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Enhancing plastic waste recycling: Evaluating the impact of additives on the enzymatic polymer degradation

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ABSTRACT

While (bio)degradation of polymers is a recognized challenge, the influence of additives on this process remains poorly understood. Their presence in commercial polyethylene (PE) may inhibit the degradation process or complicate the recycling. This study aims to develop an enzymatic degradation process for post-consumer high-density polyethylene (HDPE). The HDPE degradation was performed using laccase from *Trametes versicolor* under mild conditions of temperature and pressure. The process was developed by exploring three key conditions: (i) the biocatalytic medium; (ii) the enzymatic mediator, and (iii) the influence of the presence of additives in the polymers. The most successful enzymatic degradation system involved HDPE from which additives were removed, with a buffer used as the reaction medium and 2,2'-azinobis-(3-ethylbenzothiazoline-6-

sulfonic acid (ABTS) employed as the enzymatic mediator. This system led to a substantial 33% weight reduction of the polymer (*versus* 3% for HDPE with additive under the same conditions). The characterization of the degraded polymer revealed new bands in the Fourier transform infrared spectroscopy (FTIR) spectra, including a new carbonyl band. In addition, it also showed an increased crystallinity when compared to HDPE with additive under the same conditions. These results suggest that the enzymatic degradation of HDPE occurs through an oxidation process, with the enzyme preferentially attacking the amorphous region of the polymer.

KEYWORDS: high density polyethylene, additives, laccase, biocatalysis, degradation, plastic recycling

1. INTRODUCTION

Plastics are ubiquitous synthetic polymers, which play an indispensable role in daily life and industrial applications [1,2] due to properties such as processability, durability, light weight and low cost [3]. The global production of plastics annually exceeds a staggering 350 to 400 million tons [4], with Europe producing 57.2 million tons in 2021 [5]. Among the various types of polymers, polyolefins, particularly polyethylene (PE) and polypropylene (PP), account for approximately 41 % of total production [5]. PE stands out, representing 24 % of the total [5] due to its durability, cost-effectiveness and flexibility resulting from its basic carbon skeleton with ethylene repeating units (-[CH_2-CH_2]$_n$-). Common variations of PE include the high-density polyethylene (HDPE) [6] which is produced by a catalytic process using transition metal catalysts supported on inert porous substrates, like silica, silica-alumina or alumina [7,8]. Although the C-C backbone in polymers provides stability, it also presents a challenge for (bio)degradation

of plastics due to the lack of hydrolysable functional groups. This leads to approximately 79 % of PE waste being currently disposed and accumulated in landfills [9] where they are exposed to UV radiation from sunlight or to physical erosion, which can lead to tensile strength losses and crumbling of PE into small fragments and particles known as microplastics [10]. Therefore, there is an urgent need to develop a highly efficient and eco-friendly process for degrading PE plastics.

Numerous chemical and physical methods have been developed to control PE waste pollution [11]. However, in recent years, microbial and enzymatic degradation processes have received considerable attention due to their ability to degrade plastics under mild and environmentally friendly conditions. The biodegradation process requires the presence of specific enzymes, involving the attack of particular bonds and cleavage of the longer polymer backbone structure into smaller intermediates [3,12]. Enzymes were already reported to have notable potential for PE degradation, including laccases [EC 1.10.3.2] [13–15], manganese peroxidases (EC 1.11.1.13) [16,17], and cutinases (EC 3.1.1.74) [18]. However, in these studies, enzymes are often used in conjunction with UV irradiation or a heat treatment of PE [13,15,18]. In addition, the use of laccase mediator systems (LMS) increases the oxidation potential of laccase since the laccase first oxidizes the mediator, and the oxidized small molecules act as chemical oxidants to modify the non-phenolic subunits [19,20]. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) or 1-hydroxybenzotriazole (HBT)) and more recently (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) have been explored as mediators for the biodegradation of PE through LMS [13,14]. These innovations promise to improve the efficiency of PE degradation since they can improve laccase's oxidation potential.

Laccase and LMS have been evaluated in the enzymatic degradation of PE. Santo *et al*. [15] performed experiments with UV-pretreated branched low-density polyethylene

(LDPE) film with an average molecular weight (Mw) of 191 000 and thickness of 0.2 mm. The LDPE film incubated with laccase from *Rhodococcus ruber* C208 for 2 weeks at pH 7 and 37 °C, resulted in a 20% reduction in average molecular weight (M_w) [15]. In another work, Yao *et al*. [13] studied two recombinant laccases (from *Botrytis aclada* and *Bacillus subtilis*) and evaluated the effect of different mediators (TEMPO, ABTS and HBT) in the degradation of UV-pretreated LDPE with a thickness of 20 µm. The treated LDPE was incubated for 5 days at 30 °C, 150 rpm and the mediator was added every 24h. The best results obtained with TEMPO and laccase from *B. aclada* led a reduction of 41.0 % in M_w [13]. After the enzymatic degradation, the FTIR analyses of LDPE showed the appearance of new peaks in LDPE spectra and an increase in carbonyl and hydroxyl indices, indicating oxidative changes in PE [13]. The enzymatic degradation of a PE membrane (1 x 6 cm, 100 µm thick) was evaluated using laccase from *Trametes versicolor* and the mediator HBT [10]. After 3 days of treatment at pH 4.5 and 150 rpm with daily addition of HBT it was observed a decrease in Mw by 88.3%, a complete loss of elongation and a 60% decrease in tensile strength [14].

Polymers are typically used in combination with various additives to enhance their performance in terms of thermal, mechanical, or even color characteristics and overall appearance, such as antioxidants, plasticizers, colorants (pigments or dyes), among other compounds. Additives target heat and impact resistance, strength, stiffness, and overall appearance, while extending spanning the shelf life of the polymers [21]. Although additives have been optimized for use in conjugation with polymers, they can pose challenges during the recycling process. This limitation impacts the potential applications of recycled polymers and contributes to environmental contamination, affecting soil, air, water, and even food [22,23]. Nevertheless, most studies on the enzymatic oxidation of PE have focused on virgin polymers [9–14, 17]. Only a limited number of studies have

explored the utilization of post-consumer polymers [25] and usually do not address their effect. To the best of our knowledge, only one study focuses on the effects of additives on the enzymatic degradation of polymers [26]. This study used poly(3-hydroxybutyrate) (PHB) and several additives, such as dodecanol, lauric acid, tributyrin and trilaurin, revealing that the additives prevented the enzyme PHB depolymerase to degrade the polymer's molecules [26]. Recent research has also shown that dyes commonly used in polymer formulations, like Red 23, fuchsin base, and Sudan IV, can interact with the active sites of enzymes, such as laccase [27]. As a result, the activity of enzymes may be hindered by the presence of additives impairing the degradation of the polymers. Therefore, it is important to study the effects of these additives on biodegradation, so far neglected.

