

Ultraviolet Ozone Pretreatment Enhances Selective PETase Biodegradation of Textiles Compared to Sunlight and Alkali Treatments

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The rapid growth of the fashion industry has led to increasing textile waste, exacerbating environmental pollution and climate change. To support sustainability and circular economy goals, this study investigates the enzymatic degradation of cotton/polyethylene terephthalate (PET) mixed textiles using PETase, comparing wild-type and mutant (MUT S238F/W159H) variants. To improve enzyme accessibility, three pretreatment strategies are evaluated: alkali treatment, UV–ozone (UVO) exposure, and natural sunlight weathering. The effects are assessed by measuring textile weight loss, surface morphology (scanning electron microscopy), Fourier transform infrared spectroscopy, and yields of terephthalic acid (TPA) and mono-(2-hydroxyethyl) terephthalic acid. Alkali

treatment produces the highest weight loss, while UVO pretreatment moderately degrades textiles and significantly enhances enzymatic TPA production. In contrast, prolonged sunlight exposure has negligible effects. ¹H NMR analysis of supernatants confirms the formation of oxidized PET products following UVO exposure, indicating surface chemical modifications that increase enzymatic susceptibility. The results reveal differential effects on PET and cotton fibers, highlighting UVO as a promising, selective pretreatment for mixed textile waste. This study demonstrates the potential of combining photochemical oxidation and enzymatic processes for targeted PET degradation, contributing to more efficient textile recycling strategies.

1. Introduction

The amount of waste in the world is reaching alarming levels. In Europe, the waste is estimated at around 6.95 tons annually.^[1] The fashion industry ranks fourth highest in environmental impact (after food, housing, and mobility), significantly contributing to climate change.^[2] This negative impact stems from multiple factors: First, textile production requires enormous water resources—the water needed to produce a single cotton T-shirt is equivalent to one person's drinking needs for 2.5 years.^[3] Second, textile dyeing and finishing processes contribute ≈20% of global water pollution. Third, the fashion industry accounts for nearly 10% of global carbon emissions, exceeding the combined impact of international flights and maritime shipping.^[4] Fourth, regular washing of synthetic and mixed-fiber clothing

significantly contributes to microplastic release into water systems and ultimately oceans.^[5] Finally, textile waste in landfills not only takes centuries to decompose but also releases microplastics, chemicals, and greenhouse gases throughout this process.^[6]

To mitigate textile consumption's environmental impact, improved reuse and recycling within a circular economy model is essential. However, this remains largely aspirational—currently, less than half of used clothing is collected for reuse, and only 1% is recycled into new garments.^[7] Existing recycling technologies, including mechanical and chemical processes, are energy-intensive and struggle with processing, separating, and recycling mixed-fiber fabrics (blends of cotton, polyester, and nylon—the archetypal synthetic textiles).^[8]


Enzymatic approaches offer selectivity in breaking specific chemical bonds, enabling targeted degradation, whereas purely chemical treatments break down cotton/polyethylene terephthalate (PET) sheets into monomers without selectivity.^[9] However, PET biodegradation requires enzyme access to hydrolyzable bonds. For instance, polymer crystallinity in postconsumer PET bottles restricts enzyme access, necessitating pretreatment to enhance PETase effectiveness in degrading crystalline PET.^[10]


Similarly, pretreatment of synthetic textiles before enzymatic processing enhances degradation kinetics^[11–18] and provides alternative strategies for textile recycling and product revalorization. Various treatments have been investigated, including NaOH at –20 °C^[12], NaOH/urea solutions^[13], and mechanical milling^[7,15,17] for mixed textiles, reporting degradation of wood and cotton components, though not specifically addressing PET (Table S1, Supporting Information).

Recent innovative research has explored natural agents to facilitate controlled textile degradation for effective recycling.

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These agents include sunlight UV component) and microorganisms, pH modifiers present in the soil, and tropospheric ozone (O_3). For instance, tropospheric ozone forms when UV radiation from the sun interacts with nitrogen oxides ($NO_x = NO_2 + NO$) and volatile organic compounds.^[19]

In particular, the effects of UV and ozone on PET surface properties and crystallinity have been extensively studied (Table 1). UV with ozone (UVO) treatment modifies PET surfaces by creating polar groups during hydrolysis while causing minimal weight and strength loss.

These reports suggest that UVO is not only effective but also a dry, chemical-free, cost-efficient, and environmentally friendly approach. In contrast, natural UV sunlight appears to have an opposing effect, potentially hampering enzymatic activity.

In this study, we conducted a systematic investigation into how various pretreatment methods enhance PETase-mediated degradation of commercial cotton/PET textiles (65% PET/35% cotton). We evaluated both wild-type (WT) PETase and its enhanced S238F/W159H mutant (MUT) variant across multiple degradation parameters, including textile weight loss and production of key degradation products—terephthalic acid (TPA) and mono-(2-hydroxyethyl) terephthalic acid (MHET). Our comparative analysis examined three distinct pretreatment approaches: alkaline conditions, controlled UVO exposure, and natural sunlight exposure in Mediterranean coastal conditions (Valencia/Castellon, Spain). Results revealed that while alkaline pretreatment aggressively degraded the entire textile structure, UVO pretreatment significantly amplified PETase enzymatic efficiency without complete material destruction. Notably, extended

natural sunlight exposure produced negligible enhancement of enzymatic degradation. These findings demonstrate that the strategic combination of UVO pretreatment with engineered PETase enzymes represents a promising approach for developing targeted, efficient textile recycling technologies to address the growing global challenge of synthetic textile waste.

2. Results and Discussion

The study investigates the treatment of a polyester–cotton textile with PETase enzyme following pretreatment, followed by analysis using various techniques, including attenuated total reflection—Fourier transform infrared (ATR–FTIR), scanning electron microscopy (SEM), and high-performance liquid chromatography (HPLC), to assess the degradation of PET and cotton components and evaluate the enzyme's efficiency (Figure 1).

2.1. Weight Loss after Pretreatment and Enzymatic Incubation

Previous studies have demonstrated that NaOH pretreatment of postconsumer PET (e.g., plastic water bottles) significantly enhances biodegradation by PETase WT.^[10] In this study, textiles were incubated for 18 h at various NaOH concentrations. Low concentrations of NaOH (0.1–1 M) resulted in textile weight loss of $\approx 2 \pm 1.45\%$, whereas 10 M NaOH induced a significant $48.52 \pm 0.70\%$ weight loss (Figure 2A). In contrast, maximum

Table 1. Effect of UV action on PET degradation under different conditions.

