Caspian J. Environ. Sci.
DOI:
©Copyright by University of Guilan, Printed in I.R. Iran
Received:
Article type: Original

Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Alginate encapsulation enhances PET mass loss and PETase stability in soil microcosms

Murad Irshied Al-Maaitah[1], Farkhod Hashimov[2], Eldor Jumayev[3], Barno Abdraimova[4], Maha Mohammed Tawfiq[5], Anju Rani[6], Akilan Tamilselvan[7*], Roushan Kumar Singh[8]

[1]*Faculty of Agricultural Technology, Department of Agricultural Biotechnology and Genetic Engineering, Hourani Center for Applied Scientific Research, Al-Ahliyya Amman University, Amman, Jordan*
[2]*Department of Dermatovenereology and Cosmetology No 1, Tashkent State Medical University, Tashkent, Uzbekistan*
[3]*Department of Oral Surgery and Dental Implantology, Samarkand State Medical University, Samarkand, Uzbekistan*
[4]*Department of Biology, National Pedagogical University of Uzbekistan, Tashkent, Uzbekistan*
[5]*Department of Optics Techniques, health and medical techniques college, Alnoor University, Mosul, Iraq*
[6]*Department of Nutrition and Dietetics, Chandigarh University, Mohali, Punjab, India*
[7]*Amity Centre for Interdisciplinary Research, Amity University, Noida, Uttar Pradesh-201313, India*
[8]*Centre for Research Impact and Outcome, Chitkara University, Rajpura, Punjab, India*

**Corresponding author's Email: akilant@af.amity.edu*

ABSTRACT

Enzymatic degradation of poly(ethylene terephthalate) (PET) is a promising solution to the plastic waste problem. When applied in soil environments, however, enzyme activity may be reduced due to adsorption, proteolysis and interactions with microorganisms. This study sought to determine if encapsulation of PETase in calcium alginate beads will increase PET degradation, with minimal negative effects on soil health. In this study, PETase from *Ideonella sakaiensis* was encapsulated via ionic gelation. Non-sterile loam soil containing 500 mg PET powder per 100 g soil was divided into three treatments (control, free PETase, encapsulated PETase) with 20 independent microcosms per treatment (N = 60). Microcosms were prepared in four batches and incubated at 30 °C for 30 days. We measured PET weight loss, enzyme activity, PET molecular weight, crystallinity, carbonyl index, TPA and MHET concentrations, as well as indicators of soil health and ecotoxicity. Beads formed had a mean diameter of 849 ± 69 µm. The encapsulation efficiency was 81.7 ± 3.7%. The mass loss in degraded PET was greatest in encapsulated beads (30.1 ± 1.0%) when compared to free enzyme (22.9 ± 0.7%) and control (8.4 ± 0.8%). There was a significant difference in mass loss between treatments [$F(2,54) = 3425$, $p < .001$, $\eta p^2 = 0.992$]. The activity of the encapsulated enzyme in soil samples after 30 days (85.0 ± 2.2 U g^{-1}) was significantly higher compared with the free enzyme (46.1 ± 2.5 U g^{-1}) and control samples (30.5 ± 2.2 U g^{-1}). Molecular weight, crystallinity, carbonyl index, contact angle, concentrations of TPA/MHET, CO_2 evolution, microbial biomass, dehydrogenase activity, culturable microbes, seed germination, and earthworm survival in soil were not significantly different between treatments. In conclusions, encapsulation of PETase in alginate beads increased PET weight loss and enzyme recovery in soil under the conditions investigated. In addition, no negative effects on indicators of soil health were observed after 30 days. However, as there was no supporting molecular data, and only one time point

Caspian J. Environ. Sci.
DOI:
©Copyright by University of Guilan, Printed in I.R. Iran
Received:
Article type: Original

was sampled, it is unclear what mechanism PET degradation occurred via, and what the fate of the degradation products was.

Keywords: Polyethylene terephthalate, PETase, Enzyme immobilization, Alginate, Environmental biodegradation, Microplastics.

1. INTRODUCTION

Plastics are prevalent within modern life, however due to their durability, they cause significant issues in the environment. By 2015, 8.3 billion tons of new plastics had been produced, with approximately 79% accumulating in landfill or within natural environments (Geyer *et al.* 2017). Poly(ethylene terephthalate; PET) is heavily used in packaging for drinks, food and synthetic fibers and contributes significantly to the plastic problem. Although plastic pollution is frequently associated with marine environments, plastics have also contaminated terrestrial environments, including agricultural soils. Microplastic particles can be introduced via mulch films, sewage sludge, compost and deposition. Micrometer- to millimeter-sized PET particles are concerning in soils due to their potential persistence. They can cause changes in soil properties such as bulk density, water retention and microbial activity and therefore could affect soil function. However, the full impact of plastics in soil is still unclear (de Souza Machado *et al*. 2018; Rillig & Lehmann 2020).

The discovery of *Ideonella sakaiensis* 201-F6 has provided a biological pathway of polyethylene terephthalate (PET) degradation (Yoshida *et al.* 2016). The enzyme polyethylene terephthalate hydrolase (PETase) cleaves PET into mono(2-hydroxyethyl) terephthalate (MHET), terephthalic acid (TPA) and ethylene glycol at ambient temperatures. The MHET can then be cleaved by another enzyme (MHETase) so that the monomers can be assimilated (Yoshida *et al.* 2016). Structural analysis of PETase has allowed clarification of the active site (Joo *et al.* 2018), and targeted mutations have shown that this enzyme can be improved (Austin *et al.* 2018). Through protein engineering, several improved PET hydrolases have been generated. One example is a cutinase variant from leaf compost which can degrade most PET load in hours (Tournier *et al.* 2020). Other examples include FAST-PETase, designed using machine learning, which can degrade non-treated post-consumer plastic (Lu *et al.* 2022), and very thermostable variants (HotPETase; Bell *et al.* 2022) and computationally stabilized variants (DuraPETase; Cui *et al.* 2021). Comparison studies and metagenomic screening of environmental samples have increased the diversity of enzyme scaffolds available (Erickson *et al.* 2022; Arnal *et al.* 2023). However, many of these studies have been conducted in controlled industrial environments, meaning optimal reaction conditions such as temperature, pH and pre-treatment of substrates can occur. However, many challenges remain in enzyme use in realistic environments; stability, scalability and performance in environmental conditions are all still major challenges (Wei *et al.* 2020; Liu *et al.* 2025; Sherigar *et al.* 2025).