The main objective of this work was to develop a mild enzymatic degradation process for post-consumer HDPE with additives. Three key experimental conditions were used to evaluate the effect of additives on enzymatic degradation using laccase from *T. versicolor*. Firstly, we investigated different biocatalytic media, which included a buffer pH 4.5 and a deep eutectic solvent (DES) known for enhancing laccase's activity [28]. Secondly, we assessed the impact of laccase mediators, such as ABTS and HBT, to understand how different mediators influence the degradation process. Finally, we compared post-consumer HDPE with additive and HDPE after the removal of the additive - orange pigment 64 (PO64) - to evaluate the influence of additives on the enzymatic reaction. The extent of the degradation reactions was evaluated gravimetrically, as well as by Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analyses (TGA), Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD). These methods provide insights into the structural and chemical changes occurring during the enzymatic degradation of post-consumer HDPE with additive.

2. EXPERIMENTAL SECTION

2.1. Materials

Post-consumer HDPE packaging, containing an orange pigment 64 (PO64) additive was obtained from a commercial degreaser packaging (Mr. Muscle). All lids, labels and adhesives were removed from the packages and the HDPE with additive was washed with detergent and water to remove surface impurities. Then, the HDPE with additive was dried and cut into small square pieces (0.3 cm × 0.3 cm). A part of this sample was pretreated according to the procedure developed by Ferreira *et al*. [29] to remove the additive - PO64. The post-consumer HDPE packaging that has not been pretreated and still contains the additive (PO64) will be referred hereinafter to as “HDPE with additive”, and the pretreated HDPE, on the other hand, will be referred to as "HDPE without additive.

The chemical compounds are summarized in Table S1 in Supplementary material, which also includes their abbreviations, CAS numbers, mass purities, and suppliers. Double distilled water was used for all solubility measurements performed in the experiments. This water source was subjected to additional purification steps, including passage through a reverse osmosis system and treatment with a Milli-Q plus 185 water purifier.

2.2. Degradation of HDPE by enzymatic assays

To study the enzymatic degradation of HDPE, we used three different approaches. In approach A, we studied the effect of the biocatalytic media. Approach B focused on the presence of different laccase mediators. Approach C focused on the use of HDPE with or without additive. Figure 1 provides a comprehensive overview of these approaches. Each approach includes replicate tests (1 – which is the control composed with the biocatalytic

medium, 3 – which contains laccase, and 4 – which has laccase and ABTS) to ensure comparability of results between different approaches and systems.

In all experiments, the enzymatic degradation of 100.0 mg HDPE (weighed using a Mettler Toledo scale, within $\pm 10^{-4}$ g) was carried out in the presence of laccase from *T. versicolor* with a polymer:liquid phase ratio of 1:10 (details about the liquid phase below). This process was carried at 37 °C and 100 rpm for 14 days, using a shaker IKA KS 4000 ic control. The liquid phase of each system was changed daily, and new solutions were added, and the enzyme's activity was being monitored every day following the method described in section 2.3.1. The choice of these conditions was based on the work of Santo *et al*. [15]. The liquid phase of each system had different compositions to study the presence or absence of laccase and mediators on the degradation process. Specifically, the liquid phase in all systems consisted of 90% (v/v) biocatalytic medium with additional components such as 5% (v/v) laccase (at a concentration of 0.85 mg/mL (1.5 U)), 5% (v/v) mediator (ABTS or HBT at a concentration of 4.0 mM), and water to achieve the desired volume as exemplified in Figure 1. The composition of the systems is presented in Table S2 in the Supplementary material.

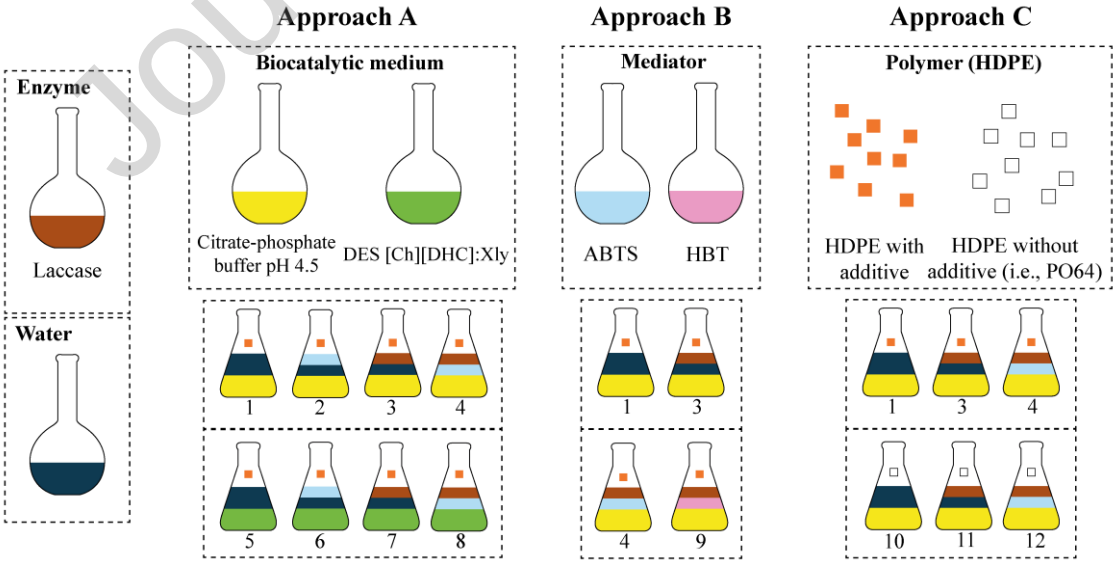

Figure 1. Representation of the enzymatic degradation of HDPE (■) with or (□) without additive, applying the enzyme (laccase), biocatalytic medium (citrate-phosphate buffer or aqueous mixture of DES) and mediator (ABTS or HBT). Three different approaches (A, B and C) were evaluated. The color presented for each solution and the size of each phase are just illustrative and does not correspond to reality.

