Efficient degradation of oxidized polyethylene containing ester bonds by cutinase or lipase in aqueous phase

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Abstract:

Polyethylene is a widely used plastic that presents challenges in degrading within natural environments. Therefore, the degradation of polyethylene is of utmost importance. Currently, physical or chemical methods are efficient but more dangerous. Conversely, biological methods are safer but less efficient. Combining the two approaches to create entirely new polyethylene degradation pathways is a viable idea. Based on the previous research, it is an effective method to add ester bonds inside polyethylene and degrade using cutinase or lipase. However, the method only detected a decrease in molecular weight and did not observe a rate of weight loss at the macroscopic level. In this research, rapid degradation of polyethylene is achieved by optimizing catalysis conditions and machine learning. Finally, the maximum weight loss up to 80.51% within 24h and a mild catalysis environment (60-80°C). Furthermore, the method's versatility was confirmed for cutinase and lipase. Overall, this research has established an effective, efficient, and mild degradation process of polyethylene, significantly contributing to the progress in polyethylene degradation.

Keywords:

Polyethylene, cutinase, degradation, weight loss

Introduction

Polyethylene, a ubiquitous used plastic[1], is widely used in the agricultural, food, construction and medical industries. However, due to its stable chemical properties, polyethylene is difficult to degrade in the natural environment and is accumulating in large quantities, polluting the environment[2]. Hence, in order to replace traditional incineration and landfill methods, the quest for safe, dependable, and efficient degradation techniques is imperative.

Physical or chemical methods have the advantage of high efficiency. The catalyst $mSiO_2/Pt/SiO_2$ was utilized for polyethylene hydrogenation after 6 h at 300□ under 1.38 MPa of H_2, resulting in the production of alkanes spanning from C_6 to C_{40}[3]. L-ZrO_2@$mSiO_2$ or a comparable material was employed for decomposing polyethylene to generate C_8-C_{40}[4]. Polyethylene can be fully converted into C_6-C_{10} at 70 °C, and the

degradation system was catalysed under Lewis acidic conditions.[5] Microwaves and $FeAlO_x$ were employed in the degradation of polyethylene to generate H_2 and solid carbon[6]. The utilization of polyethylene as a substrate for the production of olefins was achieved through dehydrogenation, which was coupled with tandem isomerizing ethenolysis for the desaturated chain[7]. Nonetheless, the scalability of the mentioned catalysts or processes on an industrial level poses a significant challenge, constituting a critical drawback of physical or chemical methodologies [8].

Biological degradation methods are safer but less efficient than the above methods[8]. When biological methods are combined with physical or chemical methods, the efficiency and practicality of the methods are effectively enhanced.[9] Yao et al. reported that the laccase-mediator system can be used to degrade LDPE with UV treatment, and the weight-average molecular weight (M_w) was reduced by approximately 40% compared with that of the original PE[10]. Latex clearing protein from the *Streptomyces* sp. Strain K30 degradation system resulted in a 42.02% reduction in the Mw of UV-treated LDPE[11]. On the other hand, more and more enzymes have demonstrated the ability to catalyze the degradation of polyethylene and yielding carbonyl, hydroxyl or carbon-carbon double bonds. Examples include laccase from *Psychrobacter* sp. NJ228[12], glutathione peroxidase from *Alternaria alternata* FB1[13] or *Rhodococcus* sp. C-2[14], and demetra or cere from *Galleria mellonella* larvae[15]. These enzymes present novel opportunities for the enzymatic breakdown of polyethylene.

In conclusion, incorporating appropriate functional groups into polyethylene proves to be a highly effective approach for accelerating its degradation process. Polymers containing ester bonds exhibit greater susceptibility to degradation compared with polyolefins, such as poly (ethylene terephthalate)[16] and poly (butylene adipate-co-terephthalate)[17]. Similarly, ester bonds can be added in polyethylene by using the Baeyer–Villiger oxidation[18, 19] and be degraded using cutinase from *Thermobifida fusca* WSH03-11. However, previous research noted that while cutinase degraded the polymer, the efficiency was limited to molecular weight loss[18]. To address this, the catalytic system was enhanced by incorporating various surfactants, which helped to

increase the solubility of polyethylene and its oxidation products in water and increased the degradation rate of the system[20, 21]. Ultimately, the weight loss of polyethylene after cutinase degradation was up to 80.51%. Moreover, the research used machine learning to determine the optimal conditions for the degradation system. The research establishes a gentle and effective method for converting polyethylene to long-chain fatty acids or fatty alcohols, as well as determining the versatility of the method in cutinase or lipases.

Materials and methods

Reagents, plasmid, and strain

Polyethylene film (PE_{film}) and pure polyethylene (PE_{pure}) were purchased from PetroChina Company Limited and Shanghai Macklin Biochemical Technology Co., Ltd, respectively. The reagents used to prepare the medium include tryptone, yeast extract, NaCl, K_2HPO_4, KH_2PO_4, kanamycin (Kan), ampicillin (Amp), and isopropyl-beta-D-thiogalactopyranoside (IPTG), all of which were purchased from Sinopharm Chemical Reagent Co., Ltd. Some surfactants, embedding agents, or organic reagents were used for enhancing the solubility of products in this study, mainly including sodium dodecyl sulfate (SDS), hexadecyl trimethyl ammonium Bromide (CTAB), Triton X-100, PEG, betaine, rhamnolipid, cyclodextrin (α, β, γ), and dimethyl sulfoxide (DMSO), and they were purchased from Sinopharm or Taobao (China) Software Co., Ltd. Some of the model substrates used for enzyme activity assays were purchased from Merck KGaA, including 4-nitrophenyl butyrate (pNPB), 4-nitrophenyl octanoate (pNPO), 4-nitrophenyl laurate (pNPL), 4-nitrophenyl myristic (pNPM), and 4-nitrophenyl palmitic (pNPP). Four plasmids expressing cutinases or lipases, which contained the genes of *Thermobifida fusca* cutinase (*Tf*Cut), *Humicola insolens* cutinase (HiC), *Thermomyces lanuginosus* lipase (TLL), *Ideonella sakaiensis* cutinase (*Is*PET), were constructed, namely pET-24a(+)-*TfCut*[16], pPIC9K-*HiC*[22], pPIC9K-*TLL*[23], and pET-21b(+)-*IsPET*[24]. The hosts used to construct plasmids or express genes in this paper included *Escherichia coli* (*E. coli*) JM109, *E. coli* BL21(DE3), and *Pichia pastoris* (*P. pastoris*) KM71.

