by [9] and [3] reported that extracellular enzymes produced by microbial strains exposed to plastic polymers during biodegradation play important roles in the plastic polymer breakdown into smaller subunits. These plastic monomers are easily assimilated into microbial cells and utilized as carbon sources, leading to the production of energy, water, and carbon dioxide. In this study lipase, manganese peroxidase (MnP), and laccase activities produced by *K. pneumoniae* in PBM were screened. Lipase activity was optimum on the tenth day with a specific activity of 2.64 U/mg (Fig.1b). Also, lipase activity produced by *Pseudomonas* spp [22]. During PET and LDPE degradation, it discovered that lipase activity was lower during polyethylene degradation. Lipase activity reported in this study was also lower compared to laccase and manganese peroxidase activities, this may be a result of the hydrophobic nature of PE derivatives that is caused by inadequate C-O bonds. There was minimal biofilm formation and adhesion required for optimum active-site binding of lipase, however, there was increased biofilm formation and adhesion by the
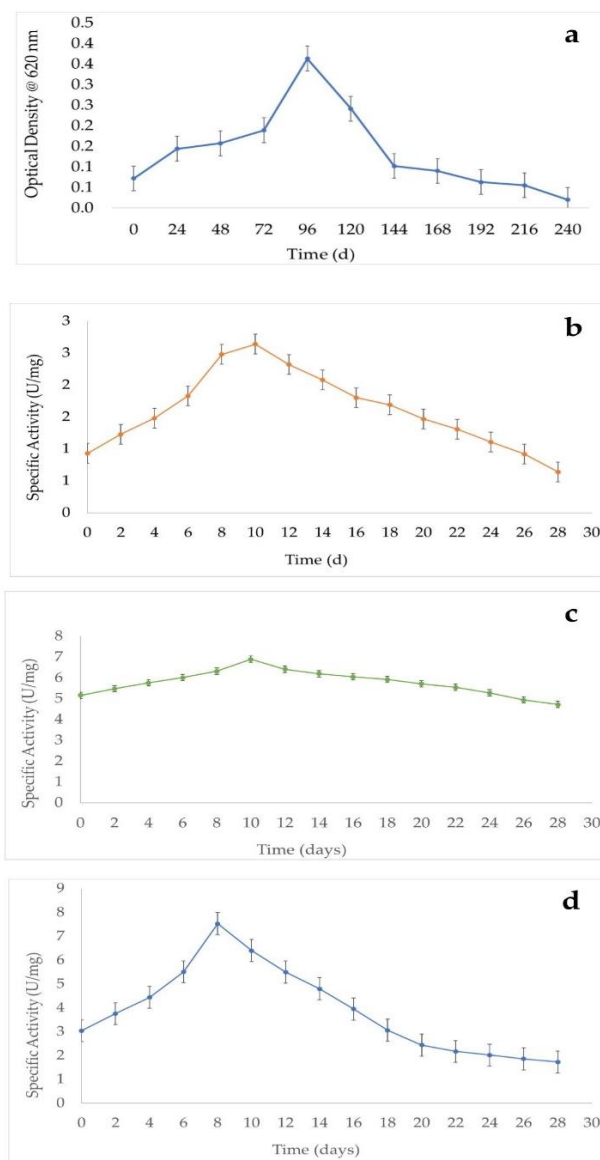

Figure 1. (a) Growth profile of *K. pneumoniae* in PBM, and Production of (b) Lipase, (c) Manganese peroxidase, and (d) Laccase from *K. pneumoniae* (*Error bars* represent Mean ± standard deviation

lignolytic enzymes. According to [23] and [15] oxidoreductases (manganese peroxidase and lignin peroxidase) are involved in the biodegradation of PE. Manganese peroxidase activity was optimum on the tenth day with 6.*89* U/mg specific activity (Fig. 1c) in PBM. A previous study by [24] reported the biodegradation of polyethylene by *Bacillus cereus* due to its ability to produce manganese peroxidase. Also, it was reported [25] that the degradation of a high molecular weight PE membrane by *P. chrysosporium* ME-446 due to MnP activity. Laccase is an important extracellular enzyme involved in lignin degradation
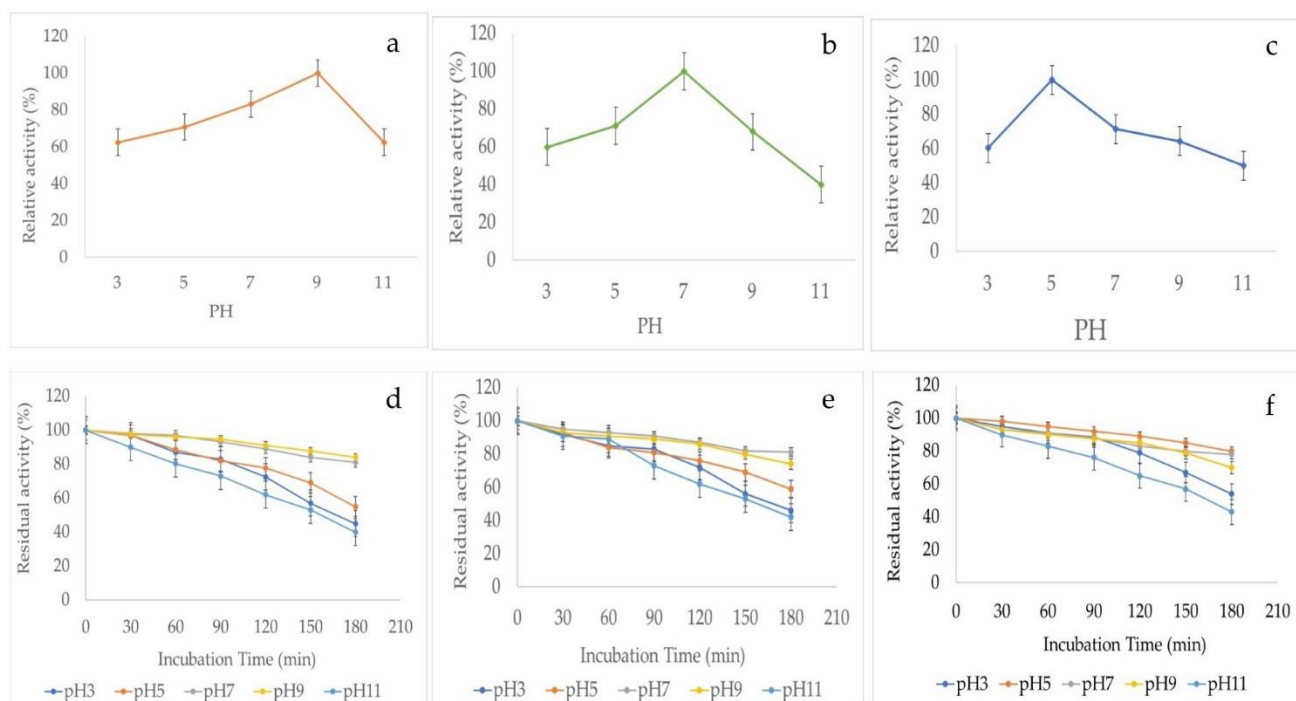
Figure 2. Effect of pH on the activity of (a) Lipase, (b) Manganese peroxidase, (c) Laccase and stability of (d) Lipase, (e) Manganese peroxidase, and (f) Laccase produced from *K. pneumonia*e (*Error bars* represent Mean ± standard deviation).