2.2.1. Approach A - Influence of the biocatalytic medium in the degradation of HDPE with PO64 additive

In this first approach, we studied the enzymatic degradation of HDPE with additive in the presence of two different biocatalytic media. Biocatalytic medium 1: consisted of citrate-phosphate buffer pH 4.5, maintained at a molar ratio of 1:2 and a concentration of 3.4 (w/w)%. Biocatalytic medium 2: consisted of an aqueous mixture of DES, specifically cholinium dihydrogencitrate and xylitol, [Ch][DHC]:Xly, with a molar ratio of 2:1 and a concentration of 50 (w/w)%. The DES was prepared according to the method described by Toledo *et al*. [28].

In the context of biocatalytic media, as shown in Figure 1, we examined four systems associated with biocatalytic medium 1 (systems 1, 2, 3, and 4) and four associated with biocatalytic medium 2 (systems 5, 6, 7, and 8). In these systems, laccase was present in systems 3, 4, 7, and 8, while systems 2, 4, 6, and 8 utilized ABTS as the enzymatic mediator.

2.2.2. Approach B – Influence of the different mediators in the degradation of HDPE with PO64 additive

In this approach, we studied the enzymatic degradation of HDPE with additive in a biocatalytic medium containing a citrate-phosphate buffer with a pH of 4.5, using ABTS

and HBT as enzymatic mediators. Laccase was present in systems 3, 4 and 9, while ABTS was used in system 4 and HBT in system 9.

2.2.3. Approach C – Influence of the presence of PO64 additive in the degradation of post-consumer HDPE

Finally, the degradation of HDPE was evaluated using HDPE with additive in systems 1, 3, and 4, and HDPE without additive in systems 10, 11, and 12. The liquid phases of these systems consisted of a citrate-phosphate buffer with a pH of 4.5 as the biocatalytic medium (in all systems), laccase (in systems 3, 4, 11, and 12), and ABTS as the enzymatic mediator (in systems 4 and 12).

2.3. Evaluation of the extension of enzymatic degradation of HDPE

2.3.1. Enzyme's activity

ABTS (1.71 mM) was mixed with citrate-phosphate buffer at pH 4.5 in a ratio of ABTS and buffer of 5:14. The laccase activity was measured daily by mixing the liquid phase of each system with the previous ABTS/buffer solution in a ratio of 1:1. The increase of the absorbance was measured at 420 nm using a Shimadzu UV-1800 spectrometer with UVProbe 2.43 software. The relative enzyme activity was calculated according to Eq. 1:

$$\%\, relative\ enzyme\ activity = \frac{\frac{enzyme\ activity_{day\ 1}}{enzyme\ activity_{initial}} + \cdots + \frac{enzyme\ activity_{day\ n}}{enzyme\ activity_{initial}}}{n} \times 100 \quad \textbf{(Eq. 1)}$$

where *n* is the number of days.

2.3.2. HDPE weight loss

The degradation yield was assessed gravimetrically according to Eq. 2:

$$\%\, weight\ loss = \frac{|weight_{final} - weight_{initial}|}{weight_{initial}} \times 100 \qquad \textbf{(Eq. 2)}$$

where $weight_{initial}$ (initial weight) and $weight_{final}$ (final weight) were determined using the Mettler Toledo scale (within $\pm 10^{-4}$ g).

2.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analyses of HDPE samples, both before and after enzymatic degradation from all approaches, was done using the Spectrum BX model of the Perkin Elmer system, with Spectra software. The results were obtained with 32 scans between 4000 cm^{-1} to 500 cm^{-1} and a of resolution of 4 cm^{-1}. All FTIR data were previously normalized for consistency.

2.3.4. Thermogravimetric Analyses (TGA)

TGA and the following characterization analyses were performed on the samples of HDPE without additive from approach C. Additionally, samples of HDPE with and without additive prior to enzymatic degradation were also analyzed. TGA of HDPE was performed using a Setaram Setsys EV 1750 thermogravimetric instrument to evaluate polymer weight loss after enzymatic degradation. Samples weighing between 2.5 mg and 8.0 mg were subjected to a temperature range of 20 to 600 °C with a heating rate of 10 °C/min under a nitrogen atmosphere. The nitrogen flow in the TGA cell was maintained at approximately 50 mL/min. To account for all gas-related effects on the sample holders, a baseline was established with an empty sample cruicible.

2.3.5. Differential Scanning Calorimetry (DSC)

A Hitachi DSC7000X and a Perkin Elmer AD6 micro-analytical balance working at atmospheric pressure and coupled to a electrical cooling unit was used to measure properties of HDPE samples, namely melting temperature (T_m), enthalpy of fusion (ΔH_f) and degree of crystallinity (χ). The degree of crystallinity can be expressed in a percentage and calculated through Eq. 3.

$$\% \chi = \frac{\Delta H_f}{\Delta H_{f^*}} \times 100 \qquad \textbf{(Eq. 3)}$$

where, ΔH_f is the enthalpy of fusion of the HDPE sample and ΔH_{f^*} is the enthalpy of fusion of a 100 % crystalline HDPE (293 J/g) [30]. DSC analyses were performed on samples of HDPE without additive resulting from Approach C. In addition, HDPE with and without additive before enzymatic degradation were also analyzed. Samples of approximately 5 mg were weighted, sealed in hermetic aluminum crucibles and heated from 70 to 170 °C at a heating rate of 2 K/min under an atmosphere of nitrogen, followed by cooling at 5 K/min and a second heating step at the heating conditions. A baseline was also determined with an empty sample holder, to eliminate the effect of the gas on the sample holders. The equipment was calibrated with several standards (decane, 4-nitrotoluene, naphthalene, benzoic acid, diphenylacetic acid, indium, tin, caffeine, lead, zinc, potassium nitrate, water, and anthracene) at 2 K/min.

2.3.6. X-Ray Diffraction (XRD)

XRD method was used to understand if the enzymatic degradation of HDPE without additive from approach C changed the crystalline structure of the initial polymer. In addition, HDPE without additive before enzymatic degradation was also analyzed. Philips

X'pert MPD instrument was used while employing CuK radiation (λ = 1.5405980 Å) at 40 kV and 45 mA. Samples were scanned using a step size of 0.1050422 ° and a time per step of 400 s in the 2θ range of 5 to 60 °.

3. RESULTS AND DISCUSSION

3.1. Effect of enzymatic degradation on the weight loss of HDPE and enzyme's activity

In this study, to assess the optimum conditions to prompt the efficient and mild enzymatic depolymerization of abundant HDPE plastic waste we have assessed the effects of biocatalytic media (approach A), different mediators (approach B) and the presence or absence of PO64 (additive - approach C) in the extent of degradation measured by the weight loss of HDPE (Figure 2, for detailed information see Table S3 in the Supplementary material). In addition, the enzyme's activity was monitored daily (see Figure S1 and Table S3 in the Supplementary material). No changes in the HDPE color were observed in any of the assays. Moreover, no weight losses were observed for the control systems, *i.e.*, for the systems with liquid phase composed of biocatalytic medium (systems 1, 5 and 10 in Figure 1), or for those ones composed of biocatalytic medium and ABTS (systems 2 and 6 in Figure 1). Therefore, only the control system 1 was replicated in all the approaches to ensure comparability of results between different approaches and systems, as mentioned before.