Enzyme activity in soil media is problematic. Free enzymes can become adsorbed onto soil components (colloids/minerals/humus), undergo degradation by proteases, may change in pH and can interact with microbes present in the environment. Therefore, only a fraction of the total enzyme activity may be retained (Burns et al. 2013). Immobilization of enzymes can overcome some of these issues, improving stability, protection and reusability of enzymes (Sheldon & van Pelt 2013; Sheldon *et al.* 2021). Immobilization of PETase onto magnetic nanomaterials allows easy recovery of the enzyme with remaining activity after several reaction cycles (Schwaminger *et al.* 2021). However, many systems involve man-made nanomaterials which may not be suitable to be introduced into open environments. Encapsulation of enzymes may provide other benefits. By encapsulating the enzyme, it may be retained close to the substrate and slowly released. In heterogeneous soils, this may be beneficial compared to homogeneous mixtures. Calcium alginate may be an ideal encapsulation material. Under milder conditions anionic alginate polymers can form hydrogel matrices through ionic crosslinking with divalent cations. This allows for simple encapsulation of enzymes without harsh chemical processes. Furthermore, alginate is biodegradable, and is generally considered non-toxic (Lee & Mooney 2012). Alginate beads have been used to encapsulate microbial inoculants in soils to protect and allow slow release (Schoebitz *et al.* 2013), suggesting that encapsulation of enzymes to increase their longevity in plastics contaminated soils may be possible.

Evidence for this application is currently limited. To our knowledge there is no direct evidence showing how effective PETase encapsulated in alginate is at degrading plastics in non-sterile soil. It is also unknown how introduction of this system into soil affects soil function and non-target species. When considering bioremediation

Caspian J. Environ. Sci.
DOI:
©Copyright by University of Guilan, Printed in I.R. Iran
Received:
Article type: Original

strategies for plastics contaminated soil in agricultural environments, it is important that any treatments not only promote efficient pollutant removal, but do not have detrimental effects on soil health.

In this study, we investigated the effectiveness of PETase from *I. sakaiensis* encapsulated in calcium alginate beads. Three treatments were compared in a blocked design, with 20 independent microcosms per treatment. We hypothesized that encapsulation of PETase would lead to more mass loss in PET than free PETase, through improving enzyme longevity in soil, with no detrimental effects on soil function and non-target species within the experimental conditions.

2. MATERIALS AND METHODS

2.1 Experimental design

We evaluated the effect of alginate encapsulation on PETase activity in soil using a controlled microcosm experiment. Three treatments were studied: (i) Control, where no enzyme was added to PET-amended soil; (ii) Free enzyme, where free PETase was added to PET-amended soil; and (iii) Encapsulated enzyme, where PETase was encapsulated within calcium-alginate beads and added to PET-amended soil. Each treatment consisted of 20 independent microcosms (n = 60). Four consecutive batches (B_1–B_4) were prepared, containing five replicates per treatment. Batches were treated as blocking factors in statistical analysis. Microcosms were incubated for 30 days at 30 °C. At the end of the incubation period, microcosms were destructively sampled.

2.2 Enzyme, polymer, and plastic

Recombinant PETase from *Ideonella sakaiensis* was expressed in *E. coli* BL21(DE3) cells, purified via Ni-NTA affinity chromatography and produced in-house. The purity of the enzyme was >95% (determined by SDS-PAGE). Low-viscosity sodium alginate (4–12 cP for a 1% solution in water at 25 °C; product code A1112, Sigma-Aldrich) was used as the carrier, and $CaCl_2$ as the crosslinking agent. PET powder (product code ES30-PD-000131, Goodfellow; nominal particle size <300 µm) was used as the substrate. The measured particle size was 301 ± 16 µm, with an initial crystallinity of 28.5 ± 1.6% and initial weight-average molecular weight (Mw) of 44.8 ± 2.3 kDa. Particle size was determined by laser diffraction, crystallinity by differential scanning calorimetry (DSC), and Mw by gel permeation chromatography (GPC) in hexafluoroisopropanol (HFIP).

2.3 Preparation and characterization of enzyme-loaded beads

PETase was encapsulated by ionic crosslinking. A solution containing 10 mg PETase and 1.5–2.5% (w/v) alginate was dripped using a syringe pump with a 22-gauge blunt needle, from a height of 5 cm, into 0.5–1.5% (w/v) $CaCl_2$ solution in 50 mM Tris-HCl buffer (pH 7.0). Encapsulation was performed at 25 °C. Beads were cured for 30 min, then washed with the same buffer. The amount of enzyme remaining in the combined supernatant and wash solutions was measured using Quick Start Bradford Protein Assay (Bio-Rad) with bovine serum albumin as standard. Encapsulation efficiency (EE) and enzyme loading (EL) were calculated as $EE\ (\%) = (E_t - E^f)/E_t \times 100$ and $EL\ (mg\ g^{-1}) = (E_t - E^f)/m^e$, where E_t is the amount of enzyme added (10 mg), E^f is the amount measured in the supernatant, and m^e is the carrier mass (g). Initial enzyme activity (U g^{-1}) was quantified in 50 mM phosphate buffer (pH 7.0) at 25 °C using 4-nitrophenyl butyrate (pNPB) as substrate. Enzymatic activity is expressed in units (U). One U corresponds to the release of 1 µmol p-nitrophenol per minute. Bead diameter was measured by optical microscopy (n ≥ 100 randomly selected beads). Zeta potential was measured using Malvern Zetasizer Nano ZS. Swelling ratios were calculated using the equation: $(W_s - W^d)/W^d \times 100$, where W_s = swollen mass, and W^d = dry mass. Cumulative release profiles of the enzyme were obtained by incubating beads in 50 mM Tris-HCl (pH 7.0) for 24 h at 30 °C. Carrier mass loss was determined as a % mass loss following incubation in non-sterile loam soil at 30 °C for 30 days. Thermal half-life, stability during storage, and reusability (number of cycles to reach 50% activity retention) were determined using modified methods from Anjani *et al.* (2007).

2.4 Soil microcosms

Soil samples (0–20 cm depth) were obtained from a field site at a research farm in Coimbatore Region, India. Soil was sieved using a 2-mm mesh sieve and used as-is. The characteristics of the sample were pH 6.81 ± 0.11, 3.14 ± 0.23% total organic matter, 60.2 ± 2.7% WHC, 0.81 ± 0.07 dS m^{-1} EC, and 15.4 ± 0.6 cmol kg^{-1} Cation Exchange Capacity (CEC). Soil pH was determined by measuring pH in a 1:2.5 ratio of soil to water. Total organic matter was quantified using the Walkley-Black method. Electrical conductivity was measured from a 1:5 ratio of soil to

Received:
Article type: Original
©Copyright by University of Guilan, Printed in I.R. Iran

water. Cation Exchange Capacity was determined via the ammonium acetate method. 100 g of soil and 500 mg of PET powder were thoroughly mixed in a 250 mL amber glass vessel. Free enzyme microcosms contained ≈0.41 mg of PETase enzyme diluted in 5 mL of 50 mM Tris-HCl (pH 7.0). Encapsulated enzyme microcosms contained the same mass of enzyme in alginate beads, divided among microcosms; excess enzyme was used during bead preparation to compensate for encapsulation loss. Control microcosms contained PET-amended soil and buffer only, without enzyme (5 mL of the same buffer). To maintain soil moisture at approx. 60% WHC, deionized water was added gravimetrically every 3 days. Microcosms were incubated in darkness at 30 °C for 30 days.