Preparation and properties of cutinase and lipase

*Tf*Cut and *Is*PET were expressed using *E. coli* BL21(DE3), and the expression conditions were 37°C enrichment culture for 2h and 25°C culture for 24h in order to express the target enzyme after the addition of 0.1mM IPTG. Kan or Amp was added throughout with 30 μg/mL or 100 μg/mL. Hic and TLL were expressed using *P. pastoris* KM71, as previously described[22, 23]. In order to exclude interference from the culture medium, all enzymes were subjected to ammonium sulfate precipitation, dialysis and freeze-drying to obtain protein powder. The enzyme active was measured using pNPB as substrate as previously described[22]. When testing the preference of different enzymes for carbon chain length, 4-nitrophenyl ester of different lengths as above are used. The proteins concentration was measured using Bradford protein assay kit, and the distribution of proteins was examined using SDS-PAGE.

Preparation of oxidized polyethylene containing ester bonds

The oxidized polyethylene containing ester bonds was prepared using meta-chloroperoxybenzoic acid (^{m}CPBA) as previously described[18], the process mainly included the dissolution of PE using a mixture of 1,2,4-trichlorobenzene and dichloroethane in the ratio of PE:solution=1g:20mL and the oxidation of PE using ^{m}CPBA in the ratio of 1:5 (PE:^{m}CPBA). PE_{film} and PE_{pure} were used as substrate. For PE_{film}, dissolving step was performed for 12h in order to fully dissolve. For PE_{pure}, the step takes only 2h[19]. In the research, oxidized polyethylene with different ester bond content was prepared and the parameters were shown in Table S1.

Construction of cutinase or lipase degradation system in aqueous phase

Cutinase or lipase degradation contained enzymes, additives (surfactants, embedding agents, or organic reagents), phosphate buffer, and oxidized polyethylene. Different factors were optimized in the system, including the type and concentration of additives, rotational speed, the type and concentration of enzyme, temperature, pH, degradation days, and the characteristic of oxidized polyethylene.

The degradation products were treated as follow. First, the products were filtered using nylon mesh. Second, the solids obtained by filtration were washed twice using 75% ethanol and water, respectively. Lastly, the solids were dried in an oven at 60

for 12h. Optimizations results were judged by weight loss ratio, and the ratio was calculated according to eq.1. Among eq.1, W was weight loss ratio, S was the weight of nylon mesh and products, s was the weight of nylon mesh, M was the weight of substrate, s_0 and s_1 was the weight of empty nylon mesh before and after drying.

$$W = \frac{S-s}{M} \times \frac{s0-s1}{s0} \qquad \text{eq1}$$

Test methods on the products of degradation system

For the degradation products, the test methods included Fourier transform infrared spectroscopy (FT-IR), high-temperature gel permeation chromatography (HT-GPC), gas chromatography-tandem mass spectrometry (GC-MS), Differential Scanning Calorimeter (DSC), and dynamic/static laser scattering instrument. FT-IR was used to analyze the ester bonds content in oxidized polyethylene with a scanning range of 4000 cm^{-1} - 400 cm^{-1} and 32 scans. Ester bond index (EBI) is defined as the ratio of the peak height at ~1735 cm^{-1} (ester bond) to the peak height at ~1465 cm^{-1} (methylene). The FT-IR data was treated using Omnic 8.2.0.387. HT-GPC was used to determine weight average molecular weight (M_w), number average molecular weight (M_n), and dW/d(LogM)-Log(M) differential distribution curve in oxidized polyethylene. The sample was dissolved in trichlorobenzene. The detail method contained detection temperature 145°C, injection volume 300 μL, elution solution dichlorobenzene, elution time 50 min, and elution flow rate 1.0 mL/min. GC-MS was used as a tool for the analysis of end products. GC conditions were a DB-5MS column, injection rate 1.0 ml/min, initial temperature 40°C, ramped up from 40°C to 250°C at a rate of 10°C min^{-1}, and a 4-minute maintenance. The MS conditions involved molecular weight detection range 33-450, an ion source temperature 70°C, and an electron energy 70. DSC was used for achieving melting temperature (T_m). The temperature ramped form 30°C to 300°C at a rate of 10°C min^{-1}. All data was treated and drawn using R.

Random forest machine learning optimizes degradation systems

Machine learning was performed using the package randomForest, and GenSA was used for optimal solution solving in R. Independent variables included EBI, pH,

degradation days, temperature, amount of enzyme addition, rotational speed, the type and concentration of additives, with the dependent variable being the weight loss rate. Among them, temperature, amount of enzyme addition, and rotational speed were taken as a logarithm for a better fit. Analysis of the input data was accomplished through the use of the vivid package, including Variable Importance, Variable Interactions, partial dependence plot (PDP), and individual conditional expectation Curves (ICE) curves[25]. The optimal conditions were analyzed using Shaply. All the code for random forests has been placed in supporting information.

Result and discussion

The preparation of cutinase and lipase

In order to determine the versatility of the degradation systems, two extensively researched cutinases and lipases were chosen for this study, mainly including *Tf*Cut, *Is*PETase, TLL, and HiC. These enzymes were selected due to their distinctive features, particularly the variance in substrate binding grooves and charge distribution (shown in Fig. 1). For the former, *Tf*Cut and *Is*PETase prossess open tunnels without significant site resistance at entry and exit points, making them more suitable for binding medium- and long-chain substrates. This aligns with their capacity to degrade ester bonds within the main chains of large molecules such as poly (ethylene terephthalate) [16, 24] and poly (butylene adipate-co-terephthalate)[17]. Conversely, TLL and HiC exhibit substrate-binding grooves with greater site resistance, aligning with the ability to degrade ester bonds in the side chain such as poly (methyl acrylate) and poly (ethyl acrylate)[22, 26] For the latter, *Is*PET and HiC are predominantly positively charged near the active site, whereas *Tf*Cut and TLL are characterized by negatively charged.

The enzymes were produced using *E.coli* BL21(DE3) or *P. pastoris* KM71. All enzyme were concentrated and analyzed through ammonium sulfate precipitation and SDS-PAGE. According to pre-laboratory, the concentrations of NH_4SO_4 were determined to be 30% (w/v), 50%, 50% and 40% for *Tf*Cut, *Is*PETase, TLL and HiC, respectively. The result of the SDS-PAGE analysis are presented in Fig S1. Greyscale

analysis using Image-J, the percentages of the four enzymes were 73.15%, 41.95%, 63.49%, and 74.61%. The specific enzyme activity (pNPB as substrate) of them were 52.5, 3.5, 456.6, and 5.0U mg^{-1} of enzyme powder for *Tf*Cut, *Is*PETase, TLL, and HiC, respectively. In comparisons with substrates of different carbon chain lengths (pNPO, pNPL, pNPM, pNPP), *Tf*Cut exhibited enhanced affinity for long-chain substrates when compared to the other enzymes, as demonstrated in Fig S2.

