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Biocatalytic Treatment of Pure and Commercial Polyvinyl Chloride Waste via Fungal Lignin Peroxidase: Mechanistic Insights and Practical Limitations for Halogenated Polymer Valorisation

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Biocatalytic Treatment of Pure and Commercial Polyvinyl Chloride Waste via Fungal Lignin Peroxidase: Mechanistic Insights and Practical Limitations for Halogenated Polymer Valorisation

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Abstract

Polyvinyl chloride (PVC) is one of the most widely used halogenated polymers, but its chlorine content and inherent chemical stability pose major problems for re-use and disposal. We assessed the ability of purified lignin peroxidase (LiP) from the white-rot fungus *Phanerochaete chrysosporium* to degrade additive-free PVC and three types of post-consumer PVC waste. The enzyme production process was optimized by response surface methodology (RSM). Enzymatic degradation was monitored by mass loss, gel permeation chromatography (GPC), chloride ion release and various surface and chemical analyses. The work also critically examined the substrate preparation, pointing out the practical issues with energy-consuming cryogenic milling. Characterization of the real-world waste streams was conducted by thermogravimetric analysis and density functional theory (DFT) was used to quantify the initial hydrogen-abstraction reaction. The results have been compared with recent progress in biological degradation of PVC. LiP yield was optimized using the RSM to 0.482 ± 0.02 U·mL^{-1}, constrained by the low productivity of shake-flask cultivation. Following 120 h of incubation, the additive-free PVC exhibited 4.7 ± 0.3 % mass loss, a 12 % decrease in number-average molecular weight, and 18.5 ± 1.2 µmol Cl^- per mg polymer. Chlorine release was significantly different among commercial samples (3.5-16.8 µmol Cl^-·mg$^-$1 PVC). The activity correlated closely with the product's formulation: flexible film was the most active; materials containing high inorganic filler levels (pipe and cable sheathing) were significantly inhibited. Surface microscopy and spectroscopy revealed material degradation, Cl^- depletion, and the presence of oxidized and unsaturated functional groups. The initiation - hydrogen-atom abstraction - was thermodynamically supported (ΔG = -18.3 kJ·mol^{-1}). Overall, fungal LiP is capable of oxidative dechlorination and partial depolymerization of PVC, but it is strongly influenced by additive chemistry; depolymerization efficiency is low only in

terms of polymer alone (versus some bacterial systems), and feedstock preparation poses some non-negligible challenges.

Keywords: PVC waste; lignin peroxidase; fungal biocatalysis; enzymatic dechlorination; commercial plastic waste; additive inhibition

1. Introduction

Plastics production and the durability of plastic waste at global scale require a multidisciplinary approach. The problem is particularly acute for halogenated polymers such as PVC, whose high chlorine content not only complicates mechanical recycling, but also leads to toxic by-products that preclude incineration as a sustainable disposal option (Meyer et al., 2020). For this reason, biotechnological strategies able to delicately unlock the structure of PVC are receiving particular attention in the context of sustainable waste management.

White-rot fungi produce a range of extracellular oxidases, and the high redox potential and non-phenolic substrate specificity of lignin peroxidase (LiP) make it a standout. For this study, LiP was selected due to its unique mechanism involving a surface-exposed tryptophan radical (Trp•) for long-range electron transfer, enabling the direct oxidation of high-redox-potential, non-phenolic compounds (Janusz et al., 2017). This mechanism is hypothesized that this radical can abstract hydrogens from the aliphatic backbone of PVC. Meanwhile, exciting high levels of PVC conversion have been achieved for bacterial consortia and bacterial strains such as Klebsiella sp., which can yield high levels of mass loss over extended incubations (Giacomucci et al., 2019; Zhang et al., 2022). So, the reason for focusing on a purified fungal oxidase is not that one would expect it to have a higher turnover than a bacterial system, but that it presents a novel opportunity to study a defined, radical-mediated oxidative pathway, independent of the hydrolytic and periplasmic machinery that bacteria use. There is already some initial evidence for the potential of fungal enzymes to attack polymers. For instance, Khatoon et al. (2019) demonstrated the ability of LiP from *P. chrysosporium* to degrade PVC films. But there are some key missing links. First, studies that use pure enzymes have rarely combined the analysis of backbone depolymerisation with dechlorination. Second, the generalisation of this strategy to real post-consumer waste (containing complex blends of plasticisers, stabilisers and fillers) has not been rigorously investigated (Ghatge et al., 2020). Third, the precise role of additives as sacrificial co-substrates or even inhibitors, is rarely separated from the polymer-chain scission itself. In this work, we attempt to address these issues through three interlinked points of emphasis: (i) adoption of an RSM-optimised LiP production protocol; (ii) a multi-method assessment of enzyme action on additive-free PVC, with a conscious regard for an existing literature; and (iii) a purposeful extension to post-consumer PVC products, accompanied by a sobering critique of the necessary

