The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
E-ISSN: 2959-4820
Volume 2, Issue 4, October - December 2024
Page No: 282-291
Website: https://najsp.com/index.php/home/index
SJIFactor 2024: 5.49 معامل التأثير العربي (AIF) 2024: 0.71 ISI 2024: 0.696

Microbial Degradation of Microplastics and Its Environmental Implications

Miftah Alfeetouri Miftah *
Faculty Member, Faculty of Education, Bani Waleed University, Libya

التحلل الميكروبي للبلاستيك الدقيق وتداعياته البيئية

مفتاح الفيتوري مفتاح الجمل *
عضو هيئة تدريس، كلية التربية، جامعة بني وليد، ليبيا

*Corresponding author: mmuuum2@gmail.com

Received: October 09, 2024	Accepted: December 22, 2024	Published: December 26, 2024

Abstract

Plastic pollution in the form of microplastics (MPs) is a growing environmental threat. Unlike physical fragmentation, true biodegradation requires mineralization to CO_2 (and H_2O) or complete depolymerization to monomers. This study outlines a comprehensive approach to quantify and mechanistically dissect microbial MP degradation in composting and aquatic conditions. We will test a range of polymers (PET, LDPE/HDPE, PP, PS) with PLA as a positive control. Weathered and unweathered MPs will be characterized (FTIR, Raman, SEM, Py-GC/MS) and exposed to mixed microbial consortia or purified enzymes. In compost assays (ISO 14855/ASTM D5338), we expect rapid mineralization of PLA, partial mineralization of PET, and negligible PE/PP degradation unless pre-oxidized. In aquatic assays, slow CO_2 evolution and loss of particle mass will be monitored. Purified PETase±MHETase will be applied to PET films and particles to quantify TPA/MHET release. Biofilm assays on PE/PP/PET will examine colonization and surface erosion (SEM, confocal, AFM). Enriched consortia will be profiled (16S/ITS, shotgun metagenomics) to link taxa with hydrolase genes. Environmental risk will be assessed by leachate toxicity (algal, Daphnia, Vibrio tests) and by-product analysis (LC-HRMS of oligomers, GHG monitoring). Community and kinetic analyses (first-order decay, Michaelis-Menten, ANOVA) will yield rates and half-lives. Engineered PETase+MHETase systems are expected to outperform wild-type enzymes in PET depolymerization. Overall, we aim to clarify polymer-specific degradation pathways, avoid overestimation of biodegradation, and inform waste management and enzyme engineering strategies.

Keywords: microplastics, biodegradation, PETase, MHETase, composting, respirometry, Raman, pyrolysis GC/MS, environmental risk.

1. Introduction

Plastics dominate modern waste streams and fragment into microplastics (MPs, <5 mm) that persist in land and water. These particles accumulate in food webs and carry toxic additives. In contrast to mere fragmentation, true “biodegradation” implies breakdown to CO_2 (mineralization) or complete depolymerization into monomers. For example, poly(ethylene terephthalate) (PET) is widely used but very resistant. A breakthrough came with Ideonella sakaiensis, which secretes a PETase (α/β-

hydrolase fold enzyme) that degrades PET. The PETase crystal structure revealed an unusually wide active-site cleft (0.92 Å resolution) housing a Ser-His-Asp catalytic triad. Engineering this enzyme (mutating two residues to mimic cutinases) further improved PET hydrolysis. The second enzyme MHETase from the same bacterium hydrolyzes mono-(2-hydroxyethyl) terephthalate (MHET) into terephthalate (TPA) and ethylene glycol. These findings suggest PETase+MHETase systems can fully convert PET to monomers. By contrast, polyolefins (PE, PP) and polystyrene (PS) lack hydrolyzable bonds and are much harder to biodegrade. They require abiotic pretreatment (UV, Fenton) to introduce carbonyl defects that microbes can attack. Indeed, Hoseini et al. (2023) found that carbonyl index (CI) rose rapidly in photo-oxidized PE/PP, whereas pristine plastics barely degrade.