2.5 Analytical determinations

PET biodegradation was quantified by recovering PET from soil samples. Samples were wet sieved (mesh size 100 µm) before being density separated in saturated zinc chloride (≈1.9 g cm^{-3}). Recovered PET was washed with de-ionized water, dried at 40 °C to a constant mass and mass determined. Recovery efficiency, determined using spiked soil (0.5% PET, n = 3), was 96.8 ± 1.4%. Percentage mass loss was calculated according to $[(m_0 - m_1)/m_0]100$, where m_0 is the initial mass (500 mg) and m_1 is the final mass. Average PET degradation rates (mg d^{-1}) were calculated by dividing the mass loss by 30. As only one measurement of PET was taken at the end of the experiment, this value represents an average degradation rate over 30 days, and is not a kinetic parameter.

PET material properties were characterized by determining % crystallinity using DSC, Mw using GPC in HFIP with poly(methyl methacrylate) standards. Carbonylation was calculated by dividing the intensity of the carbonyl peak (~1715 cm^{-1}) by the intensity of the reference peak (~1410 cm^{-1}) in ATR-FTIR spectra. Hydrophilicity was determined by measuring the contact angle of water via the drop method. TPA and MHET concentrations in the soils were extracted by 0.1 M NaOH and quantified by RP-HPLC, with detection at 240 nm. Enzyme activity in soils was determined at day 30 using the pNPB method. Enzymatic activity (in relation to dry soil mass) was calculated and presented as a percentage remaining activity, using the formula; (remaining activity/initial activity)100. The pH of soils was recorded in a 1:2.5 (w/v) soil/water ratio.

Soil quality and toxicity were assessed by measuring CO_2 production, microbial biomass C and dehydrogenase activity. The latter was determined by monitoring triphenylformazan (TPF) production from 2,3,5-triphenyltetrazolium chloride (TTC; Casida *et al.* 1964). Microbial cell numbers were measured by plating onto R2A and expressed as log (CFU g^{-1} soil). Phytotoxicity was measured by calculating a germination index (GI%). using the following formula: [(Germination of treatment × Root length of treatment) / (Germination of control × Root length of control)] × 100. Earthworm survival (*Eisenia fetida*) was assessed by placing five individuals per vessel for 14 days.

2.6 Analysis of data

All statistical analysis was conducted in Python (v3) using SciPy (v1.18) and statsmodels (v0.15). Data are displayed as mean ± SD (n = 20 for each treatment unless otherwise stated). A Two-Way ANOVA model was used for all endpoints with type II sum of squares. The treatment and batch factor were considered as fixed factors. Treatment × batch interactions were investigated for primary endpoints. Partial η^2 values were calculated to assess effect size. Post-hoc pairwise comparisons were made using Tukey's HSD. The difference in means with 95% confidence intervals (CI) are displayed as Hedges' g. Assumptions of normality of residuals and homogeneity of variance were checked using the Shapiro-Wilk test and Brown-Forsythe/Levene test respectively. When homogeneity of variance was violated (relative residual activity), a Welch's ANOVA was performed. To account for multiple hypothesis testing, Holm's correction was performed on the overall *p*-values for treatment for all endpoints (n = 43) and derived change scores. Paired *t*-tests were used to test changes in PET Mw, crystallinity and soil pH from baseline to day 30. These were confirmed with Wilcoxon signed-rank tests. Effect sizes are displayed as dz. All tests were two-sided. Significance level was set to $\alpha = 0.05$.

3. RESULTS

3.1 Characteristics of the PETase–alginate beads

Beads had a mean size of 849 ± 69 µm (range: 750–950 µm) and a zeta potential of −28.6 ± 2.4 mV. A total of 1.83 ± 0.37 mg of enzyme was found in supernatant solution meaning that encapsulation efficiency was 81.7 ± 3.7% (range: 74.0–91.4%) and enzyme loading was 20.7 ± 2.7 mg g^{-1} carrier. Initial enzyme activity was 42.5 ± 2.5 U g^{-1}, swelling was 308 ± 25%, with 18.7 ± 2.5% enzyme released cumulatively. A total of 11.6 ± 1.5% of

Caspian J. Environ. Sci.
DOI:
Received:
Article type: Original
©Copyright by University of Guilan, Printed in I.R. Iran

carrier mass was lost. High stability was observed, with 84.9 ± 1.7% of activity remaining. The formulation had a thermal half-life of 96.6 ± 4.2 h. The formulation could be reused for 5.1 ± 0.8 cycles. As all treatment groups were made using the same formulation method, there were no significant differences among treatment groups for any parameter ($p \geq 0.13$). Therefore, pooled values are presented.

3.2 Baseline comparability of the treatments

Baseline soil and PET properties were similar among all three treatments (all $p \geq .068$). However, slight numerical differences in PET particle size (308 µm encapsulated, 296 µm control) and initial Mw (45.6 kDa encapsulated, 44.1 kDa control) were noted. These differences were not significant ($p = .068$ and .083, respectively). Even when adjusted for these factors, along with the other baseline properties, the conclusions of this study were not changed (Section 3.7).

3.3 Enzymatic depolymerization of PET in soil

As shown in Table 1, PET mass loss varied significantly among treatments [$F(2,54) = 3425$, $p < .001$, $\eta p^2 = 0.992$]. Mass loss in the control samples may be due to abiotic degradation and by naturally occurring microorganisms present within soil (non-sterile) and was 8.4 ± 0.8%. Addition of free enzyme led to a much greater mass loss than in the controls (22.9 ± 0.7%; an increase of 14.5 percentage points [95% CI: 13.8–15.1]). The greatest mass loss was observed with encapsulated enzyme (30.1 ± 1.0%), an increase of 21.7 percentage points from controls [95% CI: 21.0–22.3]. Encapsulation led to an additional increase in mass loss when compared to the free enzyme (7.2 percentage points [95% CI: 6.6–7.8]; Tukey $p < .001$). This equates to 1.32 times greater mass loss over free enzyme. However, if the background mass loss in controls is taken to be additive, then mass loss in encapsulated enzyme samples was 1.50 times greater than with free enzymes. All treatments differed significantly (Table 1; Fig. 1).