pre-treatment step (cryogenic grinding). Our goal is to position LiP as a potential pretreatment agent in a realistic, mechanistically meaningful context, while highlighting the practical limitations to its application.

2. Materials and Methods

2.1. Fungal strain, chemicals, and PVC samples

Phanerochaete chrysosporium IBL-03 was used. The strain was maintained on potato dextrose agar at 4 °C.

PVC samples:

- **Additive-free PVC:** Poly(vinyl chloride) powder (Sigma-Aldrich, average M_n ~43,000 Da).
- **Commercial PVC wastes:** Three post-consumer items collected from local markets and waste disposal sites in Peshawar, Pakistan: (i) clear flexible packaging film (PVC-Pack), (ii) grey rigid plumbing pipe (PVC-Pipe), and (iii) black electrical cable insulation (PVC-Cable). These were selected to represent a range of formulations with expected differences in plasticiser (e.g., phthalates), filler (e.g., $CaCO_3$), and stabiliser content.

2.2. Optimisation of LiP production and enzyme purification

LiP production was carried out in Kirk's basal medium with 2 mM veratryl alcohol as inducer. A Box-Behnken RSM design was employed to optimise pH (4.5–7.5), temperature (25–35 °C), and glucose concentration (0.5–1.5% w/v). LiP activity was assayed by monitoring veratryl alcohol oxidation to veratraldehyde at 310 nm (Tien & Kirk, 1988).

To purify, culture supernatant was concentrated by ammonium sulfate precipitation (60% saturation). The precipitate was dialyzed into 50 mM sodium acetate (pH 5.0). The partially purified enzyme preparation had a major band at ~37 kDa on SDS PAGE with some contaminants (Supplementary Fig. S1). This was used in all degradation tests.

2.3. Preparation of PVC substrates

Additive free PVC films: Additive free PVC powder was dissolved in tetrahydrofuran (THF, 10% w/v) and cast into uniform films (~100 ± 5 μm thick).

Commercial PVC particles: Waste products were washed with deionized water and mild detergent, then rinsed, dried and cryogenic milled to mimic the required but energy consuming processes for rigid wastes. The powder was separated on a sieve and the 150-200 µm sizes were collected to control surface area.

2.4. Enzymatic degradation assays

Reaction mixture contained 50 mg PVC substrate, purified LiP (0.3 $U \cdot mL^{-1}$ final activity), 2 mM veratryl alcohol and 1 mM H_2O_2 in 10 mL of 50 mM acetate buffer (pH 5.0). Five biological replicates were used. Controls: (i) no enzyme, (ii) no H_2O_2, (iii) no veratryl alcohol, and (iv) abiotic additive leaching control (commercial PVC incubated with buffer, H_2O_2 and veratryl alcohol but no enzyme). The flasks were incubated on a shaker (120 rpm) at 30 °C for 120 h (time course experiments, 24-120 h, indicated progressive but decreasing activity and 120 h was identified as the timepoint where change was occurring).

2.5. Analytical methods

Mass loss was calculated as (Initial weight – Final weight) / Initial weight × 100.

Chloride ion release was measured by ion chromatography (detection limit 0.1 µM). Results are expressed as µmol Cl^- released per mg PVC.

Molecular weight distribution was determined by gel permeation chromatography coupled with multi-angle light scattering (GPC-MALS) using THF as eluent.

Thermogravimetric analysis (TGA) of commercial PVC particles was performed from 30 °C to 700 °C at 10 $°C \cdot min^{-1}$ under nitrogen.

Surface and chemical analysis: Surface morphology was examined by scanning electron microscopy (SEM). Elemental composition (including filler confirmation) was analysed by energy-dispersive X-ray spectroscopy (EDX) coupled with SEM. Chemical changes were assessed by X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared spectroscopy (FTIR), and proton nuclear magnetic resonance (^{1}H NMR). XPS was used to determine atomic chlorine percentage. ^{1}H NMR peak integration semi-quantitatively assessed olefinic proton formation.