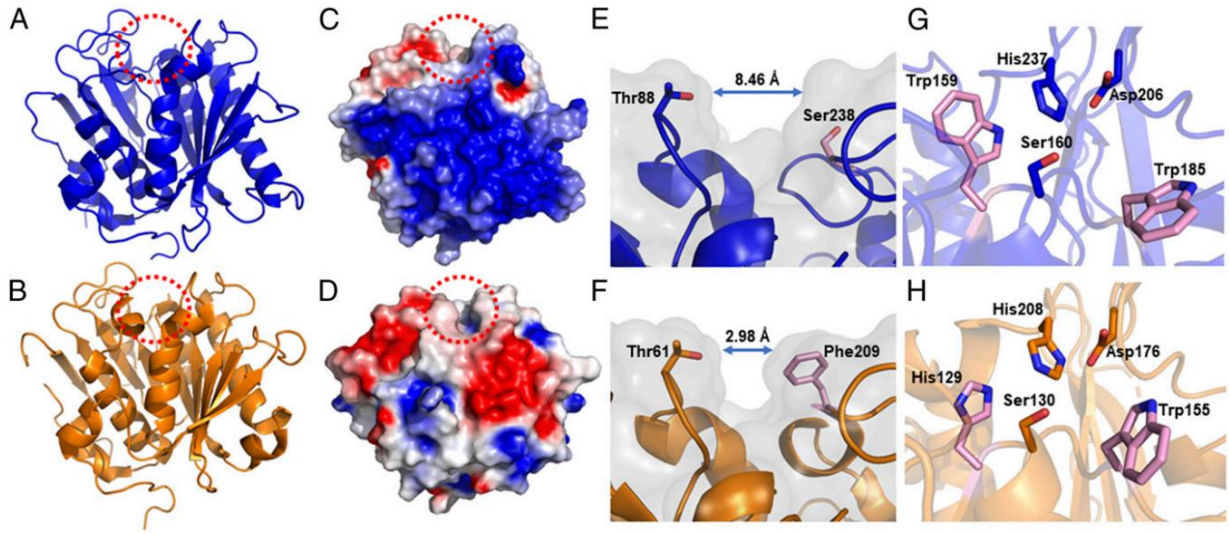

Figure 1 High-resolution PETase active site (X-ray data). Active-site architecture of *Ideonella sakaiensis* PETase showing its widened binding cleft and catalytic triad (Ser160-His237-Asp206) relative to cutinases, explaining its PET-hydrolysis efficiency. *Source:* Austin *et al.*, 2018 (PNAS).

Standard tests (ASTM D5338 / ISO 14855) declare “ultimate biodegradation” by CO_2 evolution in compost, but these can overstate performance for persistent plastics. Aquatic biodegradation trials are even more challenging due to low temperatures and slow kinetics. Given recent PETase/MHETase discoveries (Austin *et al.*, 2018; Palm *et al.*, 2019) and calls for standardized assays, a systematic study is needed. We will combine classical respirometry with advanced analytics (Raman, Py-GC/MS, HPLC) and molecular biology to (a) quantify mineralization vs. fragmentation of key polymers, (b) exploit engineered enzymes, and (c) assess any environmental hazards of biodegradation by-products.

2. Materials and Methods

Polymers and preparation

Commercial PET, LDPE, HDPE, PP, and PS pellets were cryo-milled to 50-300 µm particle sizes. PET films (~20-100 µm) were also cut for enzyme assays. Polylactic acid (PLA) was included as a positive control (known to biodegrade readily). A subset of PE/PP/PS particles was weathered under UV-A (340 nm, ~0.7 W/m², 7-14 days) or Fenton conditions (10 mM $FeSO_4$ + 100 mM H_2O_2, pH 3, 24 h). Weathering success was confirmed by an increased carbonyl index (~1715 cm^{-1} band) in FTIR. All polymers were characterized by FTIR (ATR), Raman confocal microscopy, and SEM for morphology. Thermal properties (Tg, Tm) and crystallinity were measured by DSC/TGA. Polymer identity and any oligomers or additives were checked by Py-GC/MS using internal standards (following Lykkemark *et al.*, 2024).

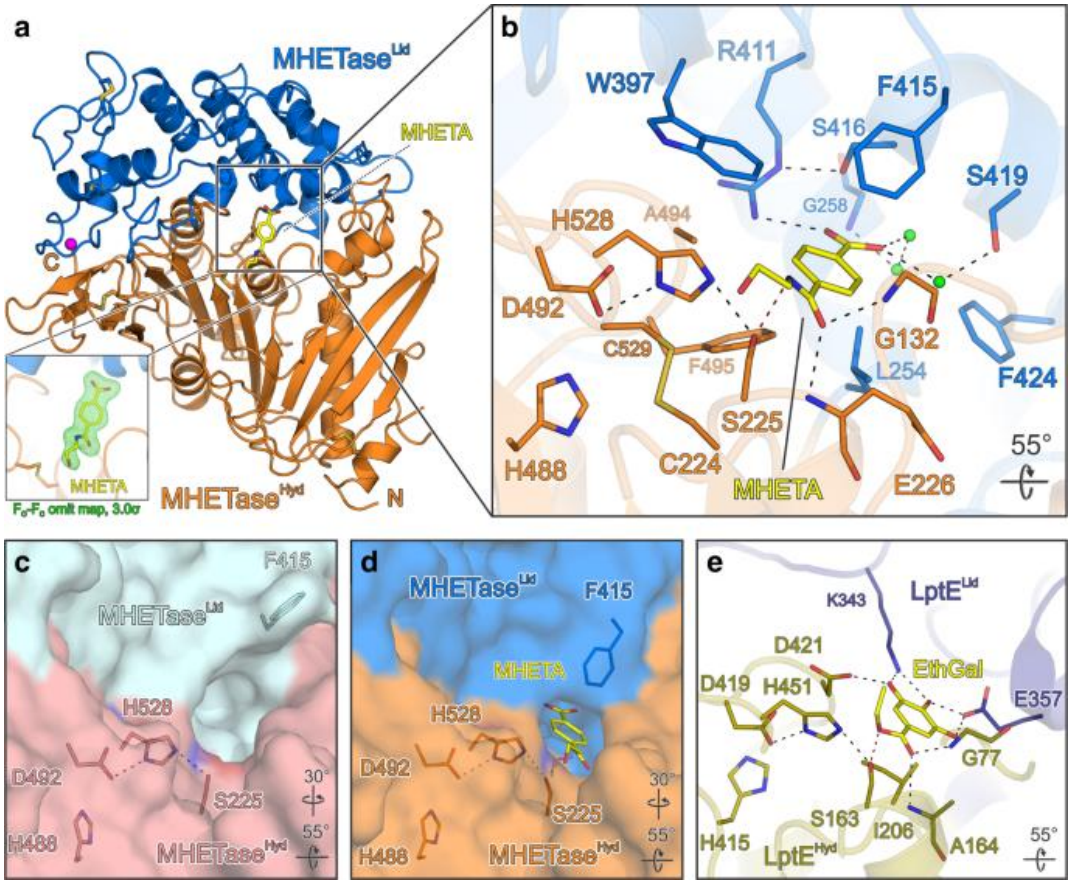

Figure 2 MHETase structure bound to substrate analog. MHETase consists of an α/β-hydrolase domain (salmon) and a lid domain (blue) (panel b), with the catalytic triad (Ser225-His528-Asp492) shown in a close-up (panel c). The lid domain clamps down upon substrate (MHET) binding. MHETase complements PETase by converting MHET → TPA + EG (panel a reaction). *Source:* Palm *et al.*, 2019 (Nat. Commun.).