Average degradation rates were 1.40 ± 0.14 mg/day in controls, and 3.81 ± 0.11 mg/day with addition of free enzyme (2.7 times greater than control) and 5.01 ± 0.16 mg/day with addition of encapsulated enzyme (3.6 times greater than control). PET remaining was therefore 457.9 ± 4.1 mg, 385.7 ± 3.3 mg and 349.6 ± 4.9 mg from an original 500 mg for controls, free enzyme and encapsulated enzyme respectively. Since mass loss, rate of degradation and remaining mass can all be transformed into each other (linear relationship), they result in identical test statistics. The pattern in treatment effect was consistent in all four batches (Fig. 1C). Batch effect ($p = .593$) and treatment × batch interaction were not significant ($p = .780$). Variation within each group was small; coefficients of variation were 3.3% and 2.9% in encapsulated and free enzyme groups respectively.

Table 1. Primary endpoints after 30 d of incubation (mean ± SD, n = 20). Different letters within a row indicate significant differences (Tukey HSD, α = .05). Test statistics are from Two-Way ANOVA (treatment + batch); relative residual activity (unequal variances, Levene $p = .002$) was tested with Welch's ANOVA. ηp^2, partial eta squared. Holm-adjusted $p < .001$ for all endpoints.

Endpoint	Control	Free PETase	Encapsulated PETase	Test statistic	*p*	ηp^2
PET weight loss (%)	8.41 ± 0.83[c]	22.86 ± 0.67[b]	30.08 ± 0.98[a]	F(2,54) = 3425	< .001	0.992
Degradation rate (mg d^{-1})	1.40 ± 0.14[c]	3.81 ± 0.11[b]	5.01 ± 0.16[a]	F(2,54) = 3425	< .001	0.992
Residual PET mass (mg)	457.9 ± 4.1[a]	385.7 ± 3.3[b]	349.6 ± 4.9[c]	F(2,54) = 3425	< .001	0.992
Residual enzyme activity (U g^{-1})	30.5 ± 2.2[c]	46.1 ± 2.5[b]	85.0 ± 2.2[a]	F(2,54) = 2973	< .001	0.991
Relative residual activity (%)	71.7 ± 5.8[c]	109.0 ± 10.1[b]	201.8 ± 15.8[a]	Welch F(2,33.2) = 619	< .001	0.963

3.4 Persistence of enzyme activity in soil

Residual enzyme activity at day 30 was significantly higher in the encapsulated PETase treatment (85.0 ± 2.2 U g^{-1}) relative to both free (46.1 ± 2.5 U g^{-1}) and control treatments (30.5 ± 2.2 U g^{-1}) ($F(2,54) = 2973$, $p < 0.001$, $\eta p^2 = 0.991$; Table 1). Residual enzyme activity in the encapsulated PETase treatment was 1.85 times greater than in free (39.0 U g^{-1} difference; 95% CI 37.2–40.7) and 3.5 times greater than after subtraction of the control. Relative to initial enzyme activity, residual enzyme activity at day 30 was 202 ± 16%, 109 ± 10%, and 72 ± 6%

Caspian J. Environ. Sci.
DOI:
©Copyright by University of Guilan, Printed in I.R. Iran
Received:
Article type: Original

in encapsulated, free and control treatments, respectively (Welch $F(2,33.2) = 619$, $p < 0.001$; Table 1). The activity detected in the control and values above 100% indicate that there is a background contribution in this soil assay, and/or that the initial and the day-30 measures are not directly comparable. This issue is considered further in the Discussion.

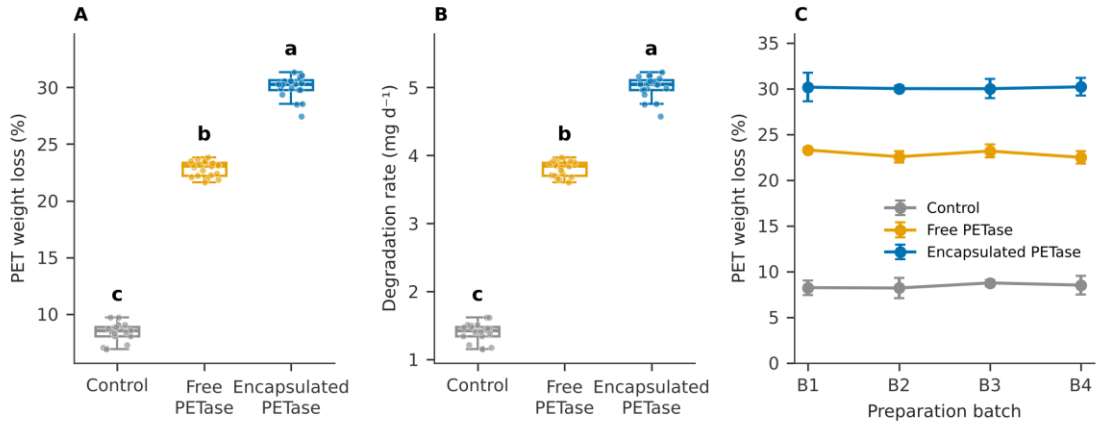

Fig. 1. PET degradation after 30 d. (A) Weight loss and (B) mean degradation rate (box: median and interquartile range; points: individual microcosms, n = 20; different letters: $p < 0.05$, Tukey HSD). (C) Weight loss by preparation batch (mean ± SD, n = 5 per point).

Across all 60 microcosms, residual enzyme activity was strongly correlated with PET mass loss ($r = 0.90$). However, this relationship seems to be driven by differences between treatments. Within each treatment, very low and non-significant correlations were observed (control, $r = .09$; free, $r = -.13$; encapsulated, $r = -.38$; $p > .10$ in all cases).

3.5 Changes in PET properties and soil chemistry

During the 30-days incubation, PET Mw declined from 44.8 ± 2.3 to 38.0 ± 1.3 kDa, a decrease of 6.76 kDa (14.9%; paired $t(59) = -20.8$, $p < .001$, $dz = -2.68$). Over the same period, crystallinity increased from 28.5% to 31.3% (+2.87 percentage points; $t(59) = 11.7$, $p < .001$), while soil pH fell from 6.81 to 6.58 (−0.24; $p < .001$; Table 2). Importantly, changes of similar magnitude occurred in all three treatments, including the control. Neither the change scores (ΔMw, $p = .068$; Δcrystallinity, $p = .255$; ΔpH, $p = .540$) nor the day-30 values for Mw, crystallinity, carbonyl index (0.409–0.423; $p = .430$), or water contact angle (77.4–78.3°; $p = 0.433$) differed significantly among treatments (Table 2). Soil concentrations of TPA (12.5 ± 0.9 mg kg^{-1}) and MHET (3.2 ± 0.4 mg kg^{-1}) were also similar across treatments ($p = 0.450$ and 0.269, respectively).