in combination with manganese peroxidases [20]. However, it has been studied for application in the bioremediation of recalcitrant polymers, including polyethylene [23, 15]. In this study, laccase activity was optimum on the eighth day with an activity of *7.53* U/mg (Fig. 1d) in the PBM. According to [26], the production of laccase increased when microbes were near polyethylene using it as a carbon source. Moreover, [21] was also able to produce crude laccase when bacterial isolates were incubated with polyethylene. Likewise, [25] observed a reduction in the molecular weight of polyethylene membranes after treatment with laccase from *Trametes spp.*

3.2. Effect of pH on activity and stability of lipase, MnP, and laccase

Enzymes are sensitive to the pH of the culture medium, as sudden alteration affects the Ionization state, activity, and substrate selectivity of the enzymes [27, 28]. The pH activity profile of lipase from *K. pneumoniae* showed lipase was active over a broad pH range (pH 3.0 to pH 11.0). However, optimum lipase activity was obtained at pH 9.0 At pH 3.0, 62 % relative activity was observed, which increased to 70 %, at pH 5.0. (Fig. 2a). Similarly, lipase exhibited optimum stability at pH 9.0 with 84 %

residual activity when incubated for 180 mins at room temperature. Forty-five percent (45 %), 55 %, 70 %, and 40 % residual activities were obtained at pH 3.0, pH 5.0, pH 7.0, and pH 11.0 respectively (Fig. 2d). Lipase was optimally active at pH 9.0 because the alkaline buffer solution helped to improve the reaction and hydrolysis of bonds present in PE and was stable at pH 9.0 because it maintained lipase activity for a longer time [29]. A previous study by [30] reported that lipase produced by bacterial strains is mostly effective in an alkaline medium. Lipase purified from *B. methylotrophicus* PS3 was also used for polyethylene degradation and was reported to be stable at pH 7.0, and was active between pH 7 to pH 9 [31].