Ref.	Substrate	Enzyme	Treatment	Results
[24]	PET film	None	UVO in ultrapure water (5 h)	-The UVO treatment induces ring opening of benzene rings of PET molecular chains, simultaneously introducing OH and $>C=O$ groups on the film.
[25]	PET film PET fibers	None	UVO cleaner (10–20 min)	-UVO-treatment introduces aldehyde and carboxylic acid groups on plastics, making them more hydrophilic.
[26]	PET film	None	UV (3–10 min) UVO (3–10 min) Ozone (25 min)	-UV causes chain scission and modification at the Surface of PET. -UVO modifies surfaces by the UV light itself or by the atomic oxygen formed by the decomposition of ozone. -Ozone-only treatment follows a different reaction pathway, resulting in a slower treatment with less chain scission.
[27]	PET fiber	None	UVO (40 min)	-Change in the surface morphology (SEM)
[28]	PET film amorphous (powder)	LC-Cutinasa 70 °C 1000 rpm 24 h	UV (mimicking sunlight) (14 d)	-UV increased PET crystallinity -Consequently, retarded enzymatic hydrolysis.
Present study	Mixed textile (65% PET/35% cotton)	PETase 30 °C 14 d pH9	UV sun (12 weeks)	-UV sun did not significantly improve enzyme activity and even slightly hindered it in PETase MUT.
Present study	Mixed textile (65% PET/35% cotton)	PETase 30 °C 14 d pH9	UVO cleaner (18 h)	-UVO synergizes with PETase activity; 18 h pretreatment boosts TPA production by enhancing enzyme accessibility to PET fibers' hydrolysable bonds.

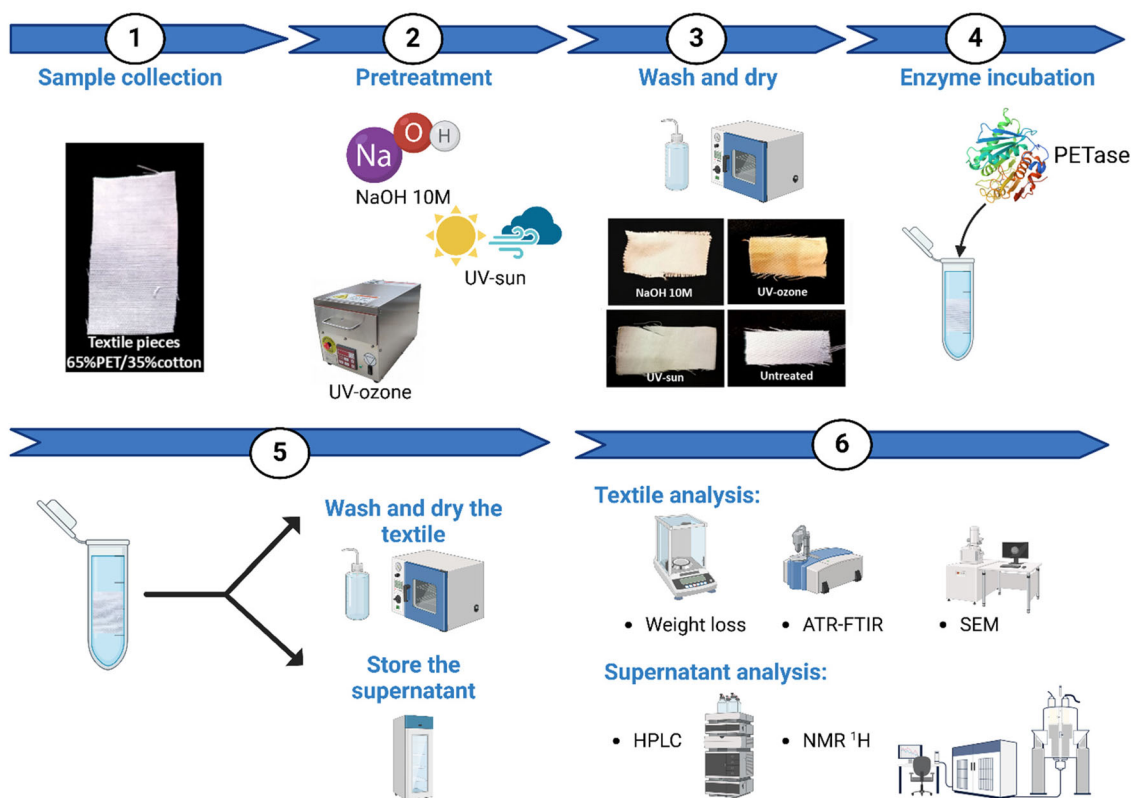


Figure 1. Schematic overview of the experimental design. A dry textile sample (65% PET, 35% cotton) is pretreated, rinsed with distilled water, vacuum-dried, and weighed. Following incubation with PETase or buffer, the supernatant is analyzed by HPLC to detect PETase degradation products. The textile is then analyzed by ATR–FTIR (to assess differential PET or cotton degradation) and SEM (to evaluate morphological changes). ATR–FTIR, attenuated total reflection–Fourier transform infrared spectroscopy; HPLC, high-performance liquid chromatography; SEM, scanning electron microscopy; UV, ultraviolet; NMR, nuclear magnetic resonance.

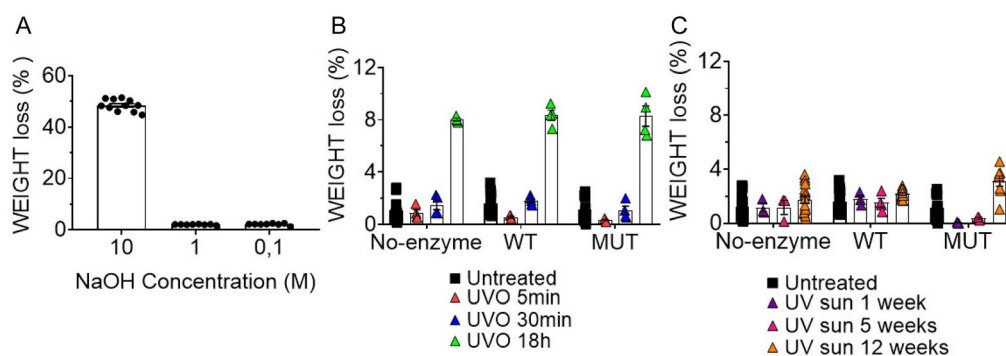


Figure 2. Textile weight loss. A) Effect of different NaOH concentrations at 30 °C for 18 h. B) Effect of varying UVO exposure times followed by enzyme or buffer treatment for 14 days. C) Effect of different UV sun exposure times followed by enzyme or buffer treatment for 14 days. For B,C): untreated (black square), UVO 5 min (red triangle), UVO 30 min (blue triangle), UVO 18 h (green triangle), UV sun 1 week (purple triangle), UV sun 5 weeks (pink triangle), UV sun 12 weeks (orange triangle). WT, PETase wild type; MUT, PETase S238F/W159H mutant.