3.6 Soil biological activity and ecotoxicity

There was no measurable effect of treatment on soil function and non-target organisms (Table 2). Carbon dioxide production was observed to be similar across the treatments (214–217 mg C kg^{-1}; $p = .578$). Microbial biomass C ($p = .830$), dehydrogenase activity ($p = 0.898$) and culturable microbial population (7.43 ± 0.11 log CFU g^{-1}; $p = .822$) were also similar. The seed germination index was 91.9 ± 2.4% ($p = .818$), and earthworm survival was 98.7 ± 2.2%. All treatments had surviving earthworms of ≥ 95% ($p = .208$). Finally, pH values were not significantly different among treatments ($p = .174$). Having 20 replicates per treatment, the statistical power of this test is 80% (two-sided $\alpha = 0.05$) and so differences between treatments in the order of 0.9 SD will be detected. Effects smaller than this cannot be ruled out.

3.7 Robustness of the analysis

Normality of residuals was violated (Shapiro-Wilk test, $p < .05$) in 12/43 response variables. Discrete or fixed variables were responsible for these violations, including those corresponding to alginate concentration, $CaCl_2$ concentration, bead size, particle size, number of reuse cycles and earthworm survival. For weight loss, the overall residuals failed the normality test ($p = .006$), however, within-treatment tests had p values of .092, .148 and .036 respectively. Heterogeneity in variance was observed in relative residual activity and CO_2 evolution ($p = .002$ and

Received:
Article type: Original

.039, Levene test respectively). Conclusions from Welch's ANOVA and Kruskal-Wallis test agreed with those from a Two-Way ANOVA for all variables.

Table 2. Secondary endpoints after 30 d by treatment (mean ± SD, n = 20; Δ, day 30 minus initial). F and p from two-way ANOVA (treatment + batch); none was significant after Holm correction.

Endpoint	Control	Free PETase	Encapsulated PETase	F(2,54)	p
PET Mw, day 30 (kDa)	38.1 ± 1.5	38.0 ± 1.4	37.9 ± 1.0	0.13	0.877
Δ PET Mw (kDa)	−5.95 ± 2.17	−6.60 ± 2.62	−7.72 ± 2.54	2.82	0.068
PET crystallinity, day 30 (%)	31.2 ± 0.9	31.6 ± 0.8	31.3 ± 0.9	1.29	0.283
Δ PET crystallinity (points)	2.93 ± 1.57	3.34 ± 2.26	2.35 ± 1.77	1.40	0.255
Carbonyl index (FTIR)	0.409 ± 0.047	0.409 ± 0.036	0.423 ± 0.029	0.86	0.430
Water contact angle (°)	77.4 ± 2.2	78.3 ± 2.6	78.3 ± 2.5	0.85	0.433
TPA (mg kg^{-1} soil)	12.58 ± 0.79	12.59 ± 1.08	12.28 ± 0.66	0.81	0.450
MHET (mg kg^{-1} soil)	3.07 ± 0.46	3.29 ± 0.36	3.24 ± 0.47	1.35	0.269
Soil pH, day 30	6.60 ± 0.08	6.58 ± 0.11	6.55 ± 0.10	1.81	0.174
Δ soil pH	−0.22 ± 0.14	−0.22 ± 0.16	−0.27 ± 0.14	0.62	0.540
CO_2 evolution (mg C kg^{-1})	213.6 ± 9.8	216.7 ± 6.2	214.5 ± 11.8	0.55	0.578
Microbial biomass C (mg kg^{-1})	311.2 ± 12.7	308.8 ± 13.2	309.3 ± 12.7	0.19	0.830
Dehydrogenase (μg TPF g^{-1} h^{-1})	14.05 ± 0.95	13.92 ± 0.79	14.00 ± 0.97	0.11	0.898
Viable count (log CFU g^{-1})	7.45 ± 0.10	7.43 ± 0.13	7.42 ± 0.12	0.20	0.822
Seed germination index (%)	91.8 ± 2.5	92.2 ± 1.8	91.7 ± 2.9	0.20	0.818
Earthworm survival (%)	98.0 ± 2.5	99.3 ± 1.8	98.8 ± 2.2	1.62	0.208

However, initial PET Mw had a nominal p value of .049 (Kruskal-Wallis), whereas in the Two-Way ANOVA p = .083. Initial PET Mw was a baseline variable so p values were not adjusted for multiple comparisons. Pairwise comparisons using Mann-Whitney U tests for all primary response variables had p values $\leq 1 \times 10^{-6}$. Adjustment for PET particle size, initial crystallinity and Mw, soil pH, organic matter, water content and batch resulted in significant treatment effects in weight loss [$F(2,48) = 2961$, $p <$ .001] and residual activity [$F(2,48) = 2270$, $p <$ .001]. Adjustment did not result in significant effects in any of the covariates. After correction for multiple comparisons (Holm-corrected p values at 43 response variables) only treatment effects in PET mass loss and activity were significant (weight loss, degradation rates, remaining PET mass, residual activity, relative residual activity).

4. DISCUSSION

4.1 Principal findings

The highest rate of PET loss was observed in reaction with calcium alginate immobilized enzyme (30.1 ± 1.0%) compared to soluble enzyme (22.9 ± 0.7%) and control (8.4 ± 0.8%). Encapsulated enzyme had significantly greater PET degradation than soluble enzyme (7.2 percentage points increase, 95% CI 6.6 to 7.8). This equates to 1.32-fold greater degradation of PET than with soluble enzyme, and 1.50-fold greater degradation of PET after subtraction of background degradation. Degradation rates were also higher in encapsulated enzyme (5.01 mg day^{-1}) than soluble enzyme (3.81 mg day^{-1}) and the control (1.40 mg day^{-1}). There was no evidence of an interaction between enzyme treatments and batches. The result remained significant in non-parametric tests, analysis with covariate adjustment, and after applying Holm corrections (43 endpoints). At day 30 enzyme activity recovered from soil was 1.85-fold higher in encapsulated enzyme samples than soluble enzyme samples (85.0 vs. 46.1 U g^{-1}) and 3.5-fold higher after background correction. None of the measured soil-health or ecotoxicity indicators differed significantly among treatments.