Preparation and properties of oxidized polyethylene

^{m}CPBA catalysis Baeyer-Villiger oxidation to introduce ester bonds into the main chain of PE[18, 19]. This reaction transforms the more resistant carbon-carbon single bonds into readily reactive ester bonds. Subsequent to ^{m}CPBA oxidization, FT-IR analysis (Fig. 2) indicated that PE_{film} and PE_{pure} can both generate ester bonds, with a higher ester bonds content in PE_{pure} compared to PE_{film}. However, due to the stochastic nature of ^{m}CPBA oxidation, different batches of oxidized polyethylene were used in this study and characterized by EBI value. Table S1 presents the range of EBI values (1.06-2.11) for the varied batches of oxidized PE examined in this study, and the FT-IR results of the oxidized PE sample were displayed in Fig S3.

The degradation system for polyethylene film with high molecular weight

To evaluate the feasibility of degradation system, PE_{film} was initially employed as experimental subjects to establish an aqueous phase system. When utilizing oxidized PE_{film} as substrate, SDS, PEG400, β-CD, and CTAB were incorporated to enhance the weight loss ratio. Specifically, the degradation system for PE_{film} comprised 40U mg^{-1} substrate of *Tf*Cut, additive (2% SDS, 10% PEG, 10mM β-CD, or 2% CTAB), phosphate buffer (pH 8.0), and oxidized PE_{film}. Following an incubating period at 60℃ for 7d, the CTAB group exhibited the had highest weight loss at approximately 19.72% (compared to the control, as illustrated in Fig. 3A). Remarkably, the inclusion of CTAB in aqueous phase system also resulted in a weight loss of 27.93% in the control sample. It meant that the phosphate buffer contained CTAB can resolve oxidized PE_{film}. Compared with other control groups, CTAB demonstrated the most significant solubilizing impact. The results of particle size distribution (Fig. 3B) revealed that the CTAB group contained more large particles, consistent with *W* result. In the FT-IR

results (Fig. 3C and detail data in Fig. S4), the EBIs of the experimental groups were consistently lower than those of the control group, implying that the presence of *Tf*Cut cleaves the ester bond. Inconsistent with the weight loss rate, the difference in EBI was not the largest in the CTAB group, possibly due to CTAB's enhanced substrate solubility. Additionally, the GC-MS results for the enzyme digests dried products illustrated that the enzyme digests from the experimental groups contained a great variety of substances, mainly monohydric alcohols, diols, binary acid, and hydroxy fatty acids, then those of the control group (shown in Fig 3D, and detail data in Table S2). The results indicated that *Tf*Cut catalyzed oxidized PE_{film} into small molecule fragments. The CTAB group displayed a broader array of substances, underscoring its efficacy. Overall, incorporating additives enhanced the performance of TfCut, with CTAB emerging as the most beneficial. It is crucial to emphasize that this section solely aims to verify the method's capability to process PE_{film} in its pristine state. Subsequently, the optimization of the degradation system was pursued using PE_{pure} as *W* was unsatisfactory with PE_{film} as the substrate. This approach enabled more precise optimization and eliminated potential interference from plasticizers.[27].

The optimization of degradation system using pure polyethylene with low molecular weight

To prevent the interference of substances such as plasticisers and antioxidants on the detection of weight loss[27], PE_{pure} was utilized as the substrate for all subsequent experiments. The optimization of the degradation system involved refining the type and concentration of additives, rotational speed, the concentration of enzyme, temperature, pH, degradation days based on the result of PE_{film}. Additives encompassed SDS, CTAB, TritonX-100, PEG, betaine, rhamnolipid, α-CD, β-CD, γ-CD, and DMSO. Following a 7d reaction period, the SDS, CTAB, TritonX-100 group exhibited the highest weight loss ratio, but the deviation from control group was not the highest, seeing Fig. 4A. The outcome can be attributed to the superior solubilizing properties of these surfactants for oxidized PE_{pure} and their impact on *Tf*Cut enzyme activity (particularly SDS and CTAB group, shown in Fig. 4B). In comparison, the rhamnolipid group displayed the most significant distinction in weight loss rate

compared to the control group, with rhamnolipid exerting a minor influence on the enzyme activity. Hence, rhamnolipid was identified as an optimal additive for further enhancement. Subsequently, experiments were conducted to explore the impact of varying rhamnolipid concentrations from 0.2% (w/v) to 8%. The results (Fig. 4C and D) showed that a rhamnolipid concentration of 1% yielded the most effective degradation performance, with minimal influence on *Tf*Cut enzyme activity. To mitigate the occurrence of the sticky wall phenomenon (seeing Fig S6) and minimize the impact of shear during the reaction, optimizing the rotational speed during incubation was deemed essential. Testing rotational speed ranging from 0 rpm to 200 rpm indicated that there was not significant effect on the results, with 200 rpm slightly increasing the weight loss rate. Subsequently, based reaction factors were optimized, including temperature (ranging from 50□ to 80□), pH (varied from 7 to 10), enzyme addition (ranging from 1 U mg^{-1} to 40 U mg^{-1} substrate), and reaction duration (spanning from 1d to 7d). The results (Fig 5) denoted the optimal conditions as 70□, pH 9.5, enzyme addition 5 U mg^{-1} substrate, and 1d reaction period. However, it was observed that *Tf*Cut enzyme activity diminished markedly under the conditions of pH 9.5 or 70°C, with less than 10% enzyme activity retained after reaction. Consequently, the *W* of oxidized PE did not meet initial expectations under the combined conditions (Fig 6A). Based on the results of the significance analysis of the machine learning modelling (see below), the decision was made to lower the temperature instead of adjusting the pH to ensure optimal weight loss rates. Notably, degradation efficiency significantly improved using the same substrate at 60°C compared with 70°C (Fig 6B). Therefore, the optimal degradation parameters encompassed 60□, pH 9.5, enzyme addition 5 U mg^{-1} substrate, and 1d reaction. The value of *W* was 80.51% under the optimal degradation conditions.

Machine learning to build models and obtain optimal solutions

Machine learning was utilized to analyze the aforementioned variables and outcomes through forest simulation[28]. The dataset incorporated details such as the type and concentration of surfactant EBI, pH, reaction day, temperature, enzyme addition, rotational speed, and *W*. Certain parameters were logarithmically transformed for

analysis, including temperature, enzyme addition and rotational speed. Before the modelling stage, the parameters ntree (number of trees to grow) and mtry (number of variables randomly sampled as candidates at each split) were optimized within ranges of 200-5000 and 1-8, respectively, through ten sets of repetitions. Following this screening process (Fig S7), ntree=4600 and mtry=7 was selected as the optimal values. The model revealed that a correlation coefficient (R^2) of 0.930 for the train set, and the test set was 0.916 (Fig 7 A-B). Furthermore, importance analyses and optimal solution calculations (genetic algorithms) were performed. The importance analyses (Fig. 7C-D, Fig S8) indicted that the surfactant type, EBI values, and pH exerted the most significant influence on weight loss rate. Notably, temperature is less important than pH according to importance analyses. This also rationalizes the choice of optimal conditions in the above process to lower the temperature rather than the pH. The results of optimal solution calculations aligning with experimental results further validated this approach (Fig. 7E). Consequently, the identified optimal conditions were retained for subsequent high-concentration substrate degradation experiments. Additionally, according to PDP results, EBI and pH have a positive effect on degrading oxidized PE (Figure S9). The shaply analysis revealed that all variables, except for ester bond content, had been optimized to enhance degradation efficiency (as shown in Figure S10). Notably, the uncontrollable nature of ester bond content highlights the need for further exploration and understanding in future investigations.