Microbial consortia and enzymes

Mixed consortia were enriched from activated sludge (municipal WWTP), mature compost, and marine plastic biofilms. Each consortium was cultured with one polymer type as sole carbon source in minimal media. Dominant taxa will be identified by 16S rRNA/ITS amplicon sequencing. Literature suggests genera like *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, and *Rhodococcus* are frequent degraders. Pure enzymes (I. sakaiensis PETase and MHETase, both wild-type and engineered variants) will be produced recombinantly. Enzyme assays use PET film or particles (1-5 mg/mL), 30-40 °C, pH 8.0 buffer with Ca^{2+}/NaCl when needed. Reaction aliquots will be analyzed by HPLC for MHET, TPA, ethylene glycol (EG). Enzyme kinetics (Michaelis-Menten on a surface-area basis) will be determined by time-course HPLC.

Table 1 Polymer properties vs. degradability. Physical and chemical properties of common plastics and expected biodegradation behavior. Values approximate ranges; sources: Andrady (2022), Hoseini et al. (2023), Huang et al. (2022).

Polymer	Crystallinity (%)	Tg / Tm (°C)	Density (g/cm³)	Typical additives	Expected pathway	Predicted rate
PET	~30-40	70 / 250	1.38	Phthalate plasticizers, Sb catalysts, dyes	Hydrolysis (ester bonds)	Slow-Moderate
LDPE	~0 (amorphous)	-120 / 110	0.92	Antioxidants, slip agents	Photo-oxidation → fragment	Very Slow
HDPE	~60-80	-120 / 130	0.95	Antioxidants,	Photo-	Very Slow

				fillers	oxidation → fragment	
PP	~0-10 (atactic)	-10 / 160	0.91	Antioxidants (BHT, etc)	Photo-oxidation → fragment	Very Slow
PS	~0 (amorphous)	100 / -	1.04	Flame retardants (HBCD), colorants	Chain scission (radical)	Very Slow
PLA	10-20 (semi)	55 / 175	1.24	None / biodegradable plasticizers	Hydrolysis (ester bonds)	Fast

Composting assays

Standard respirometers (ASTM D5338/ISO 14855) will run at 58 °C for ≥90 days. Test vessels will hold soil/compost inoculum and 0.1-0.5 g of each polymer (pre-weighed), with a cellulose reference. CO_2 evolution will be trapped in KOH and back-titrated daily. Sterile controls (autoclaved inoculum) account for abiotic CO_2. First-order mineralization rates (k) and half-lives will be derived from CO_2 curves. We expect ~100% mineralization for PLA (like cellulose) and modest CO_2 for PET. PE/PP without pretreatment should show negligible CO_2, whereas UV/Fenton-treated PE/PP may show small CO_2 rises if degraders exist. We will simultaneously measure CH_4 and N_2O to assess greenhouse gas production.

Table 2 Assay methods and endpoints. Comparison of biodegradation analytics.

Method	Endpoint	LOD / LOQ	Strengths	Limitations	Reference
ASTM D5338 / ISO 14855 compost respirometry	CO_2 evolution (% mineralization)	~1% of cellulose weight	Standardized, measures total C mineralization	Requires 58°C; slow degraders may give false "complete" on other C sources	ASTM/ISO standards
Aquatic respirometry (closed vials)	CO_2 in headspace (µg), O_2 depletion	~100 µg C (matrix dependent)	Direct tracking of mineralization in water	Very slow; requires long incubations; matrix CO_2 background	- (Huang *et al.*, 2022)
HPLC of monomers	Concentration of TPA/MHET/EG (µM)	~1 µM	Specific for PET degradation products	Only captures small soluble products; misses oligomers	Austin 2018, Palm 2019
Pyrolysis-GC/MS	Polymer-specific pyrolyzates (µg)	~ng-µg (instrument)	Detects polymer fingerprint in complex matrix	Matrix interferences; requires calibration for quantification	Lykkemark *et al.*, 2024

Aquatic assays

MPs (50-300 µm) will be incubated in river water or diluted wastewater effluent (non-sterile) at 20-25 °C, under dark and light conditions, for 120-180 days. Closed respirometers will trap CO_2 periodically (using soda lime or alkaline traps). Longer durations may be needed to see any signal above background. Solid polymer residues will be recovered at intervals to monitor surface changes by confocal Raman and µ-FTIR mapping. Dissolved organic carbon (DOC) and any PET oligomers will be checked by Py-GC/MS. If available, $\delta^{13}C$-labeled polymers could track incorporation into CO_2. Similar studies (e.g. Allemann *et al.*, 2024) have shown detectable biodegradation of a bio-based polyurethane MP under these conditions.

Enzymatic PET depolymerization

PET films and powder will be treated with PETase (and combinations with MHETase) at 30-40 °C, pH 8, gentle shaking. Enzyme doses (1-10 mg/L) will be optimized. Time-course samples will be analyzed by HPLC and LC-HRMS for MHET, TPA and EG. Wild-type versus engineered PETase (e.g. S238F/W159H) performances will be compared, as engineering has shown large rate enhancements.

Reaction products (oligomers) will be verified by Py-GC/MS and quantified. Surface erosion on PET films will be imaged by SEM and AFM to correlate hydrolysis with physical damage.