4.2 Possible reasons for the benefit of encapsulation

The increased residual activity in the encapsulated treatment may be due to protection within the alginate encapsulate. In free solution, enzymes can be adsorbed to mineral and organic surfaces, and could experience conformational change and altered activity (Quiquampoix & Burns 2007). In soil, extracellular enzymes will be subject to a greater variety of stabilizing and degrading effects (Gianfreda & Rao 2004; Nannipieri *et al.* 2012) than would occur in solution. By encapsulating the enzyme within the hydrogel, interactions with these surfaces may be minimized, as well as interactions with proteolytic enzymes. The characteristics demonstrated by this formulation are consistent with a stable, slow release system. Encapsulation efficiency was 81.7% and cumulative

Received:
Article type: Original

release was 18.7%. Mass loss of the encapsulate was 11.6%. The formulation exhibited a thermal half-life of approximately 97 h and could be reused for about five cycles. This is consistent with general advantages of enzyme immobilization (Sheldon & van Pelt 2013; Sheldon *et al.* 2021), and with the properties of ionically crosslinked alginate (Lee & Mooney 2012). The use of alginate beads for the delivery of microbial inoculants in soil systems has been shown to provide sustained release (Schoebitz *et al.* 2013). Immobilization of PETase on magnetic nanoparticles has also been shown to allow recovery of the enzyme with retained activity following multiple cycles (Schwaminger *et al.* 2021). However, it is unclear from these data whether this stability is due to protection of the enzyme or to slow release. Enzyme release and fate were not directly measured in soils. Additionally, bead size (approximately 850 μm) was larger than particle size (approximately 300 μm). It is unclear to what extent this would reduce substrate accessibility.

4.3 Interpreting background activity and relative residual activity

The results must be interpreted carefully. Although no PETase was added to the control samples, PET mass loss (8.4%) and activity (30.5 U g^{-1}) were observed. As the soil was not sterile, this is likely due to the natural microbial community in the soil, non-biological degradation processes and background hydrolase activity within the assay. This latter point is a known issue in measuring enzyme activity in soil environments (Nannipieri *et al.* 2012). Therefore, the effect of each treatment should be reported in relation to the control (net values). However, residual activity in free (109%) and encapsulated (202%) treatments was greater than 100% relative to initial activity. This may be a result of matrix effects, the normalization applied to initial (per g of carrier) and final (per g of soil) activity measurements, nonspecific esterase activity in soil degrading the pNPB substrate, or differences in experimental conditions. Absolute activity and activity relative to the control are therefore a more justifiable measure. Across treatments, a high correlation (r = 0.90) between residual activity and mass loss has been observed. However, within treatments, correlations were low and insignificant and thus the data does not show a dose–response at the microcosm scale.

4.4 Mass balance and unexplained mass loss

Despite a large effect of treatment on PET mass loss, no significant differences were seen in the molecular/chemical parameters. A decrease in PET Mw (approx. 15%), increase in crystallinity (approx. 2.9 percentage points) and decrease in soil pH (0.24 units) were observed. These changes were similar between all treatments including the control. Similarly, no differences in carbonyl index, water contact angle, TPA (approx. 12.5 mg kg^{-1}) or MHET (approx. 3.2 mg kg^{-1}) were observed between treatments. This is highlighted by considering the mass balance. An increase in PET mass loss of approx. 108 mg PET per 100 g soil in the encapsulated PETase sample compared to control equates to a total PET loss of approx. 1,080 mg kg^{-1} (or approx. 680 mg C kg^{-1}, assuming a 62.5% carbon content). However, the total amount of TPA and MHET found was approx. 16 mg kg^{-1}. Similarly, no differences in CO_2 production (approx. 214 mg C kg^{-1}) or microbial biomass carbon were found between treatments. There are a number of potential explanations for this, and it is not possible to determine which, if any, is most plausible using the current data. Firstly, given that soil microorganisms contain catabolic pathways for terephthalates (Sasoh *et al.* 2006), TPA and MHET may have been removed from the samples. However, this should have resulted in some increase in either carbon dioxide production or microbial biomass in the enzyme treatments. Secondly, because PET degrades from the surface of the particle, preferential degradation of amorphous regions would occur (Wei *et al.* 2019; Thomsen *et al.* 2022). Therefore, bulk changes in molecular weight and crystallinity may not be reflected in the particle as a whole. However, changes in surface properties (carbonyl index and contact angle) may have occurred. Finally, changes in PET quantity may have occurred due to differences in recovery efficiency. Weight loss is often used as a measurement of enzymatic PET degradation (Müller *et al.* 2005). However, the degree of weight loss may be affected by the recovery method. Loss of fine particles during sieving or density separation and adhesion of soil to PET may affect the results. The recovery efficiency of this method was 96.8 ± 1.4% (spiked soil, n = 3). Furthermore, as similar changes to Mw and crystallinity were seen in the control sample, it appears some of the changes seen may not be due to PETase. As reviewed in literature, mass loss alone is an insufficient measure of plastic biodegradation. It should be complemented by analysis of products and closed mass balances (Danso *et al.* 2019; Wei *et al.* 2020). Without such evidence, we can consider that the increased mass loss seen in encapsulated samples is a statistically robust finding; however, we cannot confirm the mechanism behind the increased mass loss.

Caspian J. Environ. Sci.
DOI:
©Copyright by University of Guilan, Printed in I.R. Iran
Received:
Article type: Original

4.5 Soil functioning and non-target organisms

No significant differences in soil respiration, microbial biomass C, dehydrogenase activity, culturable microbial abundance, germination, and earthworm survival were seen between the free and encapsulated enzyme treatments. A decrease in soil pH was observed to a similar extent in all three treatments, suggesting that this change is due to incubation rather than addition of enzyme. Despite a loss of ~12% of carrier material in these experiments, no significant increase in CO_2 production was observed. This suggests that the quantity of carbon added to the soil via the alginate matrix was too small for significant changes to be observed in community respiration in this experiment. While these results are promising, caution should still be taken when interpreting them. As there were 20 samples in each treatment, the study was powered to detect differences of ~0.9 SD, however smaller effects may not have been detected. Furthermore, only one soil type, dosage, and duration (30 days) were tested. Data on microbial community structure and other soil enzymes were not obtained. Long-term studies have found plastic types can impact enzyme activity and bacterial community structures in polymer-specific ways (Guo *et al.* 2024). It would therefore be beneficial to further investigate the impacts of PET breakdown products, enzyme and carrier on soils in longer-term experiments in different soil types.