Characteristic of degradation products of pure polyethylene at optimum conditions

After determining the optimal reaction conditions, a higher substrate concentration (increased from 5g/L to 44g/L oxidized PE_{pure} ID=10) was employed. The value of *W* was 60.33%. Various analytical techniques including FT-IR, HT-GPC, GC-MS (headspace extraction), and DSC were utilized to analyze the composition of the dried product solids. In Fig. 8A, the products displayed a significant presence of hydroxyl groups (~3000cm^{-1}), and the ester bond peak was notably lower than oxidized PE_{pure} ID=10(Fig. S3H). Additionally, the weight average molecular weight (Mw) of the products (Fig. 8B) was found to be reduced to 2522 Da compared to 261564 Da for

PE_{pure}. The melting temperature (T_m) of the products were observed at 51.9 □ and 81.01 □ (Fig. 8C), indicating the presence of both cleaved and uncleaved oxidized PE_{pure}, significantly below the T_m of PE. For products type, GC-MS results (Fig. 8D) showed that a higher concentration of dibasic fatty alcohols in both the nylon mesh intercepts and the enzyme digests dried products. In summary, the results indicated that oxidized PE_{pure} underwent degraded into smaller molecules, with *Tf*Cut demonstrating the ability to degrade high concentrations of oxidized PE_{pure}.

Application of other cutinases or lipases in degradation systems

In order to determine the generality of the degradation system, four enzymes were used to degrade oxidized PE_{pure}, include *Tf*Cut, *Is*PETase, TLL, and HiC. The rationale behind selecting these enzymes was elucidated in the initial segment of the results (Fig 1). Each enzyme operated at its optimum temperature during the reactions. *Tf*Cut and *Is*PETase functioned at 60 □, while TIL and HiC operated at 50 and 60 □. When degrading oxidized PE_{pure} ID=7, both *Tf*Cut and *Is*PETase exhibited comparable degradation capabilities. Conversely, TLL and HiC demonstrated inferior performance to *Tf*Cut at both 50 and 60 □, attributed to the larger active site of *Tf*Cut and *Is*PETase and the smaller one of the TLL and HiC. Nevertheless, all four enzymes displayed proficiency in degrading oxidized PE_{pure}, underscoring the effectiveness of the degradation system.

Conclusion

Building upon previous research, the polyethylene degradation system, which integrates BV reaction and cutinase hydrolysis reaction, was refined through optimization. By conducting experimental validation and random forest modelling, this study identified the optimal degradation conditions parameters: 60 °C, pH 9.5, enzyme addition 5 U mg^{-1} substrate, and 1d reaction duration. Under these conditions, the treatment with cutinase resulted in a remarkable weight loss of up to 80.51% of oxidized polyethylene. Moreover, the functionality of the degradation system was further refined. For example, validation of its ability to treat up to 44 g/L of polyethylene oxide with a weight loss of 60.33%, and determination of its versatility

in the face of different types of keratinases or lipases. In conclusion, this research showcases an effective, efficient and mild degradation process of polyethylene into long-chain fatty acids or fatty alcohols, with promising implications for polyolefin biodegradation.

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Fig

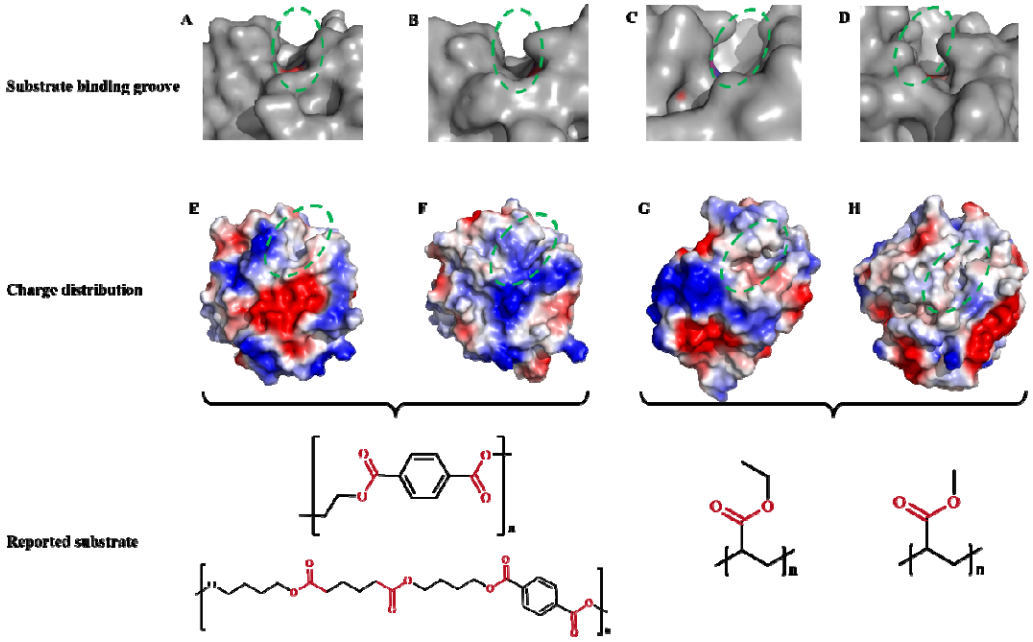

Figure 1. The structure analysis of cutinase or lipase, include *Tf*Cut, *Is*PETase, TLL and HiC. Among of them, A and E was substrate binding groove and charge distribution of *Tf*Cut, B and F was *Is*PETase, C and G was TLL, D and H was HiC. The green circle was enzyme active center. For charge distribution, blue surface was positive charge, and red surface was negative charge. For substrate, red marked were ester bonds that can be degraded by the above enzyme.