Manganese peroxidase (MnP) produced by *K. pneumoniae* also showed a wide pH activity between pH 3.0 to pH 11.0 with optimum MnP activity at pH 7.0. At pH 3.0 MnP relative activity was 62 %, increased to 70 % at pH 7.0, and declined to 40 % at pH 11.0 (Fig. 2b). Optimum manganese peroxidase stability was obtained at pH 7.0 with about 81% residual activity when incubated for 180 min at room temperature. Similarly, the residual activities of 45 %, 59 %, 74 %, and 42 % were obtained at pH 5.0, pH 9.0, and pH 11.0 respectively (Fig. 2e).
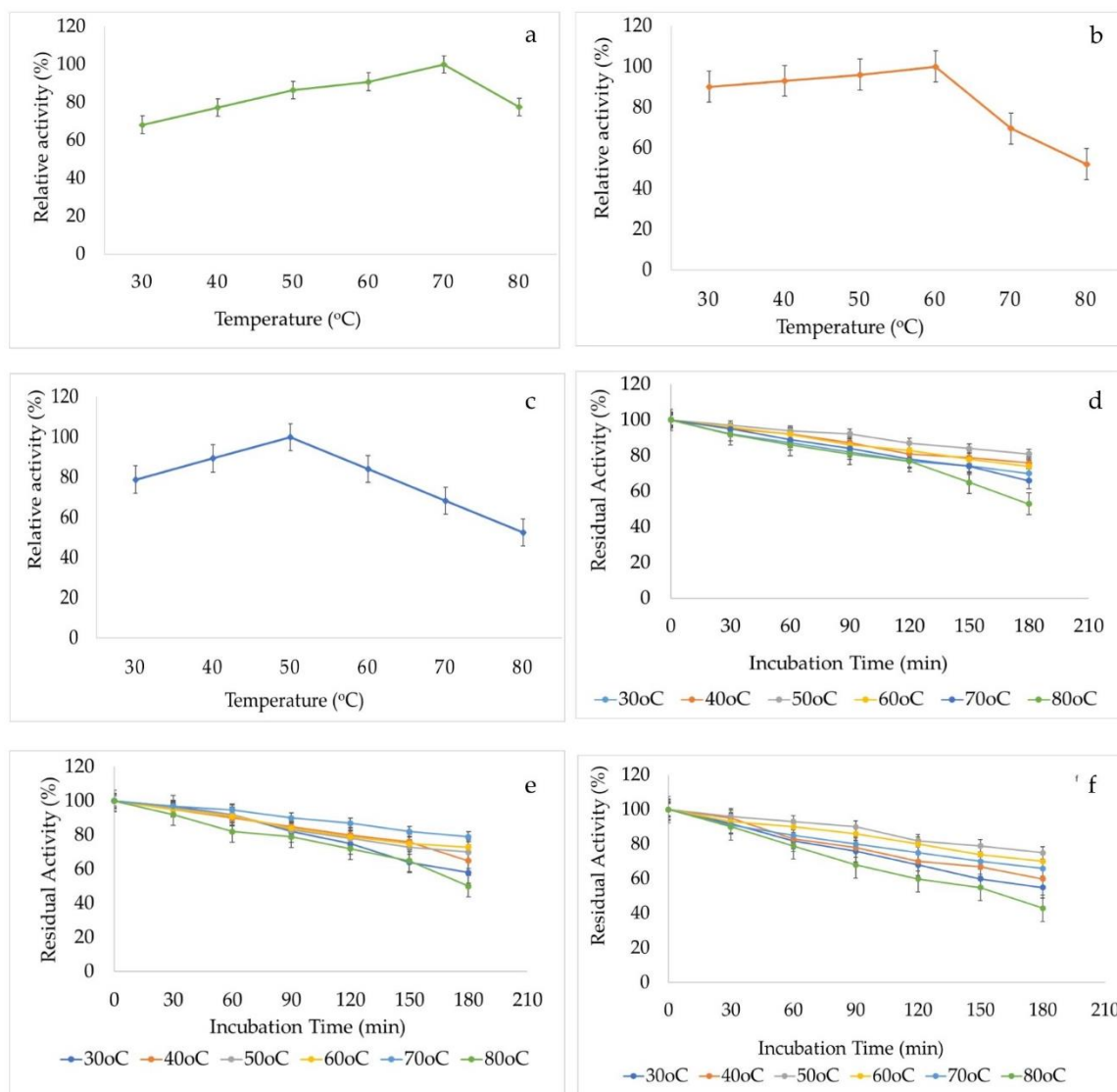
Figure 3. Effect of temperature on the activity of (a) Lipase, (b) Manganese peroxidase (c) Laccase and stability of (d) Lipase, (e) Manganese peroxidase, and (f) Laccase produced from *K. pneumonia*e (*Error bars represent Mean ± standard deviation*).

The activity and stability of MnP were optimum at neutral pH because the neutral pH aids the production of hydrogen bonds and salt bridges, which stabilizes the heme pocket of MnP and enhances MnP activity [32].

Laccase activity was optimum at pH 5.0 (Fig. 2c). Reduction in activity was recorded from pH 7.0 (71 %) to pH 11.0 (50 %). Laccase showed optimum stability at pH 5.0 with about 84% residual activity when incubated for 180 mins at room temperature. Moreover, 78%, 70 %, and 43 % residual activities were obtained at pH 7.0, pH 9.0, and pH 11.0 respectively (Fig. 2f). Laccase activity was optimum at pH 5.0 because acidic pH enhances the oxidization of

substrate by laccase, and was stable at the pH because acidic pH maintains the stability and structural integrity and catalytic potentials of laccase [33]. Studies [34] also opined that laccase with optimum activity in acidic pH is efficient in the biodegradation of recalcitrant polymers.

3.3. Effect of temperature on activity and stability of lipase, MnP, and laccase

The optimum temperature for the activity of lipase from *k. pneumoniae* was 60 °C as shown in Fig. 3a. Lipase activity increased gradually with an increase in temperature within the range of 30 °C to 60 °C. A reduction in enzyme activity was observed at temperatures above 60 °C (Fig. 3a). The enzyme

exhibited 69 % of optimum activity at 70 °C and still retained 52 % of its optimum activity at 80 °C. Lipase exhibited optimum stability at 50 °C with about 81% residual activity when incubated for 180 mins at room temperature, while 75 %, 66 %, and 58 % residual activities were obtained at 60, 70 and 80 °C respectively (Fig. 3d). Lipase activity was optimum at 60 °C because the structure of lipase is more rigid at elevated temperatures, and this helps to maintain the 3-dimensional structure of lipase, which results in maximum activity and stability [35]. The optimum temperature for lipase activity exposed to low-density polyethylene was also obtained at 50 °C [36]. Likewise, the temperature of lipase from *P. aeruginosa* EF2 used for polyethylene degradation was reported to be 50°C [36].

The effects of temperature on MnP activity of *K. pneumoniae* are illustrated in Fig. 3b. The optimum temperature for MnP activity was 70 °C. Manganese peroxidase showed optimum activity at 70 °C and 77 % as relative activity of 77 % was obtained at 80 °C (Fig. 3b). After 3 hours of incubation of the enzyme at different temperatures, the enzyme was most stable at 70 °C retaining approximately 79% of its activity, while it showed 77.5 % residual activity at temperatures of 80 °C respectively. (Fig. 3e). *K. pneumoniae* produced MnP with optimum activity and stability at 70 °C because of the presence of rigid thermophilic amino acid sequences in MnP structure, which are structurally adapted to withstand high temperatures without being denatured [37]. MnP activity was reported to increase at higher temperatures as a result of the strengthening of MnP protein molecule structure when it binds to the solid support, reducing its molecular flexibility [38].