4.6 Limitations

Other limitations must be noted. The rate of PET degradation was averaged over 30 days because PET was measured at only one time point, rather than reported as a kinetic constant. The PET powder was relatively small (~300 μm) and had moderate crystallinity (28.5%). In post-consumer PET, samples with higher crystallinity are more resistant to enzymatic degradation (Wei *et al.* 2019; Thomsen *et al.* 2022). Therefore, the effect sizes observed in this experiment may reflect a relatively favourable substrate. Since within-group variability in weight loss was low (CV ~3%), it is essential to confirm that the 20 microcosms in each treatment were independent experimental units. If they were technical rather than independent biological replicates, pseudo-replication could have inflated the apparent strength of the evidence (Hurlbert 1984). Furthermore, wild-type PETase was used at 30 °C, well below the temperatures at which engineered variants show their best performance (Bell *et al.* 2022; Lu *et al.* 2022).

4.7 Implications and outlook

This study suggests that alginate encapsulation offers a simple and degradable way to enhance the activity of plastic-degrading enzymes within non-sterile soils without causing apparent short-term impacts to soil functionality. In order to confirm the potential of alginate encapsulated enzyme in remediating plastic pollution further work is required. Long-term degradation should be investigated, closing mass balances with measurements of monomers and CO_2 (ideally with labelled PET). Enzyme release and stability should be measured directly. Stability in PET hydrolases should be improved through engineering (Cui *et al.* 2021; Bell *et al.* 2022; Lu *et al.* 2022; Arnal *et al.* 2023). The effect of increasing crystallinity or different pre-treatment of substrates should also be explored. Comparing alginate encapsulation to other delivery methods, longer-term studies in a range of soils, and field experiments including community analysis of microbes will also be important.

5. CONCLUSION

Encapsulation of PETase within calcium alginate beads resulted in greater PET mass loss (30.1%) compared to free PETase (22.9%) after 30 days of experimentation in soil microcosms. Encapsulation also resulted in higher enzyme activity recoveries from soil (85.0 U g^{-1}) compared to free enzyme (46.1 U g^{-1}). Differences between treatments were significant and consistent across different experimental batches. They were also consistent across different statistical sensitivity analyses. However, soil respiration, microbial biomass, dehydrogenase activity, culturable microorganisms, seed germination and earthworm survival were unaffected by treatment. Similarly, molecular weight, crystallinity, surface chemistry and monomer concentrations of PET material were unchanged in response to treatment. Data from monomers and CO_2 could not explain the observed mass loss; therefore, the mechanism behind the increased mass loss remains uncertain. Although alginate encapsulation may be useful in protecting plastic-degrading enzymes in soil environments, further investigation using time-resolved methods, mass balances and long-term evaluation is required to determine the value of this as a remediation method.

Caspian J. Environ. Sci.
DOI:
©Copyright by University of Guilan, Printed in I.R. Iran
Received:
Article type: Original

REFERENCES

Anjani, K, Kailasapathy, K & Phillips, M 2007, Microencapsulation of enzymes for potential application in acceleration of cheese ripening. *International Dairy Journal*, 17: 79–86.

Arnal, G, Anglade, J, Gavalda, S, Tournier, V, Chabot, N, Bornscheuer, UT, Weber, G & Marty, A 2023, Assessment of four engineered PET degrading enzymes considering large-scale industrial applications. *ACS Catalysis*, 13: 13156–13166. DOI: 10.1021/acscatal.3c02922.

Austin, HP, Allen, MD, Donohoe, BS, Rorrer, NA, Kearns, FL, Silveira, RL, Pollard, BC, Dominick, G, Duman, R, El Omari, K, Mykhaylyk, V, Wagner, A, Michener, WE, Amore, A, Skaf, MS, Crowley, MF, Mirts, AW, Stier, A, Beckham, GT & McGeehan, JE 2018, Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proceedings of the National Academy of Sciences*, 115: E4350–E4357. DOI: 10.1073/pnas.1718804115.

Bell, EL, Smithson, R, Kilbride, S, Foster, J, Hardy, FJ, Ramachandran, S, Tedstone, AA, Haigh, SJ, Garforth, AA, Day, PJR, Levy, C, Shaver, MP & Green, AP 2022, Directed evolution of an efficient and thermostable PET depolymerase. *Nature Catalysis*, 5:673–681. DOI: 10.1038/s41929-022-00821-3.

Burns, RG, DeForest, JL, Marxsen, J, Sinsabaugh, RL, Stromberger, ME, Wallenstein, MD, Weintraub, MN & Zoppini, A 2013, Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry*, 58: 216–234. DOI: 10.1016/j.soilbio.2012.11.009.

Casida, LEJr, Klein, DA & Santoro, T 1964, Soil dehydrogenase activity. *Soil Science*, 98:371–376.

Cui, Y, Chen, Y, Liu, X, Dong, S, Tian, Y, Qiao, Y, Mitra, R, Han, J, Li, C, Han, X, Liu, W, Chen, Q, Wei, W, Wang, X, Tian, W, Zhang, Y, Hou, Y, Bordes, R, Bornscheuer, UT, Wei, R, Schmid, RD & Bornscheuer, UT 2021, Computational redesign of a pETase for plastic biodegradation under ambient condition by the gRAPE strategy. *ACS Catalysis*, 11: 1340–1350. DOI: 10.1021/acscatal.0c05126.

Danso, D, Chow, J & Streit, WR 2019, Plastics: environmental and biotechnological perspectives on microbial degradation. *Applied and Environmental Microbiology*, 85:e01095-19. DOI: 10.1128/AEM.01095-19.

de Souza Machado, AA, Lau, CW, Till, J, Kloas, W, Lehmann, A, Becker, R & Rillig, MC 2018, Impacts of microplastics on the soil biophysical environment. *Environmental Science & Technology*, 52: 9656–9665. DOI: 10.1021/acs.est.8b02212.

Erickson, E, Gado, JE, Avilán, L, Bratti, F, Brizendine, RK, Cox, PA, Gill, R, Graham, R, Kim, DJ, König, G, Michener, WE, Poudel, S, Ramirez, KJ, Shakespeare, TJ, Zahn, M, Boyd, ES, Payne, CM, DuBois, JL, McGeehan, JE, Beckham, GT & Pickford, AR 2022, Sourcing thermotolerant poly(ethylene terephthalate) hydrolase scaffolds from natural diversity. *Nature Communications*, 13: 7850. DOI: 10.1038/s41467-022-35237-x.

Geyer, R, Jambeck, JR & Law, KL 2017, Production, use, and fate of all plastics ever made. *Science Advances*, 3:e1700782. DOI: 10.1126/sciadv.1700782.