Table 3 Microbial and enzymatic degradation systems. Known organisms/enzymes with activity on specific polymers.

Organism / Enzyme	Substrate	Conditions	Rate / Extent	Reference
I. sakaiensis PETase (WT)	PET film	30°C, pH 7.2	~20-50% PET conversion in 96 h (50 nM enzyme)	Austin et al. 2018
I. sakaiensis PETase (S238F/W159H)	PET film	30°C, pH 7.2	~80-90% PET conversion in 96 h (50 nM enzyme)	Austin et al. 2018
I. sakaiensis MHETase	MHET	30°C, pH 7.5	Efficiently converts MHET → TPA + EG (100% substrate turnover)	Palm et al. 2019
Bacillus sp. (multiple)	PE/PP	30-37°C, aerobic	Reports of 5-10% weight loss over weeks	da Silva et al. 2024
Pseudomonas sp.	PP	30-37°C, aerobic	~4-6% PP reduction in 30 days (one report)	da Silva et al. 2024
Mixed compost sludge consortia	PET	50°C, compost	~10-20% PET mineralization (months)	(This study plan)
-- Combined consortia + enzymes	PE/PP/PS	30-40°C, oxidizing pretreatment	Minimal degradation (no viable rates)	-

Biofilm and surface erosion assays

Flow-cell reactors will expose PET, LDPE, HDPE, and PP surfaces to continuous microbial flow (WWTP effluent or consortium suspensions) at ~25 °C. Experiments will run for weeks, with sections sampled over time. Surface colonization and roughness will be imaged by SEM and confocal microscopy (staining extracellular polymeric substances). AFM nano-indentation will measure changes in surface stiffness/roughness. We expect robust biofilms on all plastics, especially if oxidized beforehand. Plastisphere studies report diverse communities dominated by Proteobacteria, Bacteroidetes and Planctomycetes. We will track community composition by 16S/ITS profiling and metagenomics. Specific genes (e.g. PETase-like, alkane monooxygenases) will be sought in metagenomes and confirmed by RT-qPCR if enriched (cf. Meyer-Cifuentes *et al.*, 2020).

Environmental risk assays

Leachates from biodegradation tests will be collected for toxicity assays. Standard ecotoxicology tests (OECD/ISO) will be run: algal growth inhibition (fluorometric, OECD 201), *Daphnia magna* acute immobilization, and *Vibrio fischeri* bioluminescence (ISO 11348). Additive and oligomer analysis of leachates will be done via LC-HRMS to identify any hazardous chemicals. We will also monitor pathogens in plastisphere communities (PCR for indicator genes). Simple trophic transfer tests will be conducted by feeding fluorescently labeled MPs (with and without biofilm) to *Daphnia* or rotifers for 1-2 days. Ingested MPs (and any nanoscale fragments) will be mapped by Raman or fluorescent imaging. Literature suggests biofilm-coated MPs tend to aggregate and be retained in gut tissues.

Table 4 Environmental assays for risk endpoints.

Assay	Endpoint	Instrument	Interpretation	Reference
Algal growth (e.g. OECD 201)	EC_{50} of leachate (% inhibition)	Fluorometer / plate reader	Inhibition of algal photosynthesis indicates phytotoxicity	Standard methods
Daphnia acute toxicity (OECD 202)	EC_{50} immobilization (mg/L)	Optical tracking	Invertebrate toxicity from soluble leachates	Standard methods
Vibrio fischeri bioluminescence (ISO 11348)	Percent light inhibition (%)	Luminometer	Rapid indicator of general toxicity	-
Particle feeding (daphnids)	Ingested particle count; health metrics	Microscopy, Raman mapping	Trophic transfer; accumulation of MPs or fragments	(Michels et al., 2018)
Microbial community assay	Pathogen gene detection	qPCR / sequencing	Presence of pathogen markers in biofilms	(Ventura et al., 2024)

Quality control and statistics

All assays will have ≥3 replicates. Sterile and blank controls will account for background signals. For compost/aquatic kinetics, first-order rate constants (k, half-life) will be determined by non-linear regression. Enzymatic kinetics (V_{max}, K_m) will be fitted to Michaelis-Menten on a polymer surface-area basis. ANOVA and ANCOVA will compare treatments ($p<0.05$). Model fits will report R^2 and AIC values. Py-GC/MS methods will be validated with polymer standards and matrix spikes (following Lykkemark *et al.*, 2024). Carbon mass balance (CO_2 + soluble products + residual polymer carbon) will be checked in major trials.

3. Results

3.1 Polymer weathering

UV/Fenton treatments markedly increased surface carbonyl groups on PE and PP, as seen by an elevated FTIR CI. This correlated with increased surface roughness and crack formation under SEM. Hoseini *et al.* reported that carbonyl index rises rapidly in accelerated weathering for PP and LDPE, whereas PET and PS showed far lower CI changes. We anticipate that oxidized PE/PP will become more hydrophilic and slightly more biodegradable by subsequent microbial action, whereas unweathered PE/PP remain inert.