The optimum temperature for laccase activity was obtained at 50 °C. Reduction in relative activity was recorded from 80 % to 53 % at 60 °C and 80 °C (Fig. 3c). After 3 hours of incubation of enzyme solution at different temperatures, the enzyme was most stable at 50 °C retaining approximately 75% of its activity while 52.6 % residual activity was observed at temperatures of 30 °C. (Fig. 3f). Laccase was active and stable at 50 °C because high temperatures increase the kinetic energy of laccase and result in frequent and effective reactions between laccase and the substrate (PE) [39]. Laccase with 50 °C optimum activity and laccase with 60°C optimum temperature were also observed [40, 41].

3.4. Effect of metal ions and EDTA on activity of lipase, MnP, and laccase

Manganese peroxidase produced by *K. pneumoniae* increased in activity by 130 ± 0.2 %, and 170 ± 0.22 % in 10 mM Ca^{2+}, and Mn^{2+} solution (Fig. 4a) while there was a decrease in MnP activity in Mg^{2+}, Ba^{2+}, Cu^{2+} and EDTA solution (94 ± 0.3 %, 98 ± 0.2 %, 41 ± 0.3 %, and 30 ± 0.2 % respectively). Calcium ion (Ca^{2+}) increased MnP activity because of the structural stability which enhanced the interaction of MnP with PE. Likewise, MnP activity was elevated by Mn^{2+} because it facilitates the oxidation of Mn (II) to Mn (III) due to the binding site, in the active site of MnP [42]. EDTA inhibited MnP activity as a result of its binding to the metal ions present in the active site which limited the affinity of MnP for the substrate and led to the deactivation of MnP [8]. Previous studies also reported EDTA to inhibit the activity of MnP [20].

Similarly, laccase activity produced from *K. pneumoniae* was significantly enhanced by 10 mM Cu^{2+} (137 ± 0.02 %), and Ca^{2+} (120 ± 0.04 %) (Fig. 4b). The metal ions most especially Cu^{2+} increased laccase activity due to the ability to activate and stabilize laccase structure as it increases the oxidation reaction between laccase and the PE and enhances the conversion of oxygen to water [43]. Mg^{2+} and Cu^{2+} have been previously revealed to increase the activity of laccase from *Bacillus spp* [44]. In addition, Ca^{2+} enhanced laccase activity because it stabilizes the copper center of laccase enhancing the catalytic activity [45]. Laccase activity was lower in Mg^{2+}, Mn^{2+}, Ba^{2+}, Hg^{2+} solution and relative activities of 50 ± 0.04 %, 21 ± 0.04 %, 30 ± 0.04 %, and 32 ± 0.04 % were obtained respectively. Laccase activity was also significantly reduced by 1 mM, 5 mM, and 10 mM EDTA (26 ± 0.02 %, 10.6 ± 0.03 % and 10.6 ± 0.02 % respectively). These metal ions (Ba^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}) reduced the activity of Lac because when they bind to the active site of laccase they cause conformational changes, prevent substrate binding and finally inhibit Lac activity [46]. *Pseudomonas spp* was also reported to produce laccase with reduced activity in Mn^{2+}, Ca^{2+}, and EDTA solution [46].

In addition, lipase activity was elevated by 10 mM Ca^{2+} (124 ± 0.03 %), and Mg^{2+} (125 ± 0.04 %) (Fig. 4c).
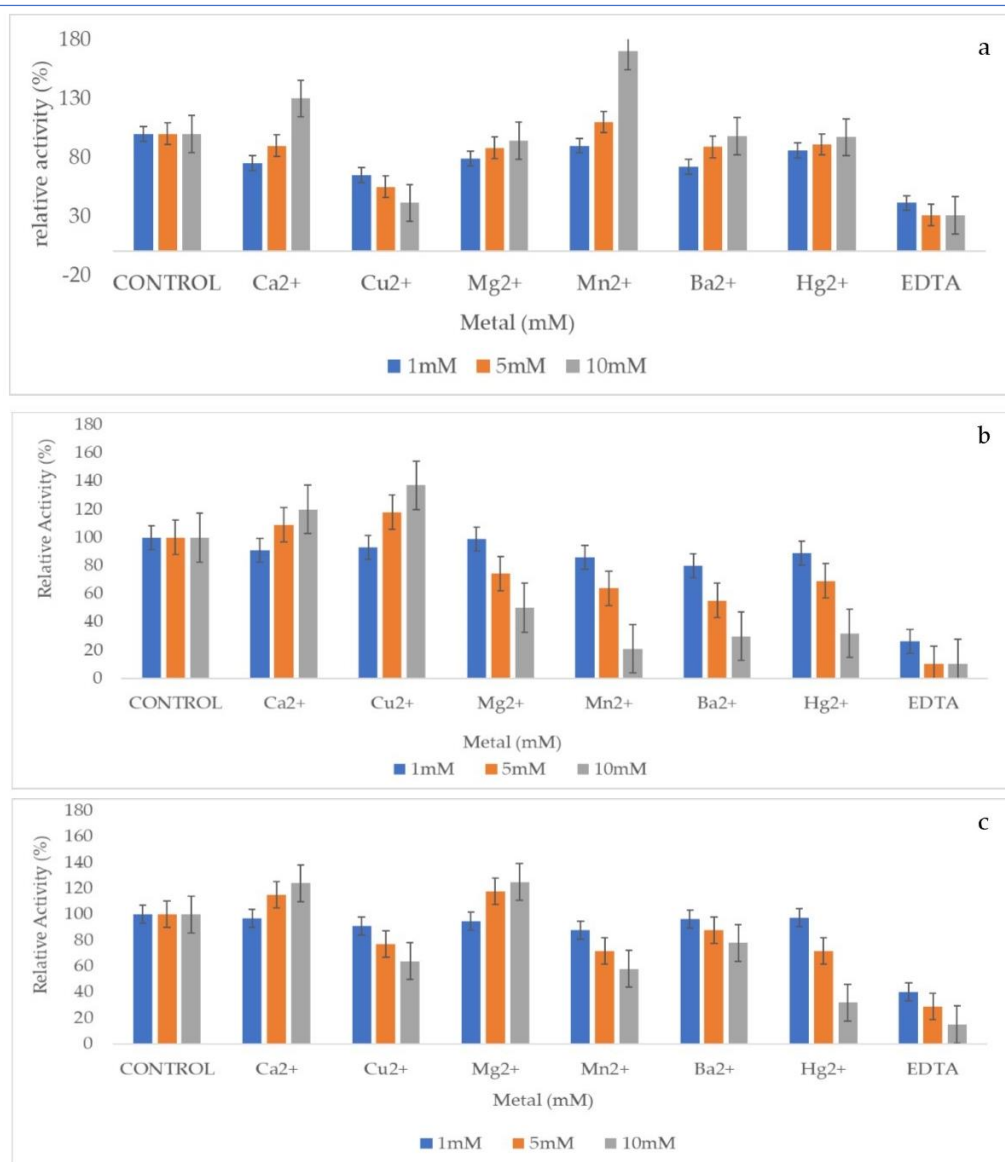
Figure 4. Effect of metal ions on (a) Manganese Peroxidase activity, (b) Laccase activity, and (c) Lipase activity *K. pneumoniae* (*Error bars* represent Mean ± standard deviation).