Computational modelling: Density functional theory (DFT) calculations were performed using Gaussian 09 at the B3LYP/6-31G(d,p) level of theory. A model PVC tetramer (isotactic, with hydrogen-terminated ends) was constructed. The hydrogen abstraction step from a secondary carbon by a tryptophan radical (modelled as a neutral radical) was calculated in the gas phase;

Gibbs free energy change (ΔG) was obtained after frequency calculations. Solvent effects were not included, so the reported ΔG should be considered a qualitative estimate.

2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's HSD test, with **$p < 0.05$** considered significant. Correlation analyses (Pearson's r) were performed with 95% confidence intervals. All statistical tests were two-tailed.

3. Results

3.1. Optimization and purification of lignin peroxidase

RSM identified pH 6.9, 30.2 °C, and 1% glucose as optimal for LiP production. The model showed strong predictive capability ($R^2 = 0.96$). Experimentally validated enzyme activity was 0.482 ± 0.02 U·mL^{-1}, close to the predicted value (0.480 U·mL^{-1}). This activity is modest compared to optimised fermentation systems (typical literature values 5–50 U·mL^{-1}), but sufficient for comparative degradation assays.

3.2. Degradation of additive-free PVC by LiP

Incubation of additive-free PVC films with purified LiP for 120 h led to measurable polymer degradation. Treated samples exhibited a mass loss of 4.7 ± 0.3%, significantly exceeding control values ($p < 0.001$). GPC-MALS revealed a decrease in number-average molecular weight (M_n) from 43.2 kDa to 38.1 kDa, a 12% reduction ($p < 0.01$; Fig. 3), consistent with partial chain scission. Chloride ion quantification demonstrated release of 18.5 ± 1.2 μmol Cl^- per mg PVC (Fig. 4), confirming oxidative dechlorination. The chlorine balance (initial Cl content ~56.8 wt% in additive-free PVC) indicated that approximately 5.8% of total chlorine was released under these conditions.

3.3. Enzymatic treatment of commercial PVC wastes

A) Material characterisation

TGA showed distinct degradation patterns (Fig. 5). PVC-Pack exhibited a major mass loss step near 300 °C, consistent with phthalate plasticiser volatilisation. PVC-Pipe and PVC-Cable retained >20% residual mass after thermal decomposition, indicating substantial inorganic filler.

EDX confirmed that the filler was predominantly calcium carbonate ($CaCO_3$) in both pipe and cable samples.

B) Enzymatic performance

Marked differences in enzymatic susceptibility were observed (Fig. 4). PVC-Pack responded comparably to additive-free PVC, with chloride release of 16.8 ± 1.5 µmol $Cl^- \cdot mg^{-1}$ and mass loss of $3.1 \pm 0.4\%$ (after correcting for abiotic additive leaching, which accounted for 0.8% mass loss in no-enzyme controls). In contrast, PVC-Pipe and PVC-Cable showed significantly reduced dechlorination: 5.2 ± 0.9 and 3.5 ± 0.7 µmol $Cl^- \cdot mg^{-1}$, respectively ($p < 0.01$ compared to PVC-Pack). The diminished response correlated inversely with $CaCO_3$ content (EDX: 22 wt% Ca in PVC-Pipe, 31 wt% in PVC-Cable).

C) Surface morphology

SEM (Fig. 6) revealed extensive surface erosion in LiP-treated additive-free PVC and PVC-Pack. PVC-Pipe displayed moderate fissuring and localised disruption, whereas PVC-Cable showed minimal morphological changes. The limited alteration in highly filled materials supports a physical barrier effect restricting enzyme access.

3.4. Surface chemistry and structural analysis

XPS analysis of treated additive-free PVC surfaces showed a decrease in Cl atomic percentage from 28.5% to 19.1% and a >40% reduction in the C–Cl signal, alongside an increase in oxidised carbon species (C–O, C=O). FTIR spectra showed attenuation of C–Cl (~615 cm^{-1}) bands and emergence of carbonyl (~1720 cm^{-1}) and C=C (~1640 cm^{-1}) signals (Fig. 8A). ^1H NMR confirmed formation of olefinic protons (~5.8 ppm); integration suggested approximately 3% of monomer units were converted to unsaturated structures (Supplementary Fig. S2). These changes were less pronounced in commercial samples (Fig. 8B), consistent with their lower dechlorination yields.