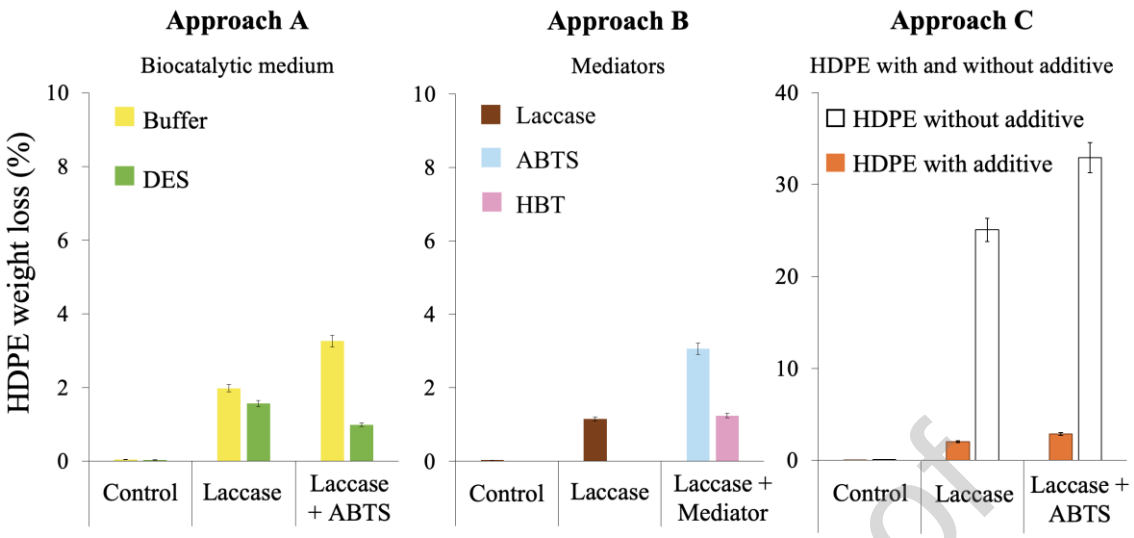

Figure 2. Weight loss (%) of HDPE as a result of different biocatalytic conditions on the enzymatic degradation of HDPE (Approaches A, B and C). Experiments were conducted at 37°C and 100 rpm for 14 days with laccase from *T. versicolor*. Approaches B and C used only buffer as biocatalytic medium. Control is composed with HDPE and biocatalytic medium.

In approach A we studied two biocatalytic media, citrate-phosphate buffer pH 4.5 and aqueous mixture of [Ch][DHC]:Xly (molar ratio of 2:1) at 50% (w/w). The choice of the buffer was based on previous studies under similar conditions [14,15]. The aqueous DES mixture was selected based on the results of Toledo *et al.* [28], who reported an important increase in laccase's activity in the presence of this DES solution. Surprisingly, in our experimental conditions, the use of DES as the biocatalytic medium did not result in an increase of HDPE weight loss (maximum observed 1.57 ± 0.09 %) compared to buffer (maximum observed 3.26 ± 0.15 %). This result can be attributed to the low relative enzyme activity after 24h of HDPE degradation, as highlighted in Figure S1 and Table S3 in the Supplementary material. The minimum average relative activity of laccase observed in the buffer systems was 72 ± 8 %, while it was almost negligible for the DES systems, contrary to expectations, which may explain the differences in polymer weight

loss. Since DES did not increase the enzyme activity as expected, only the buffer was used in the following experiments, Approaches B and C.

Additionally, we performed a series of tests to evaluate the efficacy of different mediators, namely ABTS and HBT, in combination with laccase (Approach B). As mentioned earlier, the incorporation of these compounds creates a LMS in which laccase first oxidizes the mediator and these small molecules subsequently serve as chemical oxidants to facilitate the reaction. Among the various known compounds that can form an LMS, ABTS and HBT have already demonstrated their usefulness in degrading PE with laccase, which led us to select them for this approach [13,14]. Before discussing the effects of the different mediators, it is noteworthy that the presence of ABTS resulted, as expected, in a higher weight loss (3.06 ± 0.13 %) than in its absence (1.15 ± 0.05 %), suggesting that the mediator enhances the enzyme response.

In comparative terms, evaluating the two mediators' performance, ABTS performed better than HBT, with a weight loss of 3.06 ± 0.13 % *versus* 1.24 ± 0.05 %, respectively. Also, in terms of average relative enzyme activity the ABTS system (64 ± 3 %) had twice the activity of HBT (31 ± 1 %). This underlines the correlation between higher enzyme activity and greater polymer weight loss. It is worth noting that our results are not in complete agreement with those of Yao *et al.* [13], who reported more favorable results for HBT compared to ABTS in terms of M_w reduction. Specifically, they observed a M_w reduction of 39.7% for ABTS and a 41.5% for HBT when laccase from *Botrytis aclada* was used. The observed discrepancy could be due to differences in the source of laccase (microorganism producer) used and the type of polymer used (they used LDPE films while we used HDPE bottles). Considering that HBT did not meet the performance expectations, it was excluded for further experiments (Approach C), and ABTS was selected instead as the preferred mediator.

Approach C was carried to investigate how the enzymatic degradation of HDPE is affected by the removal of additives. This effect, seldom neglected, is however of upmost importance since additives, which are commonly incorporated into polymers to improve their properties, can interact with the active site of enzymes and hinder the enzymatic degradation [26,27]. The experimental conditions of approach C were based on the best results of approaches A and B, using a citrate-phosphate buffer pH 4.5 as the biocatalytic medium and ABTS as the mediator. The removal of the additive PO64 was achieved by a pretreatment method previously developed by Ferreira *et al.* [29]. Figure 2 shows that the results obtained with HDPE without additive (e.g., laccase and ABTS = 32.90 ± 1.58%) are significantly higher than those obtained with HDPE with additive (e.g., laccase and ABTS = 2.87 ± 0.16%). It is very important to highlight that these results were obtained without UV preteatment of the post-consumer polymer, oppositely to previously reported studies where enhanced results were only achieved if UV ageing of samples of virgin polymer with additives was carried out [13,15].