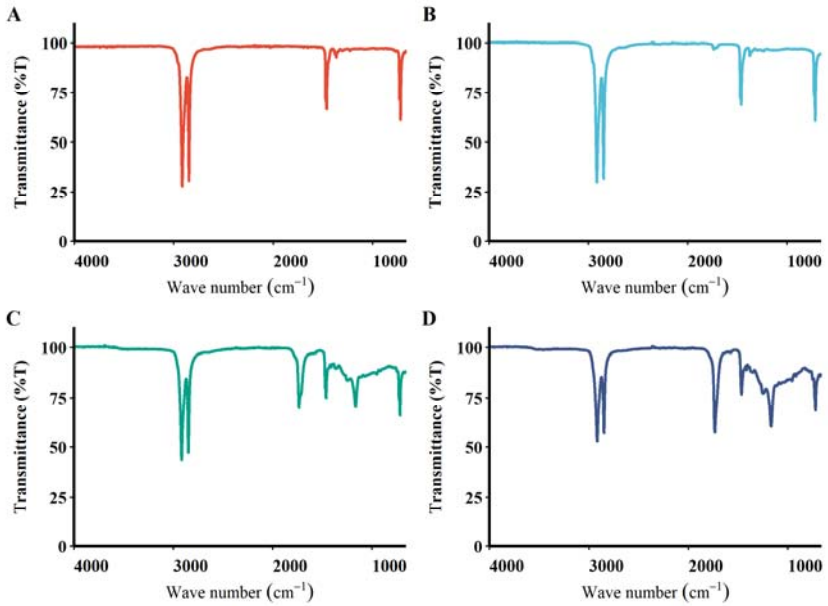

Figure 2. The FT-IR result of PE_{film} and PE_{pure}. Among them, figure A was PE_{film}, figure B was PE_{pure}, figure C was oxidized PE_{film}, and figure D was oxidized PE_{pure}.

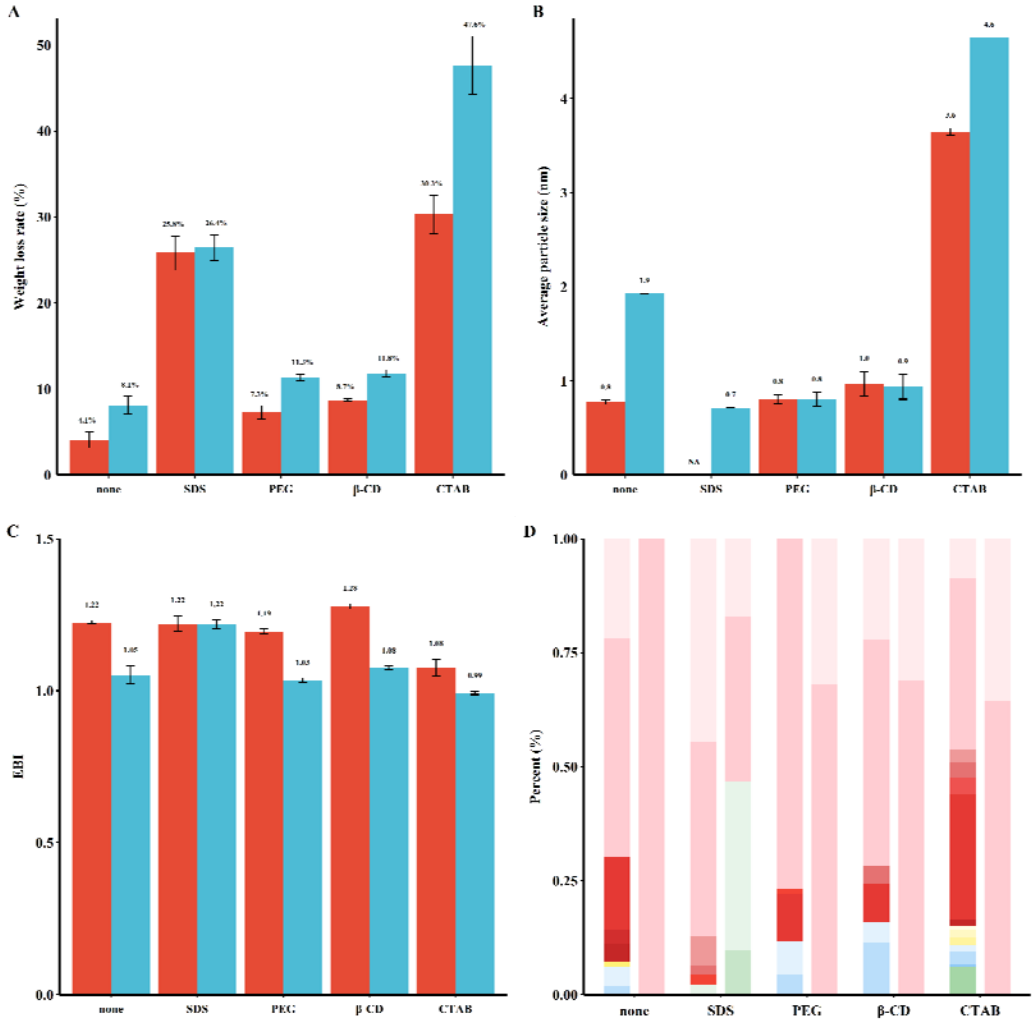

Figure 3. The result of degradation system using oxidized PE_{pure} (ID=3, figure S3 and table S1) as substrate. (A) comparison of weight loss rate of polythene, (B) changing of average particle size, (C) changing of EBI, and (D) comparison of products in the enzyme solution. In figure A-C, red column was the control group and blue column was the experimental group. In figure D, red module was monoacid, yellow module was binary acid or hydroxy fatty acid, blue module was diol, and green module was monohydric alcohols. Detail data in Table S2.