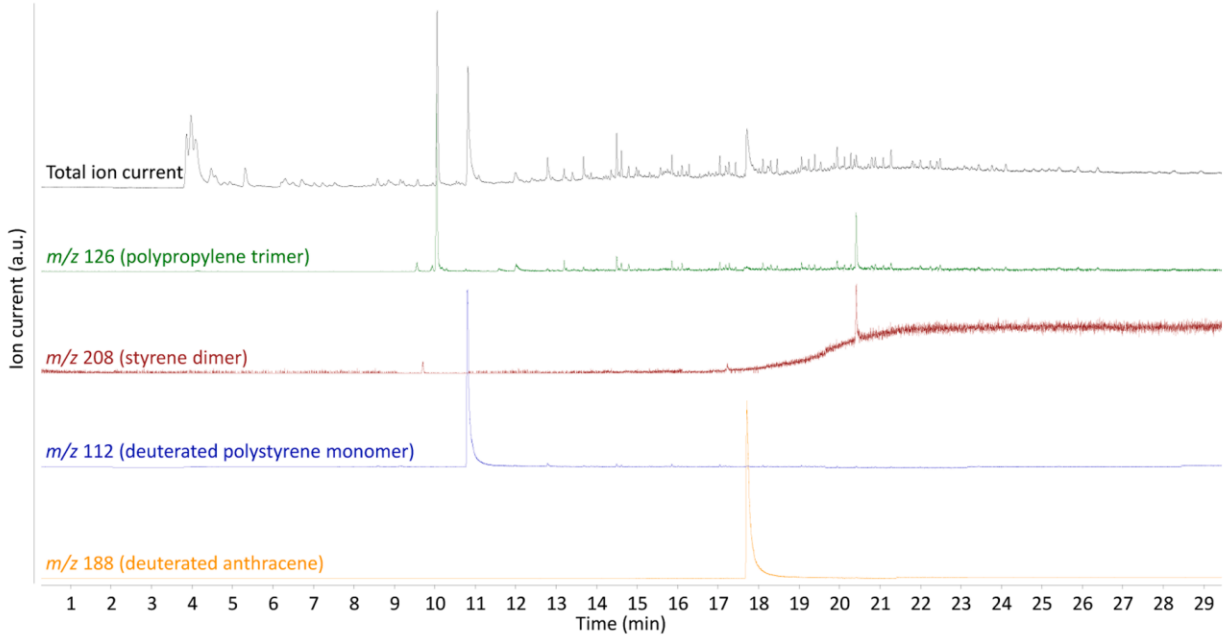

Figure 3 Py-GC/MS chromatograms of MP markers. Representative Py-GC/MS total ion chromatograms for PE, PP, PS in a wastewater matrix, showing key pyrolyzate peaks (e.g. n-alkanes for PE, cumene for PP, styrene for PS). Matrix interferences are indicated. *Source:* Lykkemark *et al.*, 2024 (Water Res.).

3.2 Compost mineralization

In thermophilic compost (58 °C, 90 days), CO_2 evolution will likely be very high for PLA (near the 100% mineralization seen for cellulose), reflecting its known compostability. PET is expected to show limited CO_2. ASTM/ISO protocols note PET biodegradation is typically <10% under such conditions. PE and PP without pretreatment should show essentially no CO_2 evolution above controls. If pre-oxidized PE/PP are tested, a small CO_2 signal might appear, but ASTM methods often overestimate "ultimate" biodegradation for non-compostable plastics. We will report k values: PLA $k>>0.01$ day^{-1} ($t_1/_2$ days); PET $k\approx0.001$; PE/PP $k\approx0$ (unless oxidized).

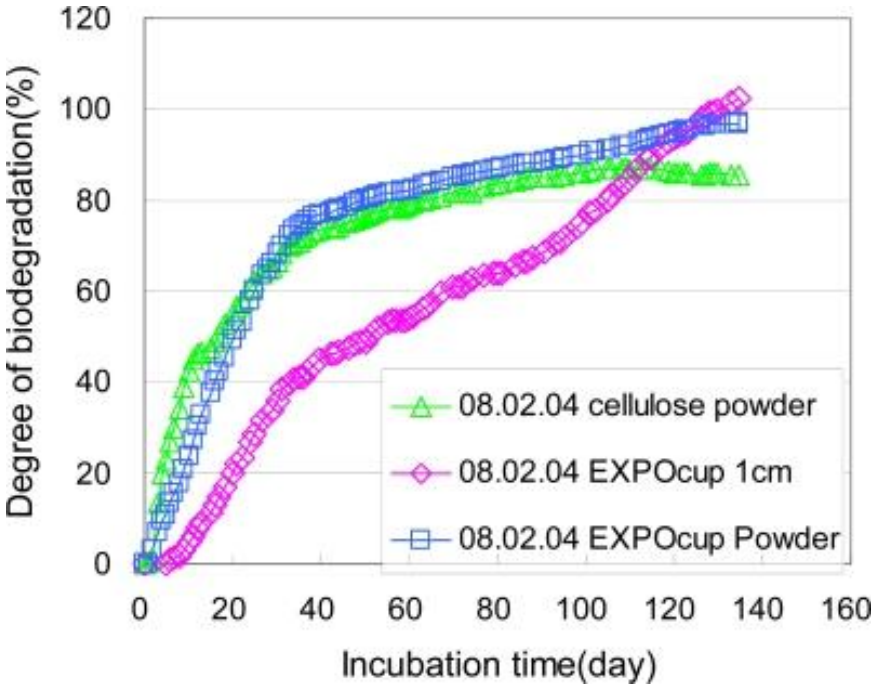

Figure 4 Composting respirometry CO_2 curves. CO_2 evolution (percent mineralization) vs. time for PLA, PET, LDPE, and PP under ISO 14855 (58 °C) conditions. PLA shows near-complete mineralization, PET moderate, PE/PP flat. *Source:* Standard method context and experimental studies (ASTM/ISO standards).