UVO exposure (18 h) resulted in an $8.03 \pm 0.11\%$ weight loss, significantly higher than that observed with 30 or 5 min UVO exposures. Importantly, enzyme incubation did not further increase weight loss (Figure 2B), with values for WT and MUT PETase of $8.35 \pm 0.40\%$ and $8.28 \pm 0.75\%$, respectively, comparable to UVO exposure alone. This finding is consistent with previous reports showing minimal weight loss after 15 min of UV treatment.^[11] Additionally, no significant impact on weight loss was

observed with temperature changes from 30 to 40 °C (Figure S2, Supporting Information).

To explore more sustainable conditions that could facilitate enzymatic degradation of textiles, we examined whether exposure to natural environmental factors could enhance PETase activity. For this, 65% PET/35% cotton textiles were exposed to natural elements (sunlight, atmospheric ozone, and wind) for 1–12 weeks during the summer of 2023 in Castellon, Spain. No

significant differences in weight loss were observed when the samples were exposed solely to sunlight for various durations (1, 5, and 12 weeks), with a maximum weight loss of $3.11 \pm 0.38\%$ (Figure 2C). Weight loss under these conditions was not significantly influenced by enzymatic activity and did not vary significantly between 2 and 12 weeks (Figure 2C). Interestingly, short sun exposure (1 and 5 weeks) followed by incubation with the MUT PETase enzyme resulted in reduced weight loss, with values of $1.14 \pm 0.28\%$ and $1.76 \pm 0.25\%$, respectively (Figure 2C).

The maximum weight loss observed with NaOH treatment can be attributed to both the strong chemical reaction and the fact that it occurs in an aqueous solution, as opposed to other pretreatments, which were conducted on dry textiles.

2.2. Selectivity of the Pretreatment

Since the textile is composed of both cotton and PET, it is crucial to determine whether the pretreatment preferentially degrades one component over the other. To address this, we examined the textile after treatment and compared the ATR–FTIR spectra (Figure 3). The spectrum of the pristine, untreated textile is shown in the top panel (red line), with pure cotton and PET spectra provided in the supplementary materials (Figure S5, Supporting Information). Characteristic peaks for each pure material are marked with asterisks on the spectra.

No new bands were observed after the pretreatments, suggesting that the reactions during the pretreatment and washing steps lead to the solubilization of reaction products. This outcome is not unexpected, as such reactions typically yield carboxylic acids and salts, which are soluble in water.

The selectivity of degradation can be qualitatively assessed by comparing the bands of the pure materials that are located close in wavenumber. These bands can serve as internal references, mitigating potential baseline correction effects. Specifically, we analyzed the bands at 1080 cm^{-1} and 1013 cm^{-1} , which are characteristics of the ester group in PET ($\text{C}=\text{O}$) and the hydroxyl group in cotton ($\text{C}-\text{O}$), respectively^[20] (inset top panel red line Figure 3).

Notably, NaOH 10 M treatment (second panel, black line) preferentially affects PET, as evidenced by a significant reduction in the intensity of the 1080 cm^{-1} band relative to the 1013 cm^{-1} band (inset, second panel, Figure 3). This observation is in agreement with the findings of Boondaeng et al.^[20] indicating that the NaOH treatment primarily targets the ester group of the PET polymer, facilitating the breakdown of the PET chains more readily than that of cotton.

UVO exposure for 18 h is a strongly oxidizing treatment that results in a slight preferential reactivity toward cotton, as indicated by the reduction in the cotton peak (inset, light green line, third panel, Figure 3). Finally, exposure to natural sunlight for 12 days produces a degradation pattern similar to that of

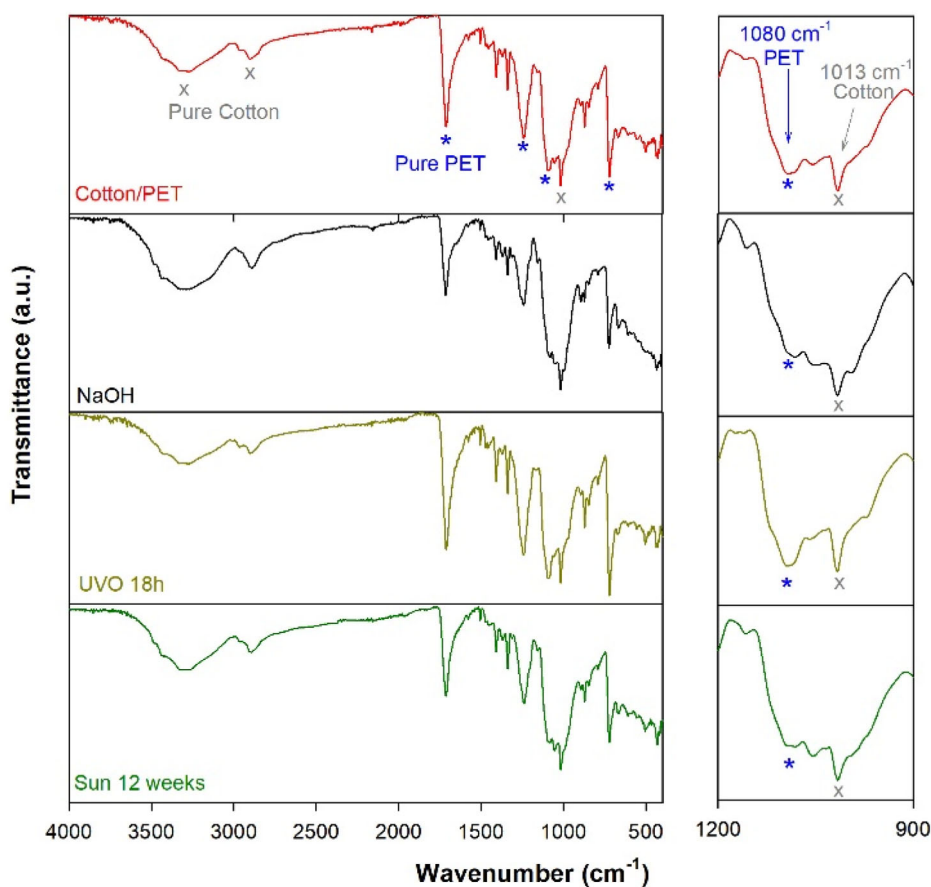


Figure 3. ATR–FTIR spectra of mixed textile. Comparison of Cotton versus PET degradation under various treatment conditions.

NaOH treatment, suggesting a preferential degradation of PET. It is important to note that preferential degradation does not imply exclusive degradation.