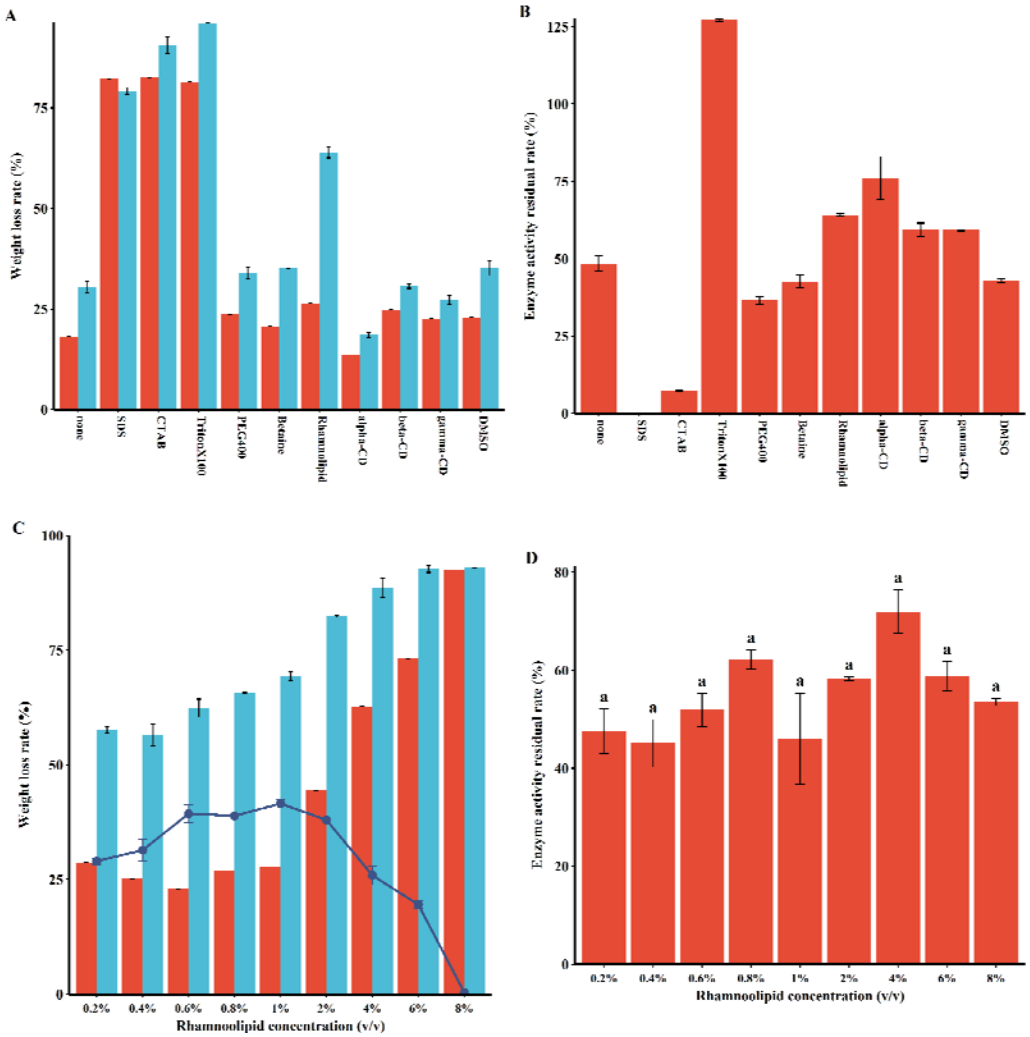

Figure 4. Additive optimization results for degradation systems using oxidized PE_{pure} (ID=4, figure S3 and table S1) as substrate. Different additives were screened using weight loss rate (A) and enzyme activity rate (B), and the concentration of optimal additive (rhamnolipid) was determined using weight loss rate (C) and enzyme activity rate (D). In figure A and C, red column was the control group, blue column was the experimental group and the dark blue line was difference in weight loss rate between control group and experimental group. In figure D, letters represent analysis of significance using one-way analysis of variance (ANOVA) followed by SNK (Student-Newman-Keuls; $P \leq 0.05$).

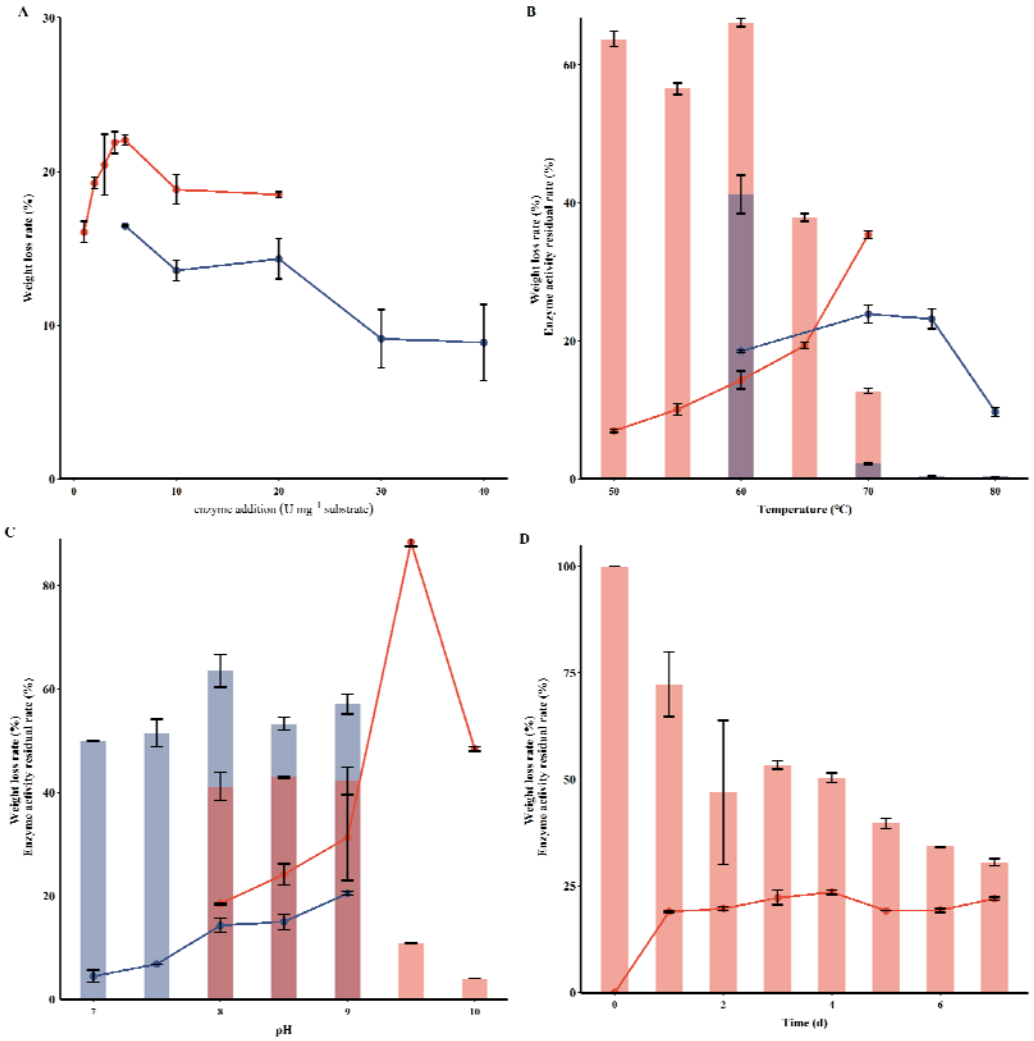

Figure 5. Optimization results for degradation systems using oxidized PE_{pure} (ID=5 (red) and 6 (blue), figure S3 and table S1) as substrate about enzyme addition, temperature, pH, and degradation time. Among them, line represents weight loss rate and column represents residual enzyme activity.