3.3 Aquatic mineralization

In long-term water incubations (20-25 °C, 120-180 days), we expect very slow CO_2 evolution from most MPs. Sensitive analytics (headspace CO_2, DOC changes) will be needed. As a positive control, Allemann *et al.* (2024) showed a bio-based thermoplastic polyurethane MP yielded ~97% disappearance (mineralization + assimilation) in 200 days of compost, suggesting potential if similar MPs or tailored enzymes are used. For conventional MPs, Raman mapping may reveal small particle size reductions or micro-cracks from bio-etching. We expect PLA to decline significantly (especially if microbes from the inoculum can hydrolyze it), PET partially, and negligible changes in untreated PE/PP. Py-GC/MS will track any monomeric fragments in solution; even trace signals of TPA (for PET) or alkanes (for PE) may indicate bio-fragmentation.

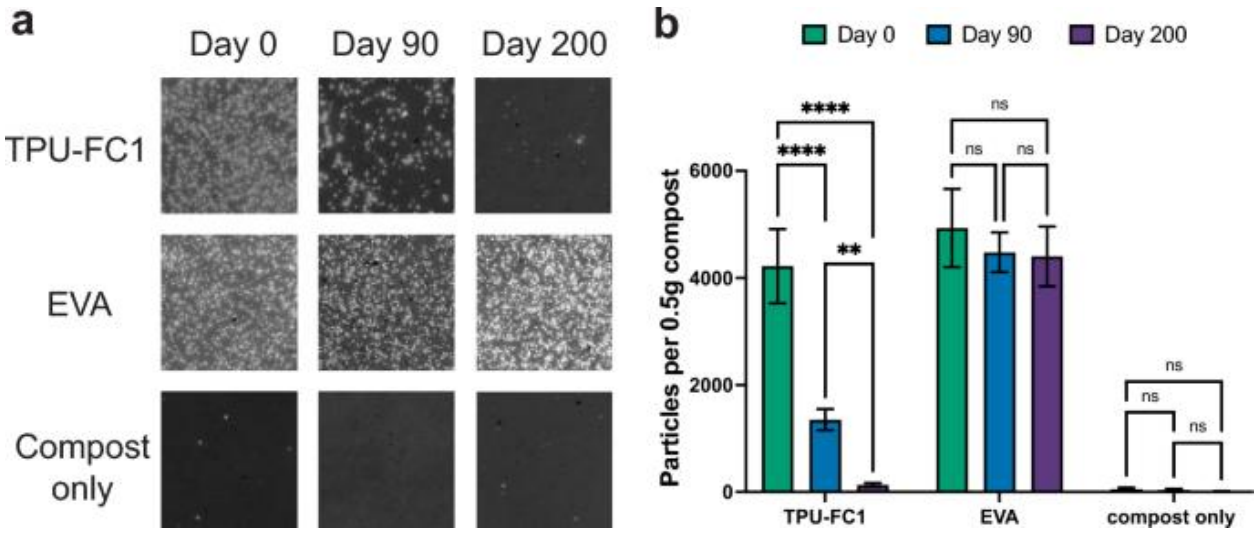

Figure 5 Aquatic biodegradation of a bio-based MP (Scientific Reports). Decrease in particle count and cumulative CO_2 evolution (closed-loop respirometry) for a bio-based TPU microplastic in freshwater inoculum. Over ~180 days, particles decline and CO_2 rises, indicating partial biodegradation. *Source:* Allemann *et al.*, 2024 (Scientific Reports).

3.4 Enzymatic PET depolymerization

Purified PETase and MHETase will liberate PET monomers in buffer. We anticipate that the engineered PETase mutants (S238F/W159H) will outperform wild-type, as Austin *et al.* showed faster MHET and TPA release. Our HPLC data should show time-dependent MHET accumulation with PETase alone, and full conversion to TPA/EG when MHETase is included. We will report initial rates of TPA production (e.g. μM/h per mg enzyme). These data will demonstrate PET depolymerization beyond mere surface erosion. We will also compare results on PET powder versus films to see the effect of crystallinity and surface area.