2.3. Morphological Alterations of Mixed Textile under Different Conditions

Next, we aimed to characterize morphologically the surface of the PET/cotton textiles under the different treatments using SEM

(Figure 4). We observed that untreated polyester and cotton fibers display a distinct appearance of a smooth surface (Figure 4A), as shown by previous reports.^[21,22] Incubation of the textile with PETase WT for 2 week period has a slight effect on the surface, with the presence of small scales at the edge of the fibers, indicating the enzyme action on the polymer surface (Figure 4B). Similar results have been reported for Lipase Cal A incubation where PET fibers in pure PET textile reduced their diameter after enzymatic action.^[11]

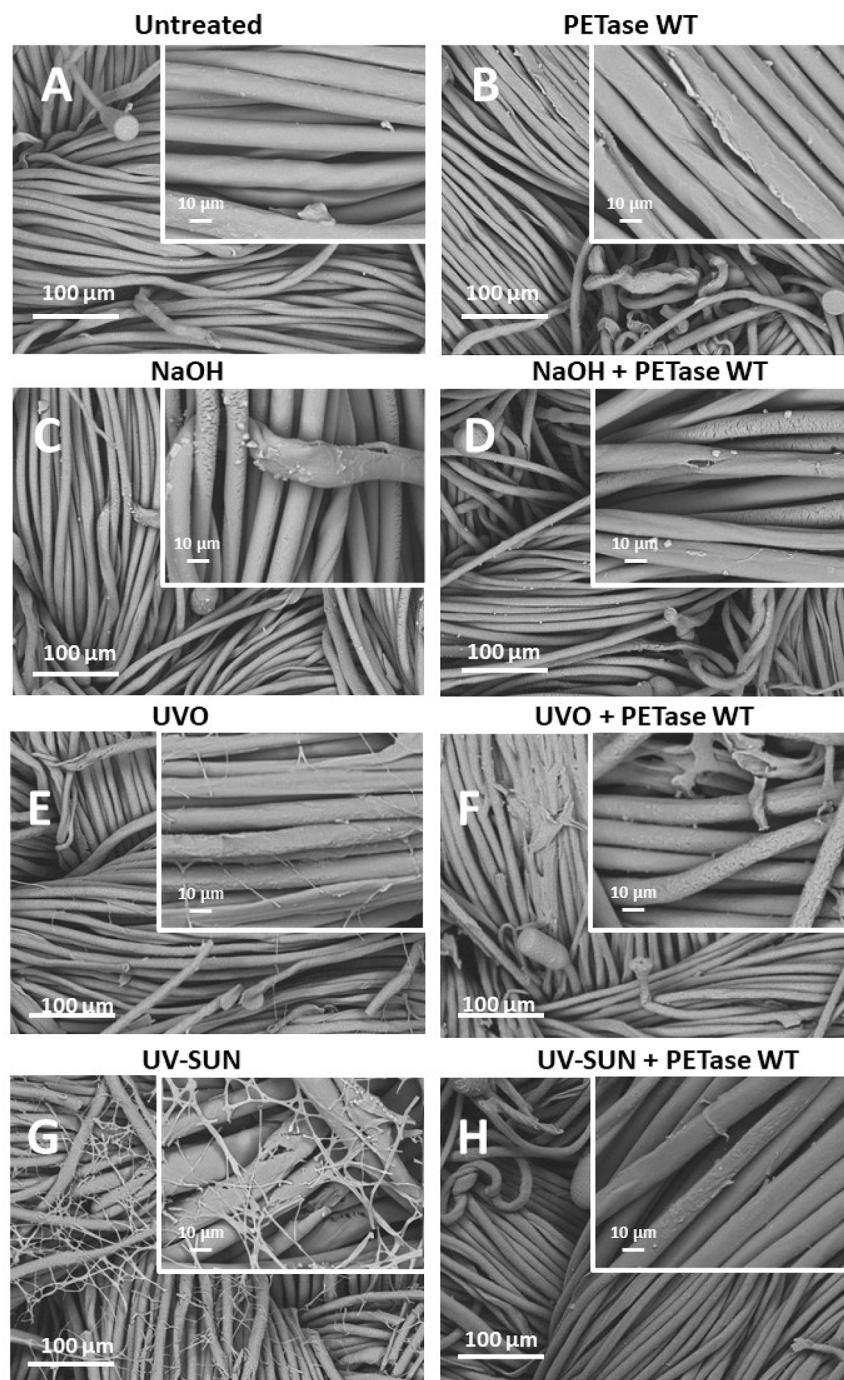


Figure 4. Comparison treatments effects on textile surface morphology by SEM. A) Untreated PET/cotton textile. B) Two week incubation with WT PETase. C) 18 h exposure to 10 M NaOH. D) 10 M NaOH (18 h) followed by 2 week WT PETase incubation. E) 18 h UVO exposure. F) UVO (18 h) followed by 2 week WT PETase incubation. G) Natural sunlight exposure (12 weeks). H) Natural sunlight (12 weeks) followed by 2 week WT PETase incubation.

Alkali treatment (10 M NaOH, for 18 h) induces evident surface alteration (Figure 4C), with distinct signs of erosion and etching, characterized by clearly defined small pits distributed at various intervals. Additionally, small deposits, absent in the untreated fiber, are observed. The sample pretreated with NaOH and incubated with the enzyme (Figure 4D) shows no added morphological differences. Our results align with previous dates showing strong surface alterations after NaOH exposure.^[11]

UVO treatment (18 h) has a strong impact on cotton/polyester textile surfaces. A uniformly roughened surface is observed on the fibers, with visible thin filaments, apparently detached from the uniform surface (Figure 4E). The combination of UVO pretreatment with enzymatic degradation leads to much more severe surface degradation, with less visible thin threads (Figure 4F). UVO exposure showed evident cracks, suggesting that this treatment goes deep into the fiber. This result agrees with previous studies that have proposed the use of UV shields to prevent textile degradation.^[23]

Finally, the samples exposed to sunlight for 12 weeks show a considerable network of thin filaments (possibly cotton fibers) that are more evident than in any other condition (Figure 4G). This might be due to the preferential attack of PET fibers, as suggested by FTIR. Similarly, to UVO exposure, PETase WT incubation after sunlight exhibits less irregularities on the surface, and a great reduction of threads compared to sunlight (Figure 4H). Although it is unclear why the cotton threads disappear with enzyme incubation in both cases (UVO and sunlight), we hypothesized that this may be due to the contribution of PETase to the detachment of cotton fibers.

2.4. Product Analysis after PETase Enzymatic Degradation

We aimed to analyze the monomer production under different conditions, to understand whether enzymatic action can improve textile recycling with targeted products, thus contributing to a circular economy.