Regarding the results obtained for HDPE with additive in the presence of enzyme and mediator (2.87 ± 0.16%), our result is consistent with those previously reported by Hou *et al.* [31], which were (3.85 ± 0.50) %. Although the authors did not specify the detailed chemical composition of the PE material under study, since they used a PE-based mulch, additives were most probably present [31]. This study, being one of the few studies focusing on the use of post-consumed PE, addresses a different grade PE, specifically a mulch film after 3 months of exposure to environmental conditions in a potato cultivation field, therefore highly subjected to UV-Visible light which could have an impact on the degradation results. The study here reported addresses instead a post-consumer HDPE packaging-grade material not subject to significant UV-visible irradiation [31]. Additionally, Hou *et al.* [31], in contrast to the current approach which makes use of a

pure enzyme, used soil microbial suspensions, where they observed that *Bacillus aryabhattai* appeared to be the main responsible for the degradation. The biocatalytic assays conditions adopted were also twice as long as those here adopted: 30 days, pH 6.65 ± 0.02, 28°C, 150 rpm.

Notably, comparing the results obtained between HDPE with additive *versus* without PO64 additive in the presence of laccase and ABTS, the weight loss reached for HDPE without additive was 11.5 times better than the weight loss for HDPE with additive. These results can be attributed to the pretreatment process in which PO64 was removed from HDPE [29]. Moreover, the results can be explained not only by the fact that the pretreatment removes the additive that could interfere with the active sites of the enzymes, but also by the loss of crystallinity of the polymer as result of the pre-treatment, making it more susceptible to enzymatic degradation [13,32].

Finally, when comparing the same experimental conditions in approaches A, B, and C for systems with laccase, all systems showed similar results, averaging around 2%. Similarly, for systems with laccase and ABTS, the results were in the same ballpark, around 3%. These slight differences could be attributed to the sensitivity of weight loss measurements. Nevertheless, the best results were achieved when using HDPE without additive with laccase (25.06 ± 1.08 %) and with laccase and ABTS (32.90 ± 1.58 %) (Figure 2).

3.2. HDPE characterization

To further characterize the degradation of HDPE by laccase and to enhance our understanding of the observed outcomes, we used techniques such as FTIR, DSC and XRD to analyze the structural changes in the recovered polymers, as discussed below. TGA was also performed, and the results can be found in the Supplementary material, Figure S2 and Table S4.

3.2.1 HDPE oxidation

FTIR was used to analyze and characterize the typical vibrational modes of the different enzymatically degraded HDPE samples under study and to confirm their oxidation. Figure 3 shows a comparison between the FTIR spectra of the samples of Approach C (HDPE with additive and HDPE without additive) treated with laccase and ABTS. The remaining FTIR spectra can be found in the Supplementary material, Figures S3 to S5.

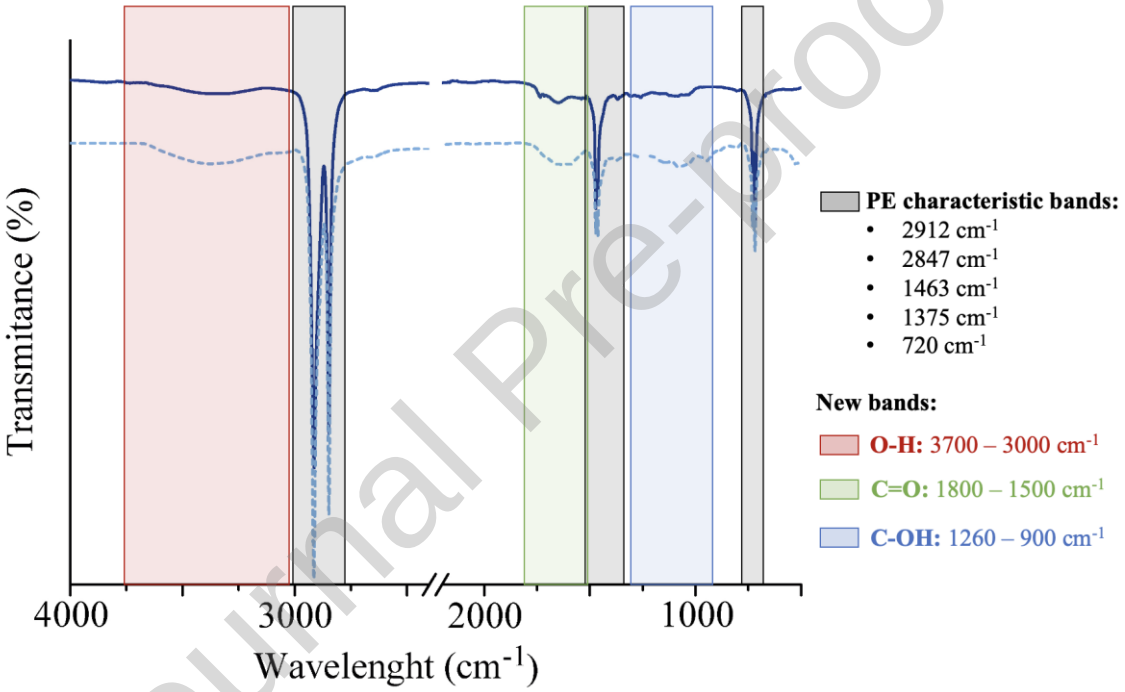

Figure 3. FTIR spectra of the samples of (**blue full line**) HDPE with additive recovered after enzymatic degradation with laccase and ABTS, and (**blue dashed line**) HDPE without additive recovered after enzymatic degradation with laccase and ABTS.

All samples show the characteristic HDPE bands as reported in the literature [29]. In particular, it is observed two absorption bands at 2912 and 2847 cm^{-1} corresponding to the asymmetric and symmetric elongation of the C-H bond of CH_2 groups, respectively;

at 1375 cm^{-1} arising from the methyl group (CH_3); and at 720 cm^{-1} corresponding to the absorption of the CH_2 groups at $n \geq 4$ which (rocking motion) [29].