The metal ions (Ca^{2+}, and Mg^{2+}) enhanced lipase activity because they help to stabilize the three-dimensional structure of lipase, ensuring that the enzyme maintains its active conformation, which is crucial for catalytic activity [47].

There was a significant reduction in lipase activity in Cu^{2+} (64 ±0.02 %), Mn^{2+} (58 ±0.02 %), and Hg^{2+} (32 ±0.03 %) solutions because they bind to the enzyme active site, hindering substrate binding, and thus inhibit the enzyme's activity [47]. EDTA also chelated the activity of lipase and 15±0.03 % relative activity was recorded.

3.5. Biodegradation study

The FTIR spectra of the untreated PE sheet showed a shift to the left in peaks from 457-719 nm (C-H out-of-plane bending vibration of the substituted amide group) to 466-719 nm when compared with the control PE sheet (Fig. 5). There was also a shift to the right at 873 nm (the skeletal C-C vibrations), 1062 nm (the alky substituted ether group); 1313 nm (the O-H plane bend); 1541 nm (the secondary amide (NH) bend); 1377 nm (the symmetric methyl (-CH3) bend), and 1313 nm (the O-H plane bend) to 773 nm, 1066 nm, 3431 nm, 1471 nm, 1303 nm, and 1176 nm respectively, when compared with the control PE sheet. In addition, new peaks were observed at the wavelength of 2918 nm (corresponding to C-C stretch aromatics) and 3443 nm (signaling the hydrogen-bonded O-H stretch of
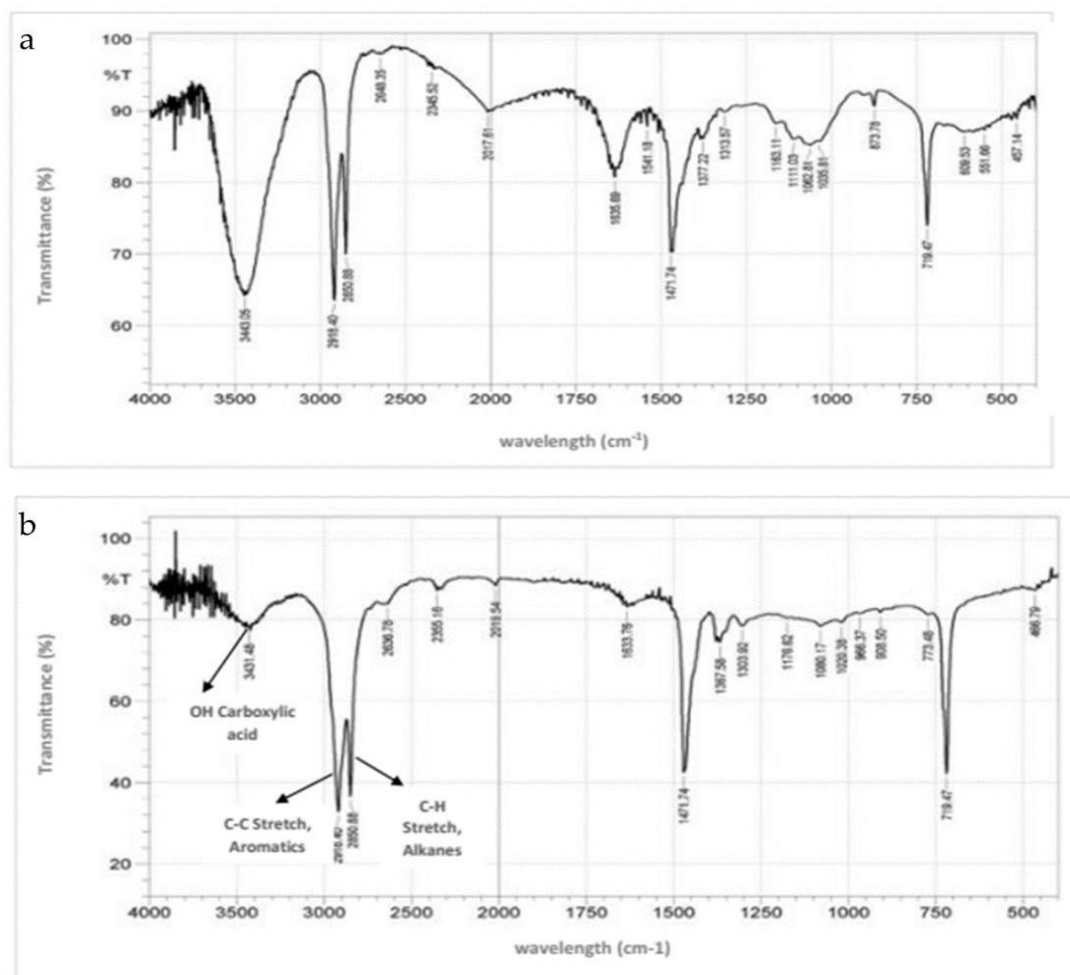
Figure 5. Showing the FTIR spectra of (a) control PE sheet, and (b) PE sheet degraded by *K. pneumoniae*

carboxylic acid).