This was evaluated by HPCL quantification of degradations products MHET and TPA. The yield was calculated with respect to initial textile weight. In addition, different enzymatic incubation times were evaluated.

PET degradation products, TPA (Figure S1A, Supporting Information), and MHET (Figure S1B, Supporting Information) were evaluated after incubation with NaOH alone or followed by enzymatic incubation. Maximum TPA yield was obtained by NaOH alone and was not improved by enzyme treatment, indicating that this severe treatment can degrade cotton and PET fibers to monomer. However, NaOH treatment did not produce MHET, being this product only found with enzymatic activity mostly with PETase MUT activity (Figure S1B, Supporting Information).

Enzyme incubation without pretreatment leads to TPA production in a time-dependent manner with slight variations depending on enzyme concentration (Figure 5, black triangles).

The impact of UVO pretreatment on PETase activity is shown for TPA (Figure 5A) and MHET (Figure 5B). Only 18 h of UVO exposure pretreatment has a notable impact on PETase activity. After 2 weeks of enzyme incubation, TPA yield reached $0.43 \pm 0.06\%$ for UVO + PETase WT; a fourfold increase above PETase WT alone ($0.10 \pm 0.02\%$) (Green triangle Figure 5A). Interestingly, at this incubation time, the plateau is not reached,

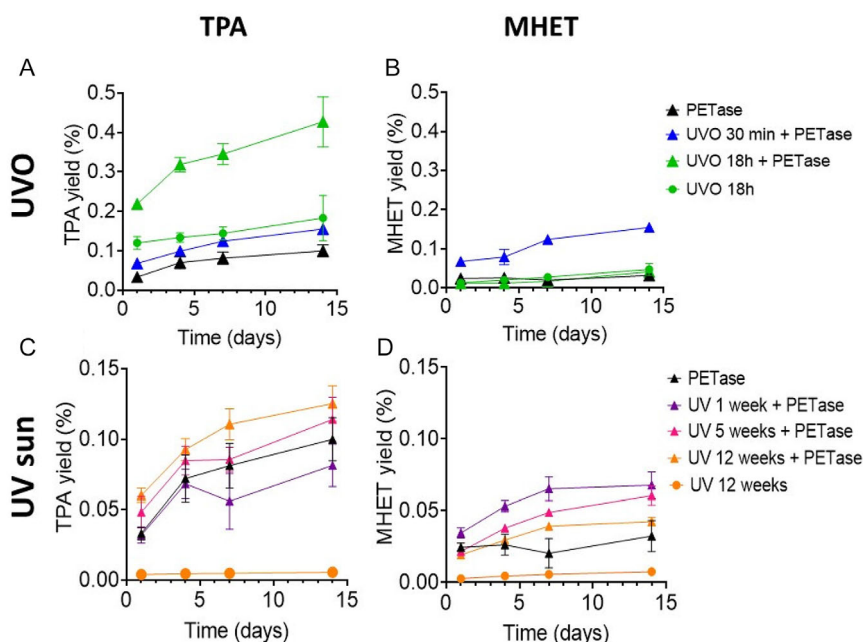


Figure 5. Effect of pretreatment on WT PETase activity (30 °C). A) TPA yield from PETase WT reaction and UVO pretreatment; B) MHET yield from PETase WT reaction and UVO pretreatment; C) TPA yield of PETase WT reaction and UV sun pretreatment; D) MHET yield from PETase WT reaction and UV sun pretreatment. Green circle, UVO 18 h; green triangle, UVO 18 h + PETase; blue triangle, UVO 30 min; black triangle, PETase alone; orange circle, UV 12 weeks; orange triangle, UV 12 weeks + PETase; pink triangle, UV sun 5 weeks + PETase; purple triangle, UV sun 1 week + PETase.

suggesting that the enzyme is still active and could increase the yield with longer incubation.

MHET production, on the other hand, was minimal under these conditions (Figure 5B), although surprisingly, 30 min exposure to UVO improved it (but not TPA) ($0.16 \pm 0.01\%$), compared to enzyme alone ($0.03 \pm 0.01\%$) (Figure 5B). These findings suggest that varying durations of UVO exposure may result in distinct effects, while longer periods (18 h) promote TPA monomer production, shorter intervals (30 min) favor MHET formation. The molecular mechanism by which shorter exposure times result in more favorable conditions for the breakdown of PET molecules into MHET remains unknown. We argue that the difference between UVO and natural conditions may rely on the different intensity of UV. As mentioned above, one hour of laboratory UVO conditions is more intense (over 4000x) than one hour of sun in summer conditions. We argue that UVO-induced chemical modifications improve enzyme accessibility to the hydrolysable bond. This was suggested by previous reports (Table 1), where UVO was shown to induce benzene ring opening within the PET molecular chains^[24] and the introduction of hydroxyl and carbonyl groups.^[24,25] Additionally, UVO can alter the surface properties of PET, making it more hydrophilic.^[25–27] Interestingly, the increased TPA production correlates with our results that suggest a favored cotton degradation (measured by FTIR) and increased roughness of the PET surface (SEM), both of which would facilitate PETase accessibility to the hydrolysable bonds of PET.

To explore sustainable conditions that would facilitate the enzymatic degradation of textiles, we analyzed TPA and MHET after exposing the mixed fibers textiles to natural elements followed by a 2 week enzyme incubation (Figure 5C,D). Twelve weeks of exposure to the sun did not result in TPA production (orange circles Figure 5C, $\approx 0.005\%$). Furthermore, it did not significantly favor PETase activity, obtaining $0.12 \pm 0.02\%$ TPA production (orange triangle Figure 5C), whereas 2 week incubation PETase WT alone resulted in a TPA yield of $0.10 \pm 0.02\%$ alone (black triangle Figure 5C).

Concerning MHET, 2 week incubation with WT PETase alone gave a very low yield ($0.03 \pm 0.01\%$) (Figure 5D), slightly incremented by 1 ($0.07 \pm 0.01\%$, purple triangle Figure 5D) and 5 weeks ($0.06 \pm 0.007\%$, pink triangle Figure 5D) of environmental conditions exposure. Remarkably, 12 week exposure did

not increase PETase WT MHET yield ($0.04 \pm 0.003\%$, orange triangle Figure 5D).

Taking together, these results indicate that environmental exposure has little or no effect on WT PETase activity. Whether much longer time of exposure will have a bigger impact is likely, but it remains to be determined. The intriguing result of the increase of MHET yield at lower sun exposure times, similar to the case with UVO pretreatment, suggests that pretreatments may modulate enzyme activity.