Importantly, for those samples treated enzymatically, their spectra show new bands near 3700 – 3000 cm^{-1} typical of the hydroxyl group elongation related to possible oxidation after the enzymatic treatment, 1800 – 1500 cm^{-1} arising from the carbonyl group and near 1260 – 900 cm^{-1} the vibrational mode of C-O stretching (typical of alcohols) [11]. However, in the case of HDPE without additive, incubated with laccase and ABTS, the differences are much more pronounced than in the system with HDPE with additive, incubated with laccase and ABTS. This difference can be attributed to polymer oxidation, since the bands at 3700 – 3000 cm^{-1} is usually associated with hydroxyl groups and those at 1800 – 1500 cm^{-1} with carbonyl groups, as observed by Yao *et al.* [13]. Similar changes were observed by Ali *et al.* [33], who also reported the presence of hydroxyl groups, carbonyl groups, and alcohols (vibrational mode of C-O stretching, at 1260 – 900 cm^{-1}). Zhang *et al.* [12] also noted the presence of carbonyl groups as a distinctive indicator of polyethylene degradation. It is worth noting that in these studies, the authors employed bacteria *Achoria grisella* (*Citrobacter freundii and Bacillus* sp.) and *Rhyzopertha dominica* (*Acinetobacter baumannii*) for the degradation, rather than using a purified enzyme, as in this study. However, the carbonyl and hydroxyl indices in the systems with laccase or laccase and mediator did not show a clear upward trend in any of the approaches studied, as reported in the literature [13].

FTIR analyses of the samples from approaches A and B (Figures S4 and S5 in the Supplementary material) showed no significant differences between them, with differences observed for samples from systems comprising laccase, mediator, and buffer as the biocatalytic medium.

3.2.3. HDPE crystallinity

DSC and XRD analyses were done to investigate the thermal behavior and crystallinity of the relevant samples, including HDPE with and without additive before enzymatic depolymerization assays, as well as samples of HDPE without additive obtained in the assays of Approach C (control, laccase, and laccase and ABTS). The corresponding results for T_m, ΔH_f and χ are presented in Table 4, and the XRD results, including the diffraction angles of the maximum peaks defined are in Figure S6 in Supplementary material.

Table 1. Melting temperature (T_m), enthalpy of fusion (ΔH_f) and degree of crystallinity (χ) of the recovered HDPE without pigment from systems using buffer as biocatalytic medium, *i.e.*, control, laccase, laccase and ABTS. HDPE with additive and HDPE without additive also are presented for comparison purposes.

Polymer	Approach	T_m (ºC)	ΔH_f (J/g)	χ (%)
HDPE with additive	---	132.62	219.11	74.80
HDPE without additive	---	130.33	181.91	62.10
	Control	130.56	182.87	62.40
	Laccase	131.11	191.45	65.30
	Laccase + ABTS	132.41	201.12	68.60

HDPE is a semi-crystalline polymer and shows well documented behavior during its melting process. All samples showed an endothermic transition during the melting process, in a range up to *ca.* 133 °C, which is consistent with the reported T_m range for HDPE of 126 to 135 °C [34]. Notably, a slight increase in the T_m values of the samples obtained after enzymatic degradation with the increasing complexity of the liquid phase in the system was observed. This is consistent with the preferential attack of the enzymes to the less stiff and more accessible domains. Moreover, HDPE with additive after

degradation showed the highest T_m value, while HDPE without additive showed the lowest value, with an increasing trend with the complexity of the liquid medium, as shown in Table 4.

The χ results also reflected the T_m trend. HDPE with additive had the highest χ value (74.8 %), while HDPE without additive had the lowest (62.10 %). The pretreatment to remove the additives contributed to the lower χ value, possibly due to dissolution/precipitation adopted [35]. Turning our focus to the post-enzymatic treated samples, the sample of HDPE without additive with laccase and ABTS has higher χ (68.6 %) compared to HDPE without additive and to the control (Table 4).

The XRD analyses (Figure S6 in Supplementary material) corroborated the essential semi-crystalline nature of all samples with two diffraction peaks at diffraction angle of ~ 21-22 ° and ~25 °. Typical of the crystalline domains and a very small amorphous halo centered around 21 °. Notably the HDPE without additive, laccase and ABTS system showed the highest diffraction angle of 22.34 °. Despite the minor differences in the diffraction angle values, a higher diffraction angle is generally associated with a decrease in crystal size. Ali *et al*. [33] also observed a decrease in the size of the crystal after biodegradation with species of *Citrobacter freundii* and *Bacillus* sp. Again, these results suggest that enzymatic degradation had a more significant effect in the laccase and ABTS system.

In summary, the results obtained from DSC and XRD, coupled with the fact that enzymes typically target the polymer's amorphous regions first [26,36], where the enzymes initially break down accessible chains in the amorphous phase and then proceed to degrade the chains in the exposed crystalline lamellae, indicate that the biocatalytic reaction (enzymatic degradation) was more extensive in the system containing laccase and ABTS, which is consistent with the observed weight loss results. This observation is consistent

with prior studies. Patel *et al.* [25] and Kumagai *et al.* [37] both reported a decreased enzymatic degradability as crystallinity increased in polymer, using PET and PHB, respectively. These observations validate our results and highlights the superior performance of the laccase and ABTS system and the crucial role of additive removal in in the biodegradation of post-consumer polymers.

4. CONCLUSION

In this work, we investigate several variables related to the enzymatic degradation of post-consumer HDPE, with a particular focus on the presence or absence of additives in the polymer. The results showed that the citrate-phosphate buffer and ABTS are the optimal biocatalytic medium and mediator for laccase from *T. versicolor*. In particular, the efficiency of enzymatic degradation stands out when HDPE without additive was used. These samples (HDPE without additive) showed a remarkable weight loss of 32.90 ± 1.58 % compared to a modest loss of only 2.87 ± 0.16 % when HDPE with additive was used. Furthermore, the HDPE without additive subjected to enzymatic degradation exhibited significant changes, manifesting new bands associated with hydroxyl and carbonyl groups in FTIR analyses, alongside the most pronounced diffraction angle (22.34 °) among all tested samples. These shifts are indicators of enzymatic polymer degradation, particularly in the amorphous domain. Our findings suggest that the pretreatment for the removal of additives can play a major role in enzymatic degradation of HDPE and we believe that this technique in the medium/long run can be an effective, ecofriendly and socially acceptable methodology for PE waste treatment.

CRediT authorship contribution statement

MISA: Investigation, Formal analyses, Writing - original draft preparation, Writing - review & editing. AFS: Conceptualization, Writing - review & editing, Supervision. GT: Investigation, Writing - review & editing. APMT: Conceptualization, Writing - review & editing, Supervision, Funding acquisition. AMF: Conceptualization, Formal analyses, Writing - review & editing, Supervision. JAPC: Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no competing financial interest.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version a xxx.