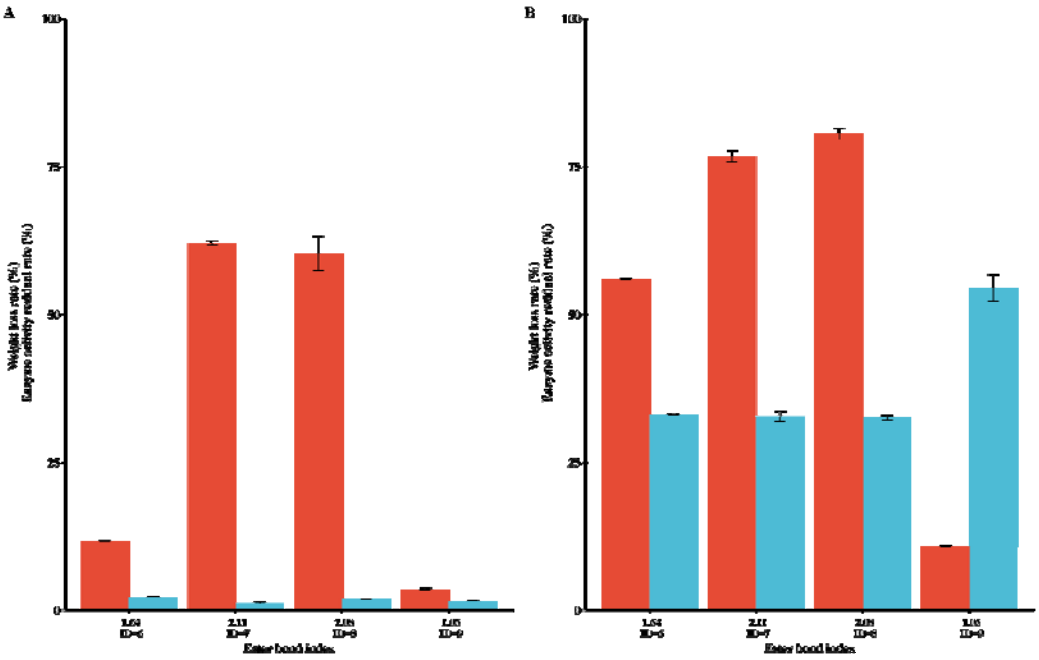

Figure 6. The oxidized PE_{pure} degradation results using optimization conditions. The reaction temperature was (A) 70 □ or (B) 60 □. Among them, red column was weight loss rate and blue column was enzyme activity residual rate. The number in the horizonal coordinate represents EBI value.

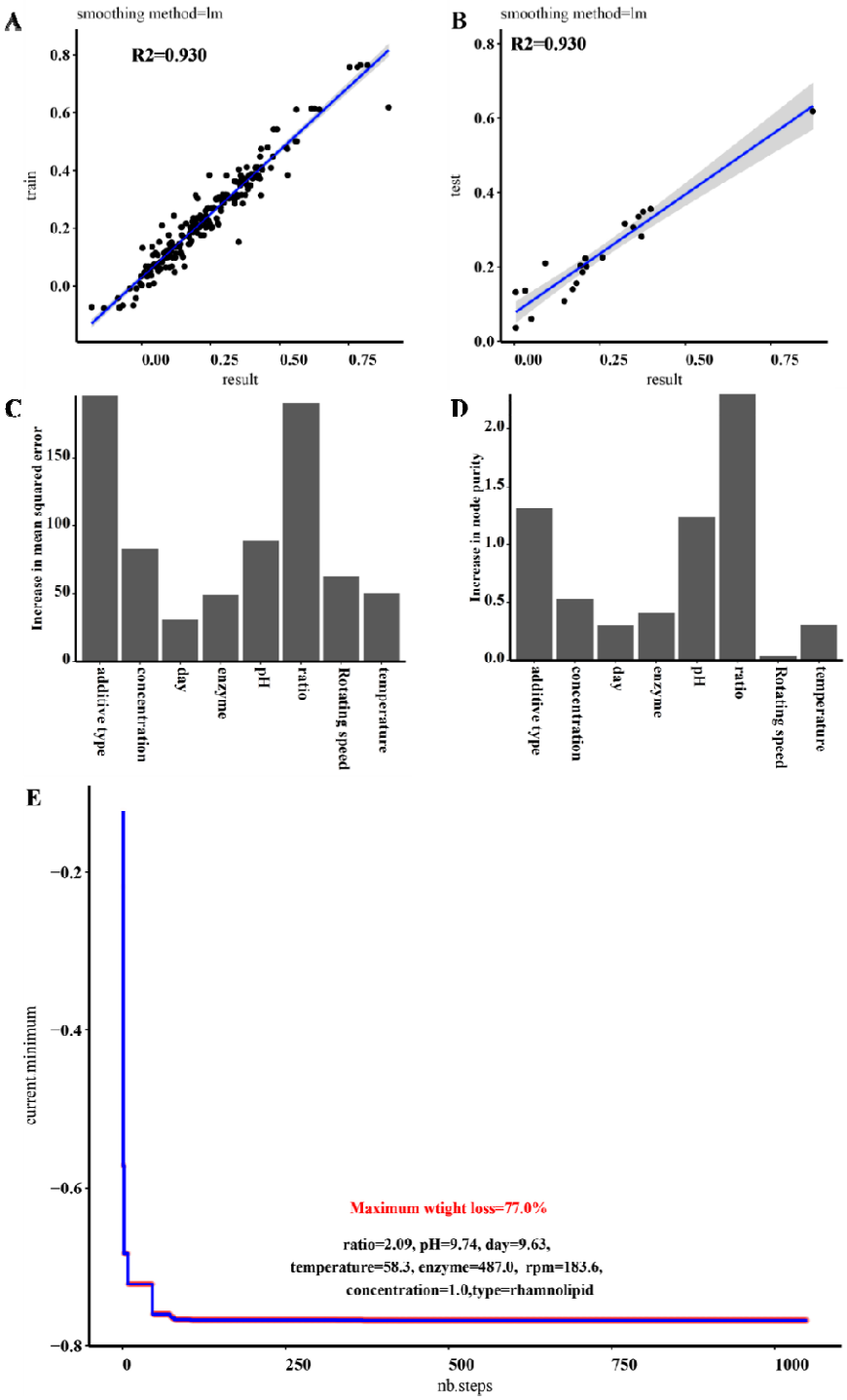

Figure 7. Random Forest fit to oxidize PE_{pure} degradation data. The fitting results for the training (A) and test (B) data are shown using R^2. Importance analyses for different factors are shown in C and D. In figure E, optimal degradation conditions were calculated using genetic algorithms.