2.5. Effect of Pretreatment on the Activity of Both PETase Enzymes in Textile Degradation

We next aimed to compare PETase WT and PETase MUT effectiveness after the maximum effect conditions UVO (18 h) and natural conditions (12 weeks) in TPA (Figure 6A) and MHET (Figure 6B) yield.

TPA yield from PETase activity with no pretreatment was similar for PETase WT ($0.10 \pm 0.02\%$) and PETase MUT ($0.13 \pm 0.02\%$) (gray columns in Figure 6A). Without enzyme, UVO 18 h had a noticeable TPA ($0.183 \pm 0.06\%$) (green column Figure 6A) whereas environmental conditions did not (orange column Figure 6A). A slight but synergistic effect was found combining UVO 18 h with PETase incubation, increasing by almost twofold PETase WT ($0.43 \pm 0.06\%$) and PETase MUT ($0.37 \pm 0.04\%$) TPA yield (green columns in Figure 6A).

In contrast, environmental conditions did not have any effect on PETase WT activity but intriguingly decreased PETase MUT activity from $0.13 \pm 0.02\%$ to $0.05 \pm 0.01\%$ of TPA yield (orange columns Figure 6A). This result would agree with Falkenstein et al.^[28] study (Table 1), where exposing PET film amorphous (powder) to UV radiation prior to enzymatic incubation hindered enzymatic activity. NMR techniques studies of chain dynamics revealed an increase in the rigidity of UV-treated polymer chains, which was associated with an intensification of surface PET crystallinity and thereby reducing the accessibility for enzymatic hydrolysis.^[28] However, in our study this reduction is only evident with PETase MUT, but not PETase WT, and the mechanism underlying this difference remains elusive.

Concerning MHET, the highest yield was observed with PETase MUT alone ($0.08 \pm 0.004\%$), a 2.5-fold higher than PETase WT ($0.03 \pm 0.01\%$, gray columns Figure 6B). MHET

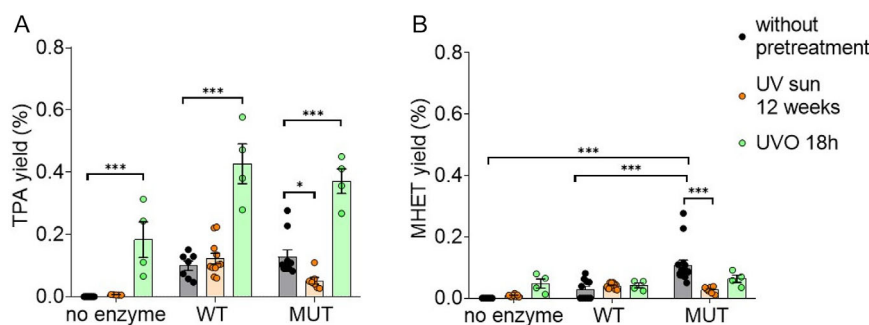


Figure 6. Effects of UVO and natural sunlight on WT and MUT PETase activity on PET degradation product yield, A) TPA and B) MHET. Gray columns, control, WT, or MUT enzyme without pretreatment; orange columns, control, WT, or MUT enzyme following 12 week exposure to natural sunlight; green, control, WT, or MUT enzyme following 18 h exposure to UVO.

production by MUT was significantly reduced by 12 week sun exposure ($0.04 \pm 0.007\%$) and did not affect PETase WT (orange columns Figure 6B). Interestingly, shorter environmental exposure time (1 week) increased MHET yield in short, but not long PETase MUT enzyme incubation, (4 days) ($0.09 \pm 0.007\%$) (Figure S4, Supporting Information). On the other hand, UVO induces a minimal amount of MHET, and this is not affected by PETase incubation (green columns Figure 6B). However, shorter intervals (30 min) also favor MHET formation by PETase MUT (Figure S4, Supporting Information). This result agrees with the observed with NaOH pretreatment (Figure S1, Supporting Information), indicating that PETase S238F/W159H mutation favors the production of MHET. PETase WT-dependent production of MHET was not affected by any pretreatment.

Taking together these results, although the mechanism is unknown, the aggressive pretreatment of UVO 18 h seems to direct enzymatic activity to TPA production. When comparing TPA production with other studies reporting textile enzyme degradations, the yield is very low (Table 2). This is due to the aggressive nature of the pretreatment used such as high pressure and high temperature^[16] or milling.^[17] These treatments may be effective; however, they are energetically costly. The research of cost-effective treatments is warranted.

To better understand the differential effect between natural sun and UVO, it is important to note that 1 h of UVO radiation has an intensity of $1152 \times 10^3 \text{ W m}^{-2}$, whereas a full day of summer sunlight has an intensity of 6408 W m^{-2} . Consequently, 1 h of UVO exposure is equivalent to 6 months of summer UV sunlight exposure (which we do not get in latitudes far from the Ecuador as sun intensity varies with seasons).

2.6. Alkaline and Photooxidative Treatments Induce Molecular and Structural Changes

To gain deeper insight into the molecular changes occurring during UVO-induced PET degradation, we analyzed the ^1H NMR spectrum of the supernatant obtained from PET/cotton textiles exposed to NaOH and UVO radiation (Figure S6, Supporting Information). The results confirm distinct structural modifications associated with each pretreatment (Figure 7).

Sodium hydroxide promotes the alkaline hydrolysis of PET through cleavage of ester bonds, leading to the formation of TPA and EG. This process enables PET to be chemically recycled into its monomers, which can be purified and reused in the production of new plastic materials.^[29] The absence of other signals indicates that, under the treatment conditions (10 M NaOH at 30°C), no cellulose degradation products from cotton were generated in concentrations detectable by this technique, suggesting minimal degradation of the cotton component. This result is consistent with the data obtained from infrared (IR) spectroscopy.

In contrast, UVO exposure leads to photo-oxidative degradation of PET, resulting in polymer chain scission, which reduces crystallinity and creates more enzyme-accessible regions and the formation of various oxygen-containing functional groups,^[30] including carboxyl, hydroxyl, and carbonyl moieties^[28,31] on the surface of PET. These modifications increase the hydrophilicity of PET, making it more accessible to PETase. This preconditioning effect of UVO treatment explains why the combination of UVO pretreatment and enzymatic activity works synergistically to enhance textile biodegradation, providing a more effective strategy for plastic waste management

Table 2. PET degradation in mixed textiles. Comparison of different treatments.