The FTIR spectra result shows that *K. pneumoniae* degraded PE (untreated) as new functional groups such as aromatic OH group and carboxylic group (-COOH) were observed in the PE sheet, which are products of the PE hydrolysis and oxidation [48]. However, this result is in contrast to the report of [49] who concluded that only pre-treated PE sheets can be degraded by microorganisms.

4. Conclusions

In conclusion, *Klebsiella pneumoniae* produced thermostable MnP, Lac, and lipase. The enzymes also showed optimum activity and stability over a broad range of pH. MnP activity was increased by Ca^{2+} and Mn^{2+}, laccase activity was enhanced by Ca^{2+}, and Cu^{2+}, while lipase activity was elevated by Ca^{2+}, and Mg^{2+}. After biodegradation over 30 days, the FTIR spectra of the PE sheet showed shiftings and formation of new

peaks at different wavelengths, revealing changes in functional groups when compared with the control. These results show the unique properties of MnP, laccase and lipase produced by *Klebsiella pneumoniae* and reveal the efficiency of *Klebsiella pneumoniae* in the PE biodegradation process. Hence, we recommend culturing *Klebsiella pneumoniae* for effective biodegradation of plastics, most especially polyethylene.

Authors' contributions

Conducted the research and wrote the manuscript, O.D.E.; Isolated the bacterium and wrote the manuscript, M.A.O.

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Availability of data and materials

The data used to support the findings of this study can be obtained from the corresponding author upon request.

Conflicts of interest

The author declares no conflict of interest.

References

1. Hiraga, K.; Taniguchi, I.; Yoshida, S.; Kimura, Y.; Oda, K. Biodegradation of waste PET: a sustainable solution for dealing with plastic pollution. Appl. Microbiol. biotechnol. 2019, 20, 49365. http://dx.doi.org/10.15252/embr.201949365

2. Liu Ren, L.M.; Zhiwei, Z.; Feifei, G.; Jian, T.; Bin, W.; Jihua, W.; et al. Biodegradation of polyethylene by *Enterobacter* sp. D1 from the guts of wax moth *Galleria mellonella.* Int. J. Environ. Res. Pub. 2019, 16, 19-41. https://doi.org/10.3390/ijerph16111941

3. Danso, D.; Jennifer, C.; Wolfgang, R.S. Plastics: microbial degradation, environmental and biotechnological perspectives. Appl. Environ. Microbiol. 2019, 10, 1109-1128. https://doi.org/10.1128/aem.01095-19

4. Taniguchi, I.; Yoshida, S.; Hiraga, K.; Miyamoto, K.; Kimura, Y.; Oda, K. Biodegradation of PET: Current status and application aspects. Catalyst. 2019, 9, 4089–4105. https://doi.org/10.1021/acscatal.8b05171

5. Zuriash, M.N.; Nurelegne, T.S.; Mesfin, T.G. Isolation and screening of low-density polyethylene (LDPE) bags degrading bacteria from Addis Ababa municipal solid waste disposal site. Ann. Microbiol. 2023, 73, 6. https://doi.org/10.1186/s13213-023-01711

6. Hou, L.; Xi, J.; Liu, J.; Wang, P.; Xu, T.; Liu, T.; et al. Biodegradability of polyethylene mulching film by two pseudomonas bacteria and their potential degradation mechanism. Chemosphere. 2022, 286, 131758. https://doi.org/10.1016/j.chemosphere.2021.131758

7. Gajendiran, A.; Subramani S.; Abraham, J. Effect of *Aspergillus versicolor* strain JASS1 on low-density polyethylene degradation. Mat. Sci. Eng. 2017, 263:022038. https://doi.org/10.1088/1757-899x/263/2/022038

8. Ogunjemite, O.E.; Dada, O.; Tosin, A.O. Characterization of thermostable ligninolytic enzymes from *penicillium italicum* during biomineralization of polyethylene. Asian J. Microbial. Biotechnol. 2023, 8(1), 59–69. https://doi.org/10.56557 /ajmab/2023/v8i18195

9. Pathak, V.M.; Navneet, O. Review on the current status of polymer degradation: A microbial approach. Biores. Biopro. 2017, 4, 15. https://doi.org/10.1186/s40643-017-0145-9

10. Maal, K.B.; Delfan, A.S.; Salmanizadeh, S. Isolation identification of *Klebsiella pneumonia* and *Klebsiella oxytoca* bacteriophages and their applications in waste water treatment and coliform's phage therapy. Res. J. Environ. Sci, 2014. 8(3), 123–133. http://doi:10.3923/rjes.2014.123.133.