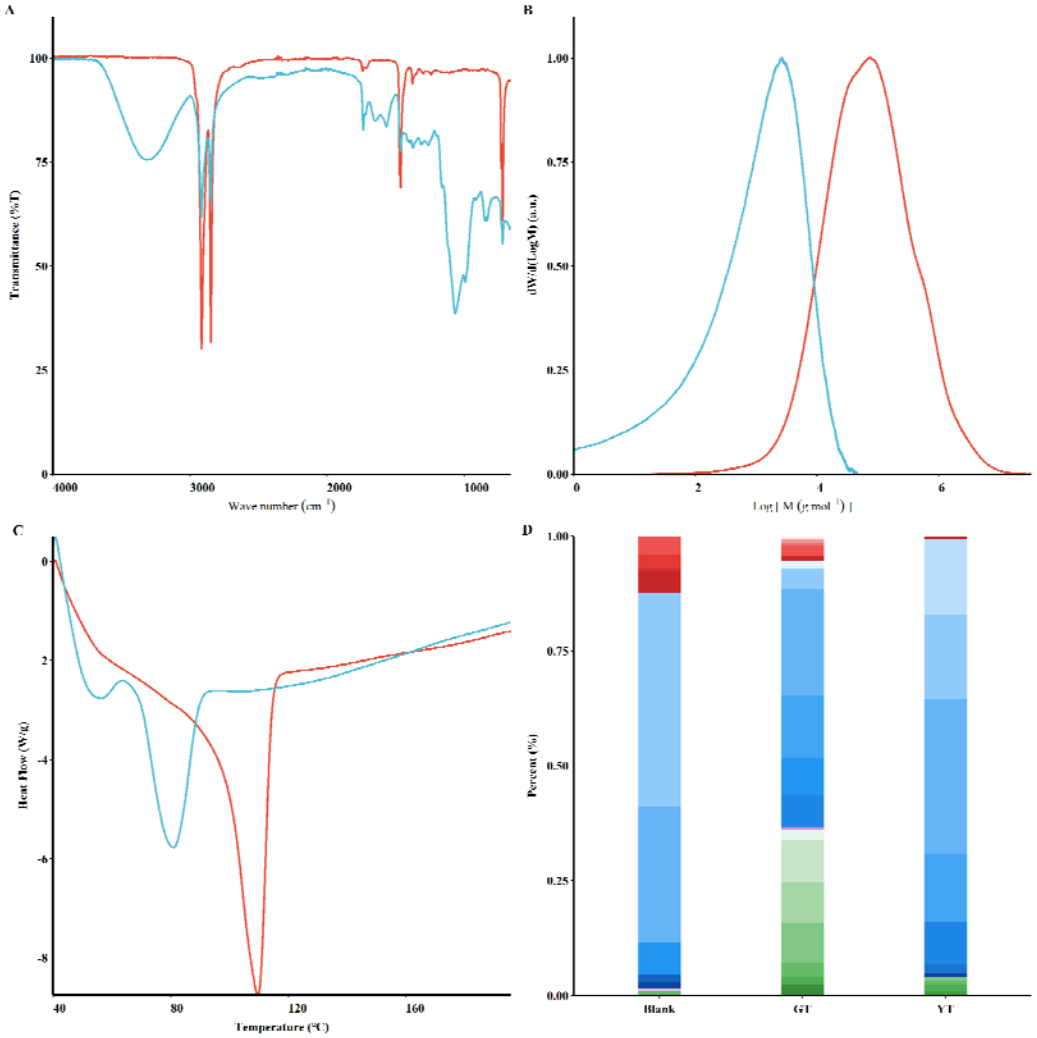

Figure 8. Characteristic of degradation products of oxidized PE_{pure} (ID=10, figure S3 and table S1). The result includes FT-IR (A), GPC (B), DSC (C), and GC-MS (D). In figure A-C, red line and blue line represents PE_{pure} and degradation products, respectively. In figure D, Blank was a control group liquid, GT was experimental group solid, and YT was experimental group liquid. red module was monoacid, yellow module was binary acid or hydroxy fatty acid, blue module was diol, and green module was monohydric alcohols.

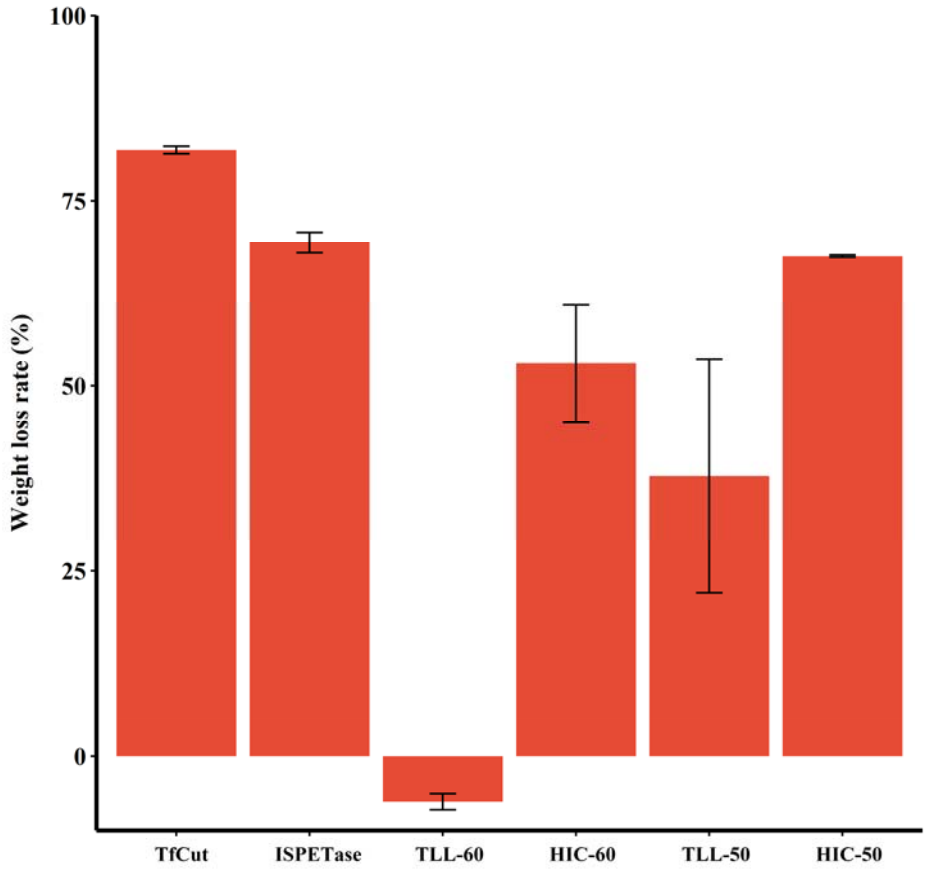

Figure 9. Results of degradation of oxidized PE_{pure} (ID=7, figure S3 and table S1) using different cutinase or lipases. The number in the horizontal coordinate represents the reaction temperature.