REFERENCES

[1] C. Sonnendecker, J. Oeser, P.K. Richter, P. Hille, Z. Zhao, C. Fischer, H. Lippold, P. Blázquez-Sánchez, F. Engelberger, C.A. Ramírez-Sarmiento, T. Oeser, Y. Lihanova, R. Frank, H.-G. Jahnke, S. Billig, B. Abel, N. Sträter, J. Matysik, W. Zimmermann, Low Carbon Footprint Recycling of Post-Consumer PET Plastic with a Metagenomic Polyester Hydrolase, ChemSusChem. 15 (2022). https://doi.org/10.1002/cssc.202101062.

[2] Q. Jiang, Z. Li, Z. Cui, R. Wei, K. Nie, H. Xu, L. Liu, Quantum mechanical investigation of the oxidative cleavage of the c–c backbone bonds in polyethylene model molecules, Polymers (Basel). 13 (2021). https://doi.org/10.3390/polym13162730.

[3] Z.H. Qin, J.H. Mou, C.Y.H. Chao, S.S. Chopra, W. Daoud, S. yuan Leu, Z. Ning, C.Y. Tso, C.K. Chan, S. Tang, Z.J. Hathi, M.A. Haque, X. Wang, C.S.K. Lin, Biotechnology of Plastic Waste Degradation, Recycling, and Valorization: Current Advances and Future Perspectives, ChemSusChem. (2021). https://doi.org/10.1002/cssc.202100752.

[4] S. Ghatge, Y. Yang, J.H. Ahn, H.G. Hur, Biodegradation of polyethylene: a brief review, Appl Biol Chem. 63 (2020). https://doi.org/10.1186/s13765-020-00511-3.

[5] P. Europe, Plastics - the Facts 2022, 2022. https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/ (accessed September 27, 2023).

[6] J.M. Restrepo-Flórez, A. Bassi, M.R. Thompson, Microbial degradation and deterioration of polyethylene - A review, Int Biodeterior Biodegradation. 88 (2014) 83–90. https://doi.org/10.1016/j.ibiod.2013.12.014.

[7] Y. V. Kissin, Catalysts for the Manufacture of Polyethylene, Handbook of Industrial Polyethylene Technology. (2016) 25–60. https://doi.org/10.1002/9781119159797.ch2.

[8] B.M. Weckhuysen, R.A. Schoonheydt, Olefin polymerization over supported chromium oxide catalysts, Catal Today. 51 (1999) 215–221. https://doi.org/10.1016/S0920-5861(99)00046-2.

[9] Z. Yao, H.J. Seong, Y.S. Jang, Environmental toxicity and decomposition of polyethylene, Ecotoxicol Environ Saf. 242 (2022). https://doi.org/10.1016/j.ecoenv.2022.113933.

[10] A. Sivan, New perspectives in plastic biodegradation, Curr Opin Biotechnol. 22 (2011) 422–426. https://doi.org/10.1016/j.copbio.2011.01.013.

[11] B. Agostinho, A.J.D. Silvestre, J.A.P. Coutinho, A.F. Sousa, Synthetic (bio)degradable polymers – when does recycling fail?, Green Chemistry. 25 (2023) 13–31. https://doi.org/10.1039/D2GC02726G.

[12] Y. Zhang, Y. Lin, H. Gou, X. Feng, X. Zhang, L. Yang, Screening of Polyethylene-Degrading Bacteria from Rhyzopertha Dominica and Evaluation of Its Key Enzymes Degrading Polyethylene, Polymers (Basel). 14 (2022). https://doi.org/10.3390/polym14235127.

[13] C. Yao, W. Xia, M. Dou, Y. Du, J. Wu, Oxidative degradation of UV-irradiated polyethylene by laccase-mediator system, J Hazard Mater. 440 (2022) 129709. https://doi.org/10.1016/j.jhazmat.2022.129709.

[14] M. Fujisawa, Degradation of polyethylene and nylon-66 by the laccase-mediator system, J Polym Environ. 9 (2001) 103–108. https://doi.org/10.1023/A:1020472426516.

[15] M. Santo, R. Weitsman, A. Sivan, The role of the copper-binding enzyme - laccase - in the biodegradation of polyethylene by the actinomycete Rhodococcus ruber, Int Biodeterior Biodegradation. 84 (2013) 204–210. https://doi.org/10.1016/j.ibiod.2012.03.001.

[16] H. V. Sowmya, Ramalingappa, M. Krishnappa, B. Thippeswamy, Degradation of polyethylene by Penicillium simplicissimum isolated from local dumpsite of Shivamogga district, Environ Dev Sustain. 17 (2015) 731–745. https://doi.org/10.1007/s10668-014-9571-4.

[17] Y. Iiyoshi, Y. Tsutsumi, T. Nishida, Polyethylene degradation by lignin-degrading fungi and manganese peroxidase, Journal of Wood Science. 44 (1998) 222–229. https://doi.org/10.1007/BF00521967.

[18] D. Kong, L. Wang, X. Chen, W. Xia, L. Su, F. Zuo, Z. Yan, S. Chen, J. Wu, Chemical-biological degradation of polyethylene combining Baeyer-Villiger oxidation and hydrolysis reaction of cutinase†, Green Chemistry. 24 (2022) 2203–2211. https://doi.org/10.1039/d2gc00425a.

[19] O. V. Morozova, G.P. Shumakovich, S. V. Shleev, Y.I. Yaropolov, Laccase-mediator systems and their applications: A review, Appl Biochem Microbiol. 43 (2007) 523–535. https://doi.org/10.1134/S0003683807050055.

[20] R. Hilgers, J.P. Vincken, H. Gruppen, M.A. Kabel, Laccase/Mediator Systems: Their Reactivity toward Phenolic Lignin Structures, ACS Sustain Chem Eng. 6 (2018) 2037–2046. https://doi.org/10.1021/acssuschemeng.7b03451.

[21] K. Bhunia, S.S. Sablani, J. Tang, B. Rasco, Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage, Compr Rev Food Sci Food Saf. 12 (2013) 523–545. https://doi.org/10.1111/1541-4337.12028.

[22] J.N. Hahladakis, C.A. Velis, R. Weber, E. Iacovidou, P. Purnell, An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling, J Hazard Mater. 344 (2018) 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014.

[23] S. Ügdüler, K.M. Van Geem, M. Roosen, E.I.P. Delbeke, S. De Meester, Challenges and opportunities of solvent-based additive extraction methods for plastic recycling, Waste Management. 104 (2020) 148–182. https://doi.org/10.1016/j.wasman.2020.01.003.

[24] C. Zhang, Y. Mu, T. Li, F.J. Jin, C.Z. Jin, H.M. Oh, H.G. Lee, L. Jin, Assembly strategies for polyethylene-degrading microbial consortia based on the combination of omics tools and the “Plastisphere,” Front Microbiol. 14 (2023). https://doi.org/10.3389/fmicb.2023.1181967.