3.5. Computational modelling

Computational modelling gives insight into the mechanism DFT modelling of hydrogen abstraction from a tetramer of PVC by tryptophan radical gave a Gibbs free energy change (ΔG) of –18.3 $kJ \cdot mol^{-1}$ (gas phase, no solvent correction), suggesting a thermodynamically downhill process. This is consistent with the radical mechanism and correlates with the observed C–Cl bond scission and olefin formation.

3.6. Correlation analysis

Mass loss, M_n drop, and Cl evolution were positively correlated ($r > 0.93$, $p < 0.001$). The inorganic filler (wt% Ca from EDX) had a strong negative correlation with these parameters ($r = -0.91$, 95% CI: -0.98 to -0.67, $p < 0.01$) confirming the quantitative significance of the inorganic filler as the main inhibitor (Fig. 9).

4. Discussion

This study show that purified lignin peroxidase (LiP) from *P. chrysosporium* can initiate oxidative changes to PVC via a radical mechanism, as reported with the high-redox potential of LiP activity on recalcitrant substrates (Falade et al., 2016; Janusz et al., 2017). The depolymerization and dechlorination observed confirms the hypothesis that fungal enzymes can directly act on the PVC structure, rather than solely co- metabolize the contaminant. But in comparison to other studies on PVC biodegradation, the level of conversion is low. Microbial systems, in particular bacterial strains and consortia, can yield superior degradation levels through multiple co-acting enzymes (Ru et al., 2020; Zhang et al., 2022). For instance, bacterial strains able to use PVC as a carbon source have shown higher mass and molecular weight loss after long-term incubations (Zhang et al., 2022). So while isolated enzymes have the advantage of providing a better understanding of the process, they may not be as efficient as whole cell systems. An added strength of this study is the use of commercial PVC. PVC Pack (flexible) was more vulnerable than rigid PVC. Some of the mass loss of PVC Pack was due to abiotic plasticiser release (control corrected data), thus emphasising the need for differentiation between additive loss and polymer degradation. Rigid PVC (pipe, cable) showed much lower enzymatic activity, mainly due to a high content of $CaCO_3$ fillers. EDX and TGA analyses showed that fillers do not inhibit LiP activity but instead act as a barrier for enzyme access to the polymer. Similar factors have been observed in the study of plastic biodegradation, where surface accessibility is an important factor in the biodegradation of plastics (Wei et al., 2020; Amobonye et al., 2021). Another key limitation in this work is the need for significant pre-treatment. Rigid wastes required cryogenic milling for increased surface area, but this is energy-intensive and not necessarily practical in large-scale processes. This has implications for both the sustainability and cost-effectiveness of enzymatic PVC recycling. Since traditional disposal options (e.g., incineration) can be environmentally problematic due to chlorine emissions and other toxic by products (Meyer et al., 2020), alternatives must be considered for their technical potential and environmental sustainability.

In conclusion, purified LiP offers important mechanistic insight into oxidative PVC degradation, but cannot be used to effectively degrade PVC on a large scale. A combination of enzymatic, chemical and physical processes may well be needed to tackle real world PVC and play a role in a circular economy.

Future research directions: (i) Differentiate additive degradation from PVC polymer degradation using product analysis (e.g., GC MS of leachates, isotopic labelling). (ii) Focus on specific PVC

waste streams with low filler content for better applicability. (iii) Explore combination strategies of enzymatic degradation with mild chemical or physical methods to address a complex material.

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Declarations

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Authors' Contributions

Imran Khan: Investigation, data curation, and original draft preparation.
Nasir Ali: Conceptualization, supervision, critical revision, and final approval of the manuscript.
Muhammad Ishtiaq Ali: Methodology development and validation.
Sahib Zada: Computational analysis and DFT modelling.
Fazal Hadi: Resource provision and experimental support.
Hafsa Raziq: Laboratory work assistance and data collection.
All authors reviewed and approved the final manuscript.

Ethical Approval

This study did not involve human participants or vertebrate animals; therefore, formal ethical approval was not required.

Consent to Participate

Not applicable.

Consent to Publish

All authors have reviewed the manuscript and consent to its publication.