Ref.	Substrate	Treatment	Enzyme conditions	Yield % (TPA)	Results
[9]	New cotton/PET bed sheets	NaOH (2.5 M, 150 min, 90°C)	–	100	NaOH completely degraded PET.
[11]	100% PET textile	NaOH or UV excimer lamp (15 min) or enzyme lipase Cal A	Lipase Cal A 90 min at 50°C and pH 4.2	–	Alkali: Severe morphological degradation UV: Maximum polar groups with minimal strength/weight loss Enzymatic: Nonuniform degradation with variable fiber diameter
[16]	PET fiber with a viscosity of 0.62 dL g^{-1} .	Water-based PET hydrolysis (250°C , 39ba) and enzyme HiC	HiC (1 or 2 mg ml^{-1}) 24 h at 50°C and 600 rpm	12.34 (6.5 mm)	Pretreatment: 85% TPA. Enzymatic hydrolysis: 97% TPA
[17]	65/35 Postconsumer PET/cotton textile	Premilling 5 min and enzyme HiC	HiC 7 d at 55°C and pH 7.3	8.2 ± 0.2	Postconsumer textile contaminants and inherent structural differences in specific textile types may contribute to lower TPA yields.
Our study	65/35 Postconsumer PET/cotton textile	NaOH 10 M and enzyme PETase WT or UVO 18 h and enzyme PETase WT or UV sun 12 weeks and enzyme PETase WT or enzyme PETase WT alone	PETase 14 d at 30°C and pH 9	8.15 ± 3.22 0.43 ± 0.06 0.12 ± 0.02 0.10 ± 0.02	NaOH pretreatment: Nonselective degradation of both PET and cotton components UVO pretreatment: Enhances PETase effectiveness; significantly increases TPA yield Natural sunlight: Minimal effect on PET degradation; primarily affects cotton component

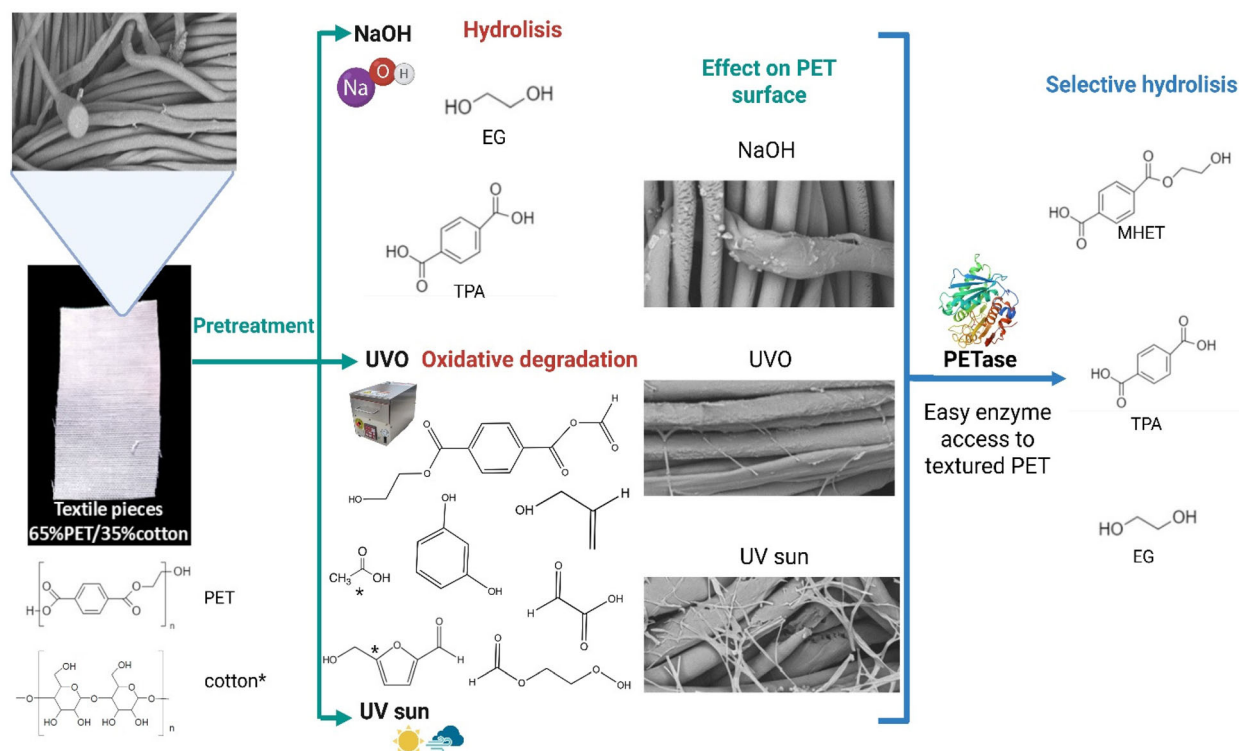


Figure 7. Mechanism of PET/cotton textile degradation. The textile was exposed to various pretreatment conditions, resulting in visible alterations on the PET surface. NaOH pretreatment directly induces PET hydrolysis into simple monomers TPA and EG, which remain unaffected by subsequent PETase application. In contrast, PETase alone acts on intact PET, generating intermediates such as MHET. UVO and UV pretreatments modify the PET structure to generate intermediate products that remain susceptible to further enzymatic degradation by PETase. Cotton components undergo minimal degradation under these conditions.

Also, UVO irradiation can induce cleavage of glycosidic bonds and oxidation of hydroxyl groups in cellulose from cotton, generating low molecular weight compounds.^[32]

Our ¹H NMR analysis of supernatants from UVO-treated PET/cotton composites confirmed the formation of low-molecular-weight degradation products originating from both polymers.

Although no distinct signals were observed in the aldehyde region, a small peak at δ 8.2 ppm suggests the presence of formate or possibly overlaps with TPA signals. Additional resonances at δ 2.1 ppm may correspond to the methyl group of acetic acid, although contributions from PET degradation products cannot be excluded. Peaks at δ 3.5–4.0 ppm may indicate the presence of ethylene glycol (EG) or hydroxylated degradation products from cellulose, though signal overlap with the buffer complicates precise interpretation. In the broader region of δ 6.0–8.0 ppm, signals likely arise from compounds derived from both PET and cotton, including sugar-derived species such as hydroxymethylfurfural, with a notable resonance at δ 6.7 ppm observed exclusively in UV-treated samples.

For PET specifically, the spectrum exhibits shifts at δ 7.8–8.1 ppm consistent with oxidation of the aromatic ring and the formation of carboxylic acid groups. Additional signals at δ 4.3–4.5 ppm indicate polymer chain scission and a reduction in intact ester linkages. Resonances at δ 3.7 ppm further support the release of free EG, a known product of PET backbone fragmentation. Broad signals in the aliphatic region (δ 1.2–2.0 ppm)

are attributed to low-molecular-weight degradation products or partially oxidized intermediates.

These findings demonstrate that UVO treatment effectively induces photooxidative degradation in both PET and cellulose components, resulting in the release of low-molecular-weight compounds detectable by NMR. The structural alterations caused by UVO treatment suggest significant molecular degradation, which may enhance the susceptibility of PET to subsequent enzymatic breakdown.