Gianfreda, L & Rao, MA 2004, Potential of extra cellular enzymes in remediation of polluted soils: a review. *Enzyme and Microbial Technology*, 35: 339–354. DOI: 10.1016/j.enzmictec.2004.05.006.

Guo, W, Ye, Z, Zhao, Y, Lu, Q, Shen, B, Zhang, X, Zhang, W, Chen, SC & Li, Y 2024, Effects of different microplastic types on soil physicochemical properties, enzyme activities, and bacterial communities. *Ecotoxicology and Environmental Safety*, 286: 117219. DOI: 10.1016/j.ecoenv.2024.117219.

Hurlbert, SH 1984, Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54: 187–211. DOI: 10.2307/1942661.

Joo, S, Cho, IJ, Seo, H, Son, HF, Sagong, HY, Shin, TJ, Choi, SY, Lee, SY & Kim, KJ 2018, Structural insight into molecular mechanism of poly(ethylene terephthalate) degradation. *Nature Communications*, 9: 382. DOI: 10.1038/s41467-018-02881-1.

Lee, KY & Mooney, DJ 2012, Alginate: properties and biomedical applications. *Progress in Polymer Science*, 37: 106–126. DOI: 10.1016/j.progpolymsci.2011.06.003.

Liu, F, Wang, T, Liu, X, Xu, N & Pan, X 2025, Efficient biodegradation and upcycling of polyethylene terephthalate mediated by cell-factories. *Frontiers in Microbiology*, 16: 1599470. DOI: 10.3389/fmicb.2025.1599470.

Received:
Article type: Original
©Copyright by University of Guilan, Printed in I.R. Iran

Lu, H, Diaz, DJ, Czarnecki, NJ, Zhu, C, Kim, W, Shroff, R, Acosta, DJ, Alexander, BR, Cole, HO, Zhang, Y, Lynd, NA, Ellington, AD & Alper, HS 2022, Machine learning-aided engineering of hydrolases for PET depolymerization. *Nature*, 604: 662–667. DOI: 10.1038/s41586-022-04599-z.

Müller, RJ, Schrader, H, Profe, J, Dresler, K & Deckwer, WD 2005, Enzymatic degradation of poly(ethylene terephthalate): rapid hydrolyse using a hydrolase from t. fusca. *Macromolecular Rapid Communications*, 26: 1400–1405. DOI: 10.1002/marc.200500410.

Nannipieri, P, Giagnoni, L, Renella, G, Puglisi, E, Ceccanti, B, Masciandaro, G, Fornasier, F, Moscatelli, MC & Marinari, S 2012, Soil enzymology: classical and molecular approaches. *Biology and Fertility of Soils*, 48: 743–762. DOI: 10.1007/s00374-012-0723-0.

Quiquampoix, H & Burns, RG 2007, Interactions between proteins and soil mineral surfaces: environmental and health consequences. *Elements*, 3:401–406. DOI: 10.2113/GSELEMENTS.3.6.401.

Rillig, MC & Lehmann, A 2020, Microplastic in terrestrial ecosystems. *Science*, 368: 1430–1431. DOI: 10.1126/science.abb5979.

Sasoh, M, Masai, E, Ishibashi, S, Hara, H, Kamimura, N, Miyauchi, K & Fukuda, M 2006, Characterization of the terephthalate degradation genes of comamonas sp. strain e6. *Applied and Environmental Microbiology*, 72: 1825–1832. DOI: 10.1128/AEM.72.3.1825-1832.2006.

Schoebitz, M, López, MD & Roldán, A 2013, Bioencapsulation of microbial inoculants for better soil–plant fertilization. a review. *Agronomy for Sustainable Development*, 33:751–765. DOI: 10.1007/s13593-013-0142-0.

Schwaminger, SP, Fehn, S, Steegmüller, T, Rauwolf, S, Löwe, H, Pflüger-Grau, K & Berensmeier, S 2021, Immobilization of pETase enzymes on magnetic iron oxide nanoparticles for the decomposition of microplastic PET. *Nanoscale Advances*, DOI: 10.1039/d1na00243k.

Sheldon, RA & van Pelt, S 2013, Enzyme immobilisation in biocatalysis: why, what and how. *Chemical Society Reviews*, 42:6223–6235. DOI: 10.1039/C3CS60075K.

Sheldon, RA, Basso, A & Brady, D 2021, New frontiers in enzyme immobilisation: robust biocatalysts for a circular bio-based economy. *Chemical Society Reviews*, 50:5850–5862. DOI: 10.1039/D1CS00015B.

Sherigar, A, Sriramakrishnan, J, Ganla, RK, Lin, C, Raval, R & Selvaraj, S 2025, Biocatalytic innovations in pETase for sustainable polyethylene terephthalate plastic recycling. *Discover Applied Sciences*, 7:1126. DOI: 10.1007/s42452-025-07764-x.

Thomsen, TB, Hunt, CJ & Meyer, AS 2022, Influence of substrate crystallinity and glass transition temperature on enzymatic degradation of polyethylene terephthalate (PET). *New Biotechnology*, 69: 28–35. DOI: 10.1016/j.nbt.2022.02.006.

Tournier, V, Topham, CM, Gilles, A, David, B, Folgoas, C, Moya-Leclair, E, Kamionka, E, Desrousseaux, ML, Texier, H, Gavalda, S, Cot, M, Guémard, E, Dalibey, M, Nomme, J, Cioci, G, Barbe, S, Chateau, M, André, I, Duquesne, S & Marty, A 2020, An engineered PET depolymerase to break down and recycle plastic bottles. *Nature*, 580: 216–219. DOI: 10.1038/s41586-020-2149-4.

Wei, R, Breite, D, Song, C, Gräsing, D, Ploss, T, Hille, P, Schwerdtfeger, R, Matysik, J, Schulze, A & Zimmermann, W 2019, Biocatalytic degradation efficiency of postconsumer polyethylene terephthalate packaging determined by their polymer microstructures. *Advanced Science*, 6: 1900491. DOI: 10.1002/advs.201900491.

Wei, R, Tiso, T, Bertling, J, O'Connor, K, Blank, LM & Bornscheuer, UT 2020, Possibilities and limitations of biotechnological plastic degradation and recycling. *Nature Catalysis*, 3: 867–871. DOI: 10.1038/s41929-020-00521-w.

Yoshida, S, Hiraga, K, Takehana, T, Taniguchi, I, Yamaji, H, Maeda, Y, Toyohara, K, Miyamoto, K, Kimura, Y & Oda, K 2016, A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*, 351: 1196–1199. DOI: 10.1126/science.aad6359.