[25] A. Patel, A.C. Chang, A. Mastromonaco, M. Acosta Diaz, S. Perry, O. Ferki, C. Ayafor, U. Abid, H.-W. Wong, D. Xie, M.J. Sobkowicz, Aqueous buffer solution-induced crystallization competes with enzymatic depolymerization of pre-treated post-consumer poly (ethylene terephthalate) waste, Polymer (Guildf). 285 (2023) 126370. https://doi.org/10.1016/j.polymer.2023.126370.

[26] N. Yoshie, K. Nakasato, M. Fujiwara, K. Kasuya, H. Abe, Y. Doi, Y. Inoue, Effect of low molecular weight additives on enzymatic degradation of poly(3-hydroxybutyrate), Polymer (Guildf). 41 (2000) 3227–3234. https://doi.org/https://doi.org/10.1016/S0032-3861(99)00547-9.

[27] V. Pande, T. Joshi, S.C. Pandey, D. Sati, V. Pande, S. Chandra, M. Samant, Molecular docking and molecular dynamics simulation approaches for evaluation of laccase- mediated biodegradation of various industrial dyes, J Biomol Struct Dyn. 40 (2021) 1–11. https://doi.org/10.1080/07391102.2021.1971564.

[28] M.L. Toledo, M.M. Pereira, M.G. Freire, J.P.A. Silva, J.A.P. Coutinho, A.P.M. Tavares, Laccase Activation in Deep Eutectic Solvents, ACS Sustain Chem Eng. 7 (2019) 11806–11814. https://doi.org/10.1021/acssuschemeng.9b02179.

[29] A.M. Ferreira, I. Sucena, V. Otero, E.M. Angelin, M.J. Melo, J.A.P. Coutinho, Pretreatment of plastic waste: Removal of colorants from hdpe using biosolvents, Molecules. 27 (2022) 98. https://doi.org/10.3390/molecules27010098.

[30] P. Olesik, M. Godzierz, M. Kozioł, J. Jała, U. Szeluga, J. Myalski, Structure and mechanical properties of high-density polyethylene composites reinforced with glassy carbon, Materials. 14 (2021) 1–10. https://doi.org/10.3390/ma14144024.

[31] L. Hou, J. Xi, X. Chen, X. Li, W. Ma, J. Lu, J. Xu, Y.B. Lin, Biodegradability and ecological impacts of polyethylene-based mulching film at agricultural environment, J Hazard Mater. 378 (2019) 120774. https://doi.org/10.1016/j.jhazmat.2019.120774.

[32] Y. Ruan, F. Hu, J. Zhao, Y. Li, J. Ling, J. Cao, L. Zhang, Degradation of polyethylene particles by Trichoderma harzianum and recombinant laccase cloned from the strain, J Appl Polym Sci. 140 (2023) e54599. https://doi.org/10.1002/app.54599.

[33] S.S. Ali, T. Elsamahy, D. Zhu, J. Sun, Biodegradability of polyethylene by efficient bacteria from the guts of plastic-eating waxworms and investigation of its degradation mechanism, J Hazard Mater. 443 (2023) 130287. https://doi.org/10.1016/j.jhazmat.2022.130287.

[34] T. Furukawa, H. Sato, Y. Kita, K. Matsukawa, H. Yamaguchi, S. Ochiai, H.W. Siesler, Y. Ozaki, Molecular structure, crystallinity and morphology of polyethylene/ polypropylene blends studied by Raman mapping, scanning electron microscopy, wide angle X-ray diffraction, and differential scanning calorimetry, Polym J. 38 (2006) 1127–1136. https://doi.org/10.1295/polymj.PJ2006056.

[35] W. Chen, Y. Yang, X. Lan, B. Zhang, X. Zhang, T. Mu, Biomass-derived γ-valerolactone: efficient dissolution and accelerated alkaline hydrolysis of polyethylene terephthalate, Green Chemistry. 23 (2021) 4065–4073. https://doi.org/10.1039/d1gc00665g.

[36] A. Göpferich, Mechanisms of polymer degradation and erosion, The Biomaterials: Silver Jubilee Compendium. 17 (1996) 117–128. https://doi.org/10.1016/B978-008045154-1.50016-2.

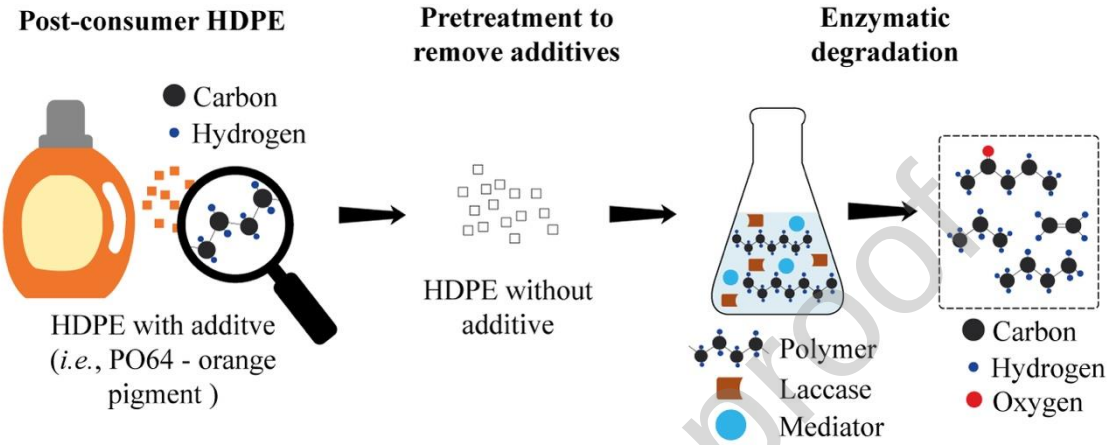
[37] Y. Kumagai, Y. Kanesawa, Y. Doi, Enzymatic degradation of microbial poly(3-hydroxybutyrate) films, Makromol. Chem. 193 (1992) 53–57. https://doi.org/https://doi.org/10.1002/macp.1992.021930105.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Graphical abstract

For Table of Contents Use Only



Synopsis: Enzymatic degradation of HDPE without additive using mediator.

Highlights

- HDPE without additive treated with laccase and ABTS lost 33% of its weight
- Additive removal boosted HDPE biodegradation 11.5× *vs.* HDPE with additive
- Laccase-driven degradation leads to structural changes in HDPE
- Enzymatic degradation may provide an alternative to conventional treatment of plastic waste.