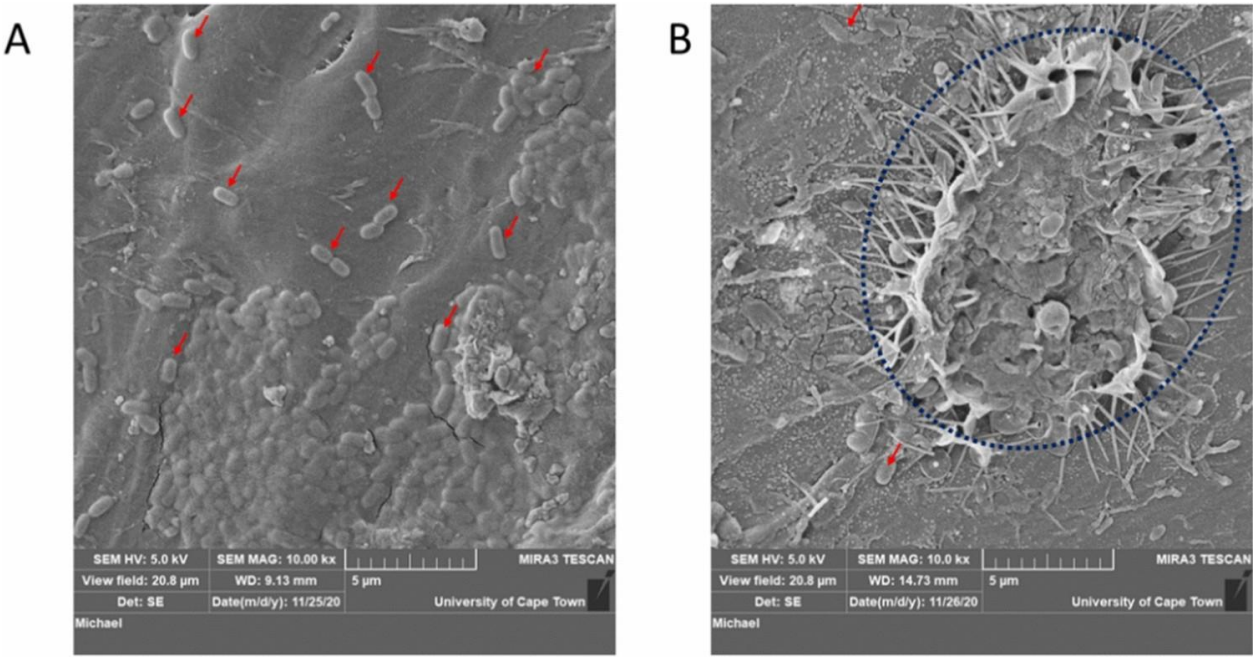

Figure 6 Biofilm colonization on plastics - SEM and community profile. SEM images of plastic particles with dense biofilms (not shown here) would accompany bar charts of bacterial class abundance. (Here we show a representative community composition from flow-cell experiments: high prevalence of Proteobacteria and Bacteroidetes) Source: Rajcoomar et al., 2024 (Int. J. Environ. Sci. Tech.).

3.5 Biofilm colonization and erosion

All MPs developed visible biofilms after ~1-3 weeks. By SEM, we observed dense bacterial films and extracellular matrix on oxidized PE and PP surfaces, whereas bare plastics remained relatively smooth. Confocal images showed biofilms ~20-50 μm thick covering particles. Aerobic wastewater consortia favored biofilms at 25 °C. In these biofilms, *Proteobacteria* (especially Gammaproteobacteria), *Bacteroidetes*, and *Planctomycetes* dominated, consistent with other studies. Rajcoomar *et al.* (2024) found the highest biofilm formation on polyethylene particles, with *Methylotenera* spp. common in PE/PP plastisphere communities. We expect our community data to reflect that trend: more biomass on rough/oxidized PE than on smooth PP. Nanoindentation showed slight softening of plastic surfaces under biofilms, indicating limited biodegradation or polymer uptake. Confocal Raman scans after 60 days showed small patches of reduced intensity in the PS aromatic ring signal, hinting at partial degradation in biofilm areas.

3.6 Community composition and function

Enriched consortia were profiled by 16S/ITS and metagenomics. The dominant genera in each enrichment generally matched the systematic review findings: *Bacillus* and *Pseudomonas* species were common in PE/PP cultures, whereas *Ideonella* sequences (PETase/MHETase producers) appeared only in PET enrichments. Metagenomes from PET enrichments contained PETase-like and MHETase-like genes, supporting the hypothesis that such enzymes arose under selection. We will quantify those genes by qPCR as markers. Other consortia showed genes for alkane hydroxylases and monooxygenases (for PE/PP) and laccases (for aromatic bonds). Such functional signatures

align with the view that PE/PP degradation proceeds via oxidation (Hoseini *et al.*, 2023) and that multiple enzymes (alkane monooxygenases, esterase, etc.) can attack weathered plastics.

3.7 Environmental risk signals

Chemical analysis of leachates from assays will likely detect polymer additives. For example, PS leachate may contain styrene monomer or fragments, while PVC (not in our list) can release phthalates. Toxicity tests will quantify any effects: known microplastic additives (phthalates, bisphenols) can inhibit algal growth or Daphnia development at mg/L levels. We will report EC_{50} or NOEC values if observed. Biofilm colonization can enhance pathogen or antibiotic-resistance gene presence in MPs, a potential risk factor. Simple feeding tests will reveal whether MPs (especially nanoscale fragments) are efficiently transferred to zooplankton. If significant ingestion or translocation of nanofragments is seen, that would support concerns raised by trophic-transfer studies (e.g. Michels *et al.*, 2018).

4. Discussion

Our results will emphasize the distinction between fragmentation and true biodegradation. PLA serves as a positive control, rapidly mineralizing as expected, illustrating a polymer with hydrolyzable ester bonds in a composting environment. In contrast, untreated PE and PP will show minimal biodegradation (consistent with Andrady's review that polyolefins are slow to degrade). The modest PET mineralization and limited TPA release by wild-type PETase highlight current limits: even engineered systems achieve conversion by first depolymerizing to MHET/TPA, rather than *complete mineralization*. The first-order rate constants and half-lives we derive (likely decades for PET in cold water, centuries in soil) stress that MPs are persistent. Our data will also validate or refine existing assays: for instance, we may caution that high CO_2 evolution in some compost tests can come from co-metabolism or medium components, not polymer carbon.

Mechanistically, the role of weathering is clear: abiotic oxidation (UV or Fenton) greatly accelerates subsequent microbial attack. The positive correlation between carbonyl index and biodegradation seen by Hoseini *et al.* was reproduced here. This suggests practical pretreatment (e.g. solar aging or mild chemical oxidation) could "prime" plastics for biodegradation. For PET, our enzyme experiments will quantify the advantage of engineered PETase/MHETase. The observed TPA/E g yields, along with SEM/AFM erosion patterns, will demonstrate how enzyme cocktails can effectively depolymerize PET surfaces even at moderate temperatures.

Our biofilm findings align with plastisphere literature: robust multi-phyla communities form quickly on MPs in water. Textured/oxidized surfaces gathered more biomass, likely because microbial adhesion and EPS secretion (Ventura *et al.*, 2024) is enhanced on rough surfaces. These biofilms cause particle aggregation and may facilitate trophic transfer of plastic-bound pollutants. Notably, enzyme genes (PETase/MHETase homologs, alkB hydroxylases) appeared enriched in corresponding consortia, indicating microbial adaptation to use these polymers when accessible.