3. Conclusions

Our study demonstrates the critical importance of effective pretreatment methodologies for enhancing the enzymatic degradation of PET in complex textile matrices. We systematically compared UVO exposure, alkali treatment, and natural sunlight conditions, revealing significant differences between their mechanisms and efficacy. We concluded that UVO pretreatment enhances selective biodegradation of mixed textiles.

Alkali treatment (NaOH) degraded PET and cotton components nonspecifically, causing substantial weight loss without enhancing enzymatic selectivity. Although NaOH effectively depolymerizes PET and partially degrades cellulose under harsh conditions, the moderate temperature parameters used in this study (30 °C) limited the extent of degradation for both polymers, preserving textile integrity while still allowing some chemical modification.

Laboratory UVO treatment induced nonspecific weight reduction but demonstrated synergistic effects with subsequent PETase activity. Notably, UVO exposure duration significantly influenced degradation product profiles, with extended treatment (18 h) favoring complete depolymerization to TPA, while shorter exposure (30 min) yielded primarily MHET. This time-dependent product selectivity represents a significant advance in controlling PET degradation pathways.

Our (NMR) nuclear magnetic resonance data suggest that the combined action of UV light and ozone greatly improve enzyme accessibility and activity by: 1) generation of oxygen-containing functional groups, increasing the surface hydrophilicity of the polymer; 2) structural changes within the polymer matrix, i.e., cleavage of ester bonds, leading to partial depolymerization that yield smaller fragments, and 3) reduced PET crystallinity by promoting chain scission and increasing molecular disorder.

Natural environmental conditions (sunlight UV, atmospheric ozone, precipitation) applied for up to 12 weeks showed minimal enhancement of WT PETase activity and slight inhibition of MUT PETase variants, despite inducing visible morphological alterations. FTIR analysis indicated that solar exposure may preferentially affect cotton rather than PET components, suggesting material-specific photodegradation mechanisms that must be considered in environmental fate models. We argue that the difference between UVO and natural conditions may rely on the different intensity of UV. As mentioned above, one hour of laboratory UVO conditions is more intense (over 4000x) than one hour of sun in summer conditions has an intensity of $1152 \times 103 \text{ W m}^{-2}$, whereas a full day of summer sunlight in Castellon area has a mean intensity of 6408 W m^{-2} .

WT and MUT PETase enzymes produced distinctly different degradation product profiles, highlighting the potential for designing enzymes with tailored activities for specific recycling applications—a crucial consideration for developing circular economy solutions for textile waste.

UVO pretreatment shows the most promise for enhancing PETase-mediated degradation of PET in textile materials. This finding has significant implications for developing scalable, energy-efficient recycling technologies. Further research is needed to elucidate the precise mechanisms of bond alterations and develop optimized strategies using UVO or extended sunlight exposure as cost-effective pretreatment approaches for textile recycling.

This study presents one of the first comprehensive and systematic comparisons of UVO exposure, alkali treatment, and natural sunlight for enhancing PETase activity in textile biodegradation. Notably, the inclusion of UVO pretreatment as a central variable is particularly novel, as prior work has largely focused on chemical or thermal strategies, with limited attention to advanced photochemical oxidation techniques.

A key strength of this work lies in its detailed mechanistic analysis of UVO-induced modifications to PET textiles at both the chemical and structural levels. By examining changes in surface functionalization, hydrophilicity, and crystallinity—critical factors governing enzymatic accessibility and activity—the study offers valuable insights into why UVO pretreatment outperforms conventional approaches, providing a foundation for rational process optimization.

Importantly, the use of real-world textile samples, as opposed to model PET powders or films, enhances the practical relevance of the findings. This choice addresses the additional challenges posed by the heterogeneous composition and structure of commercial textile waste, making the results more applicable to real recycling scenarios.

Furthermore, the study highlights the synergistic effect of UV light and ozone in promoting PET breakdown—a combination that remains largely underexplored in the context of enzymatic recycling. This synergy opens new perspectives for the development of integrated UVO–enzyme treatment strategies that could significantly improve the efficiency of plastic waste processing and contribute meaningfully to addressing the global plastic pollution crisis.

4. Experimental Section

Detailed descriptions of all materials and methods used in this study are provided in the **Supporting Information Appendix: Experimental Details**.

Enzyme Expression and Purification

Both PETases (WT and MUT) were expressed in a heterologous system using *Escherichia coli* cells and purified following the protocol described by Giraldo-Narcizo et al.^[10] The enzyme concentrations were determined using a Nanodrop Spectrophotometer.

NaOH Pretreatment of PET-Cotton Textile

Textile samples were immersed in NaOH solutions (0.1, 1, and 10 M) for 24 h at 30 °C under agitation.

UVO Pretreatment

A UVO Cleaner (Model No. 42-220, Jelight Company, Inc.) emitting light at 253.7 nm with an irradiation power of $28\text{--}32 \text{ mW cm}^{-2}$ was used to irradiate textile pieces ($\approx 15 \text{ mg}$). Scoured samples were exposed for 5 or 30 min on both sides and for 18 h under an excimer lamp, maintaining a distance of 5 mm.

UV Sunlight Pretreatment

Textile pieces were exposed to natural atmospheric conditions for up to 3 summer months in the Castellon area, Spain.

Enzymatic Degradation of PET

Untreated and pretreated textile pieces were incubated in 1 mL glycine-NaOH buffer (50 mM, pH 9.0) containing purified enzymes (2 mg enzyme/g PET) at either 30 or 40 °C for 1–14 days.

Analysis

After incubation, textile pieces were rinsed with distilled water and vacuum-dried for 20 h at 40 °C. Weight loss was measured, and samples were analyzed by ATR–FTIR and SEM. The supernatant was centrifuged, filtered, and analyzed by HPLC to quantify PET degradation products. The yields of MHET and TPA were expressed as percentages at different incubation times. Proton nuclear magnetic resonance

(¹H NMR) spectra of the supernatants were obtained using an AVANCE III HD spectrometer (Bruker, Germany) operating at 400 MHz, with DMSO-d₆ as solvent.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Sariah Giraldo-Narcizo: data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing—original draft (equal); writing—review and editing (equal). **Antonio Guerrero:** conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (lead); project administration (lead); resources (supporting); supervision (lead); writing—original draft (equal); writing—review and editing (lead). **Ana Maria Sanchez-Perez:** conceptualization (lead); data curation (lead); formal analysis (equal); funding acquisition (lead); project administration (equal); supervision (lead); writing—original draft (lead); writing—review and editing (lead).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: environmental conditions • PETase • textiles • terephthalic acid • UV–ozone

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