11. Awasthi, S.; Srivastava, P.; Singh, P.; Tiwary, D.; Mishra, P.K. Biodegradation of thermally treated high-density polyethylene (HDPE) by *Klebsiella pneumonia* CH001. Biotech. 2017, 7(5), 332. https://doi. org/10.1007/s13205-017-0959-3

12. Ijeoma, V.; Nwaguma, C.; Blaise, C.; Gideon, C.O. Isolation, characterization, and application of biosurfactant by *Klebsiella pneumoniae* strain IVN51 isolated from hydrocarbon-polluted soil in Ogoniland, Nigeria. Biores. Biopro. 2016, 3, 116-138. https://doi.org/10.1186/s40643-016-0118-4

13. Peil, G.H.S.; Kuss, A.V.; Rave, A.F.G.; Villarreal, J.P.V.; Hernandes, Y.M.L.; Nascente, P.S. Bio-prospecting of lipolytic microorganisms obtained from industrial effluents. Ann. Brazil. Acad. Sci. 2016. https://doi.org/10.1590/0001-3765201620150550

14. Maurya, A.; Amrik, B.; Sunil, K.K. Enzymatic remediation of polyethylene terephthalate (PET)–based polymers for effective management of plastic wastes: an overview. Front. Bioeng. Biotechnol. 2020, 19, 245-255. https://doi.org/10.3389/fbioe.2020.602325

15. Ogunjemite, O.E.; Zige, T.; Momoh, A.O. Production and characterization of thermostable lignolytic enzymes produced from *Staphylococcus saprophyticus* exposed to low-density polyethylene. J. Biochem. Inter. 2022, 9(4), 18-27. https://doi.org/10.56557/jobi/2022/v9i47593

16. Chris, L.P.; Yao, B.; Ji, Y. Lignin biodegradation with laccase-mediator systems. Front. Energy Res. 2015, 51, 123-134. https://doi.org/10.3389/fenrg.2014.00012

17. Azeko, S.T.; Etuk-Udo, G.A.; Odusanya, O.S.; Malatesta, K.; Anuku, N.; Soboyejo, W.O. Biodegradation of linear low-density polyethylene by *Serratia marcescens* subsp. *marcescens* and its cell-free extracts. Waste Biom. Val. 2015, 6, 1047–1057. https://doi.org/10.1007/s12649-015-

9421-0.

18. Ramnatha, D.; Eiteljoerg, I.; Trotscha, E.; Fred, G. Identification of lipolytic enzymes isolated from bacteria indigenous to Eucalyptus wood species for application in the pulping industry. Biotechnol. Rep. 2017, 15, 114–124. https://doi.org/10.1016/j.btre.2017. 07.004.

19. Kheti, N.K.; Rath, S; Thatoi, H. 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. Sus. Chem. Environ. 2023, 2, 100009. https://doi.org/10.1016/j.scenv.2023.100009

20. Olajuyigbe, F.M.; Ehiosun, K.; Jaiyesimi, K.F. Preliminary study towards enhanced crude oil biodegradation reveals congeneric total peroxidases with striking distinctions. Adv. in Enz. Res. 2016, 3, 66-74. http://dx.doi.org/10.4236/aer.2015.33007

21. Christabel, N.M.; Gabriel, M.; Mabel, I.; Huxley, M.M. Molecular characterization of low-density polyethene (ldpe) degrading bacteria and fungi from Dandora dumpsite, Nairobi, Kenya. Inter. J. Microbiol. 2018, 18: 416-434. https://doi.org/10.1155/2018/4167845

22. Morgan, V.; Gayle, C.; Cameron, R.; Natasja, A.S.; Jay, L.M. *Pseudomonas* isolates degrade and form biofilms on polyethylene terephthalate (PET) plastic, Inter. J. Microbiol. 2019, 11, 104-127. https://doi.org/10.1101/647321

23. Krueger, M.C.; Harms, H.; Schlosser, D. Prospects for microbiological solutions to environmental pollution with plastics. App Microbiol. Biotech. 2015, 99, 8857–8874. https://doi.org/10.1007/s00253-015-6879-4

24. Sowmya, H.V.; Krishnappa, M.; Thippeswamy, B. Degradation of polyethylene by *Trichoderma harzianum*. Inter. J. Adv. Polymer. Sci. Technol. 2015, 4: 28–32. https://doi.org/10.1007/s10661-014-3875-6

25. Sunil, G.; Youri, Y.; Jae-Hyung, A.; Hor-Gil, H. Biodegradation of polyethylene: a brief review. App. Bio. Chem. 2020, 63, 27. https://doi.org/10.1186/s13765-020-00511-3

26. El-Morsy, E.; Ahmed, E. Biodegradative activities of fungal isolates from plastic contaminated soils. Mycosphere. 2017, 8(8), 1071–1086. https://doi.org/10. 5943/mycosphere/8/8/13

27. Ezugwu A.L.; Ottah, V. E.; Eze, S.O.O.; Chilaka, F.C. Effect of pH, various divalent metal ion and different substrates on glucoamylase activity obtained from *Aspergillus niger* using amylopectin from tiger nut starch as carbon source. African. J. Biotechnol. 2016, 15(21), 980-988. https://doi.org/10.5897/ajb2015.14886

28. Daichi, T.; Yasuhito, Y.; Go, I.; Takahiro, M.; Tetsushi,

M. Abiotic factors promote cell penetrating peptide permeability in enterobacteriaceae models. Front. Microbiol. 2019, 10, 2534. https://doi.org/10.3389/fmicb. 2019.02534

29. Anil, K.; Srijana, M.; Neeraj, K. A broad temperature active lipase purified from a psychrotrophic bacterium of *Sikkim himalaya* with potential application in detergent formulation. Front. Bioeng. Biotechnol. 2020, 25. https://doi.org/10.3389/fbioe.2020.00642

30. Leelatulasi, S.; Raja, N.Z.; Raja, A.R.; Abu, B.S.; Fairolniza, M.; Shariff, P.C.; et al. Isolation, characterization, and lipase production of a cold-adapted bacterial strain *Pseudomonas* sp. LSK25 isolated from signy island, antarctica. Molecules. 2019, 24, 715. https://doi.org/10.3390/molecules24040715