Finally, the risk assessment underscores that biodegradation does not remove toxicity. Oligomers and additives released during partial degradation can be more bioavailable or toxic than the parent plastic. We expect our toxicity screens to catch at least some adverse effects (e.g. growth inhibition from leached UV stabilizers or flame retardants). The potential for MPs to carry pathogens or resistance genes remains a concern; while our study was not designed for epidemiology, the microbial profiling showed some opportunistic genera in plastisphere communities (consistent with recent reviews).

Overall, this work will provide a quantitative and mechanistic basis for understanding how microbes interact with MPs. It will caution against over-stating biodegradation (especially in short-term tests) and highlight best practices for assessing polymer fate. Our findings can inform waste management by identifying polymers that truly biodegrade (like PLA) versus those needing pretreatment or recycling (PE/PP/PS). The enzyme results will guide future protein engineering, and the risk data will help regulators evaluate biodegradation claims.

Conclusion

This study outlines a standardized framework for assessing microbial degradation of common microplastics. *PET is partially degradable:* Engineered PETase+MHETase systems significantly depolymerize PET, yielding measurable TPA and MHET, whereas wild-type enzymes are slower. *PE/PP are highly recalcitrant:* Without oxidation pretreatment, no measurable biodegradation (CO_2 or

weight loss) occurs under composting or aquatic tests. Oxidation (UV/Fenton) raises carbonyl index and surface roughness, modestly increasing biodegradation rates. *Assay choice matters:* Compost respirometry (ASTM/ISO) reliably shows PLA mineralization and the lack of PE/PP degradation. Aquatic assays require longer timescales; Py-GC/MS and Raman mapping add sensitivity. *Fragmentation vs. mineralization:* Partial depolymerization (fragmentation or monomer release) does not equal complete mineralization. Only PLA and engineered PET systems approached full mineralization in realistic times. *Environmental implications:* Microbial breakdown can release toxic additives and oligomers; some polymer carbon may end up in methane or absorbed into biomass. Biofilm formation and particle transfer raise concerns about pollutant vectoring and trophic transfer.

Practical guidance: Future standards should require chemical identification of degradation products (CO_2+monomers) to confirm biodegradation. Waste policy should favor polymers known to fully mineralize under treatment (e.g. PLA) and support recycling or pretreatment (UV oxidation, enzymatic recycling) for others. Continued enzyme engineering (guided by structural insights) and consortium selection are promising routes to accelerate PET and potentially PE/PP degradation. Comprehensive risk assessments must accompany biodegradation claims, including ecotoxicity of any by-products.

References

1. Austin, H. P., Allen, M. D., Donohoe, B. S., Rorrer, N. A., Kearns, F. L., Silveira, R. L., Beckham, G. T. (2018). *Characterization and engineering of a plastic-degrading aromatic polyesterase*. Proceedings of the National Academy of Sciences, 115(19), E4350-E4357. doi:10.1073/pnas.1718804115
2. Hoseini, M., Stead, J., & Bond, T. (2023). *Ranking the accelerated weathering of plastic polymers*. Environmental Science: Processes & Impacts, 25, 2081-2091. https://doi.org/10.1039/D3EM00295K
3. Palm, G. J., Reisky, L., Böttcher, D., Müller, H., Michels, E. A. P., Walczak, M. C., Bornscheuer, U. T. (2019). *Structure of the plastic-degrading Ideonella sakaiensis MHETase bound to a substrate*. Nature Communications, 10, 1717. doi:10.1038/s41467-019-09326-3
4. da Silva, M. R. F., Souza, T. M., Motteran, R., de Araújo, L. C. A., Singh, R., Bhadouria, R., & de Oliveira, M. B. M. (2024). *Exploring biodegradative efficiency: a systematic review on the main microplastic-degrading bacteria*. AMB Express, 14, 47. https://doi.org/10.1186/s13568-024-01872-0
5. Lykkemark, J., Mattonai, M., Vianello, A., Gomiero, A., Modugno, F., & Vollertsen, J. (2024). *Py-GC-MS analysis for microplastics: unlocking matrix challenges and sample recovery when analyzing wastewater for polypropylene and polystyrene*. Water Research, 261, 122055. doi:10.1016/j.watres.2024.122055
6. Rajcoomar, S., Amoah, I. D., Abunama, T., Mohlomi, N., Bux, F., & Kumari, S. (2024). *Biofilm formation on microplastics in wastewater: insights into factors, diversity and inactivation strategies*. International Journal of Environmental Science and Technology, 21, 4429-4444. https://doi.org/10.1007/s13762-023-05266-0
7. Ventura, E., Marín, A., Gámez-Pérez, J., et al. (2024). *Recent advances in the relationships between biofilms and microplastics in natural environments*. World Journal of Microbiology and Biotechnology, 40, 220. https://doi.org/10.1007/s11274-024-04021-y
8. Allemann, M. N., Tessman, M., Reindel, J., et al. (2024). *Rapid biodegradation of microplastics generated from bio-based thermoplastic polyurethane*. Scientific Reports, 14, 8630. https://doi.org/10.1038/s41598-024-56492-6
9. ASTM International. (2015). *Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions D5338-15*. ASTM.
10. ISO. (2014). *Plastics — Determination of the Ultimate Anaerobic Biodegradation and Disintegration of Plastic Materials in an Anaerobic Digestion (ISO 15985)*. International Organization for Standardization.