31. Mobarak, O.E.; Kasra-Kermanshahi, R.; Moosavi-nejad, Z. Partial purification, characterization and immobilization of a novel lipase from a native isolate of Lactobacillus fermentum. J. Microbiol. 2021, 3 (2), 92-98. https://doi.org/10.18502/ijm.v13i6.8093

32. Verónica, S.J.; Elena, F.F.; Francisco, J.M.; Antonio, R..; Angel, T.; Martínez, F.J.; Ruiz, D. Improving the pH-stability of versatile peroxidase by comparative structural analysis with a naturally-stable manganese peroxidase. Plos One. 2021, 10(11), e0143267. https://doi.org/10.1371/journal.pone.0143267

33. Yue, S.; Zi Lu, L.; Bo-Yang, H.; Qing-Jun, C. Purification and characterization of a thermo- and pH-stable laccase from the litter-decomposing fungus *gymnopus luxurians* and laccase mediator systems for dye decolorization. Front. Microbiol. 2021, 12, 2021. https://doi.org/10.3389/fmicb.2021.672620.

34. Muthusamy, G.; Soichiro, F.; Toshiki, H.; Young-Cheol, C. Biodegradation of aliphatic and aromatic hydrocarbons using the filamentous fungus Penicillium sp. CHY-2 and characterization of its manganese peroxidase activity. Royal. Soc. Chem. Adv. 2017, 7, 207- 216. https://doi.org/10.1039/c6ra28687a

35. Nivedita, J.; Pundrik, J. Thermostable α-amylases and laccases: Paving the way for sustainable industrial applications. Processes. 2024, 12(7), 1341. https://doi. org/10.3390/pr12071341

36. Gururaj, P.; Subramanian, R.; Ganesan, N.D.; Pennathur, G. Process optimization for production and purification of a thermostable, organic solvent tolerant lipase from *Acinetobacter* sp. AU07. Brazilian. J. Microbiol. 2016, 47, 647– 657. https://doi.org/10.1016/ j.bjm.2015.04.002

37. Plácido, J.; Capareda, S. Ligninolytic enzymes: A biotechnological alternative for bioethanol production. Bioresource. Bioprocess. 2020, 2(23). https://doi.org/10.1186/s40643-015-0049-5.

38. Bilal, M..; Iqbal, M..; Zhang, X. Characteristic features and dye degrading capability of agar-agar gel immobilized manganese peroxidase. Inter. J. Biol. Macromol. 2016, 6, 28-40. https://doi.org/10.1016/j.ijbiomac.2016.02.014

39. Mahuri, M.; Paul, M.; Thatoi, H. A review of microbial laccase production and activity toward different biotechnological applications. Sys. Microbiol. Bioman. 2023, 3, 533–551. https://doi.org/10.1007/s43393-023-00163-6

40. Vignesh, R.; Madhusudhanan, J.; Gandhiraj, V.; Deepika, R. Screening and characterization of laccase from fungal isolates. WVSU. Res. J. 2022, 11(1), 36-50. https://doi.org/10.59460/wvsurjvol11iss1pp36-50

41. Wang, C.; Min, Z.; De-Bin, L.; Dai-Zong, C.; Lei, L.; Xing-Dong, W. Isolation and characterization of a novel *Bacillus subtilis* WD23 exhibiting laccase activity from forest soil. Adv. Mat. Res. 2016, 9(34), 496-5502. https://doi.org/10.4028/www.scientific.net/amr.113-116.725

42. Zhang, D.; Kong, L.; Wang, W.; Xia, C.; Yao, J.; Wu, W. Degradation of UV-pretreated polyolefins by latex clearing protein from *Streptomyces* sp. Strain K30. Sci. Total Environ. 2021, 806, 150779. https://doi.org/10.1016/j.scitotenv.2021.150779

43. Wei, R.; Zimmermann, W. Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? Micro. Biotechnol. 2017, 68, 123-134. http://dx.doi.org/10.1111/1751-7915.12710

44. Arregui, L.; Ayala, M.; Gómez-Gil, X. Laccases: Structure, function, and potential application in water bioremediation. Microbial Cell Fact. 2019, 18 (200). https://doi.org/10.1186/s12934-019-1248-0

45. Ivan, B.; Erica, E.F.; Sergio, R.; Daniela, M. Biocatalysis with laccases: an updated overview. Catalysts. 2021, 11(1), 26. https://doi.org/10.3390/catal11010026.

46. Agrawal, V.; Chaturvedi, P; Verma, K. Fungal laccase discovered but yet undiscovered. Biores. Biopro. 2018, 5(1), 4-17. https://doi.org/10.1186/s40643-018-0190-z

47. Rodríguez, J.L.; Hidalgo Manzano, I.A.; Muriel-Millán, L.F. A novel thermo-alkaline stable GDSL/SGNH esterase with broad substrate specificity from a deep-Sea *Pseudomonas* sp. Marine Biotechnol. 2024, 26, 447–459. https://doi.org/10.1007/s10126-024-10308-w

48. Li, R.; Wei, M.; Gao, Y.; Ren, B.; Yu, K.; Nie, H.; Xu, L.; Liu, W. Biodegradation of low-density polyethylene by M*icrobulbifer hydrolyticus* IRE-31. J. Environ Manag. 2020, 263, 106-116. https://doi.org/10.1016/j.jenvman.2020.110402

49. Spinal, M.L.; Tummino, A.; Poli, V.; Prigione, V.; Ilieva, P.; Cocconcelli, E.; et al. Low density polyethylene degradation by filamentous fungi. Environ. Pollution. 2021, 274, 116548. https://doi.org/10.1016/j.envpol.2021.116548