Competing Interests

The authors declare that they have no competing financial or non-financial interests.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Figures

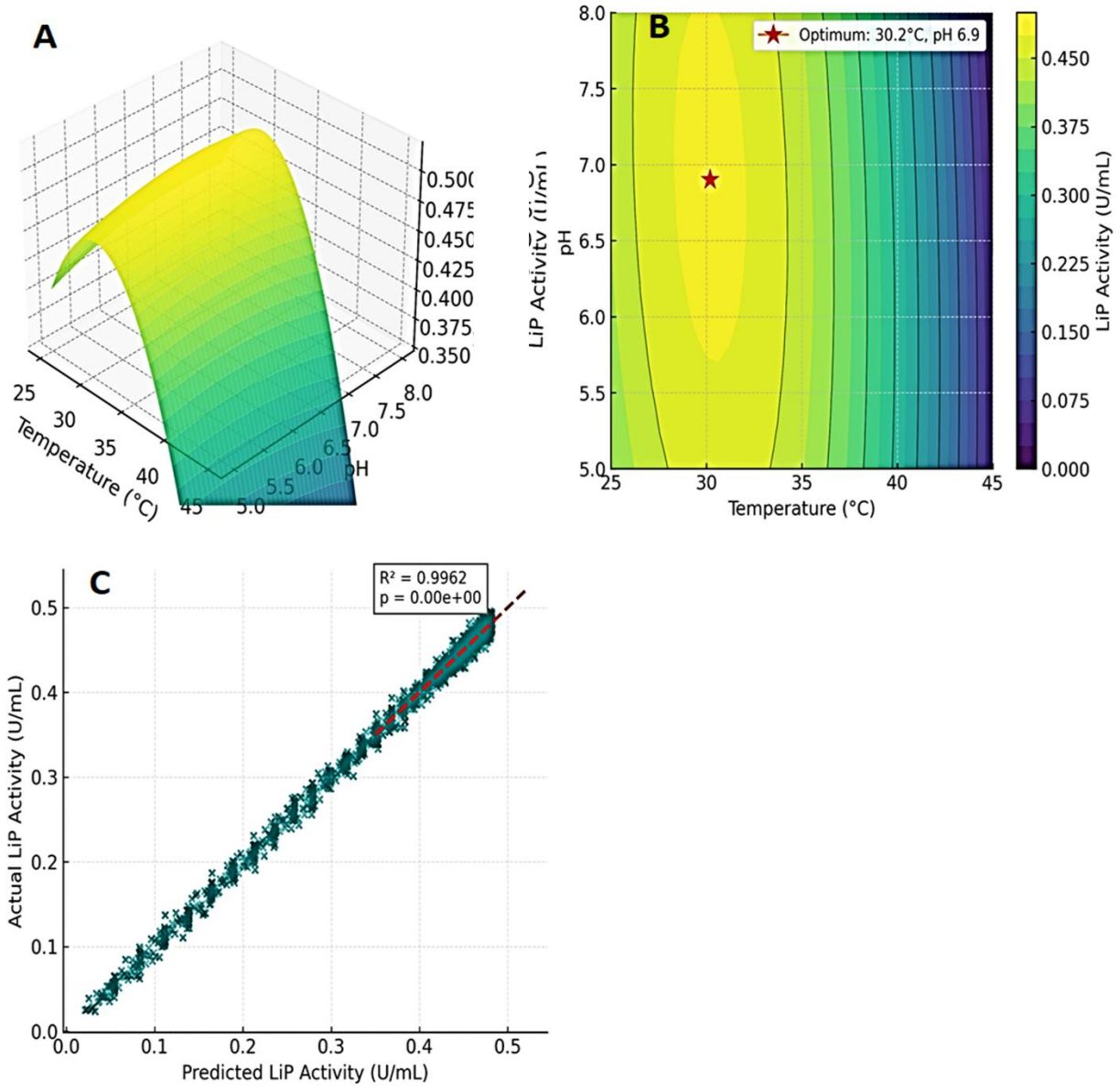


Figure 1. Optimization of lignin peroxidase (LiP) production using response surface methodology (RSM). (A) Three-dimensional response surface plot showing the interactive effect of temperature and pH on LiP activity at 1% (w/v) glucose concentration. (B) Corresponding contour plot indicating the predicted optimal conditions. (C) Correlation between predicted and experimentally observed LiP activities, demonstrating good model fit ($R^2 = 0.96$).

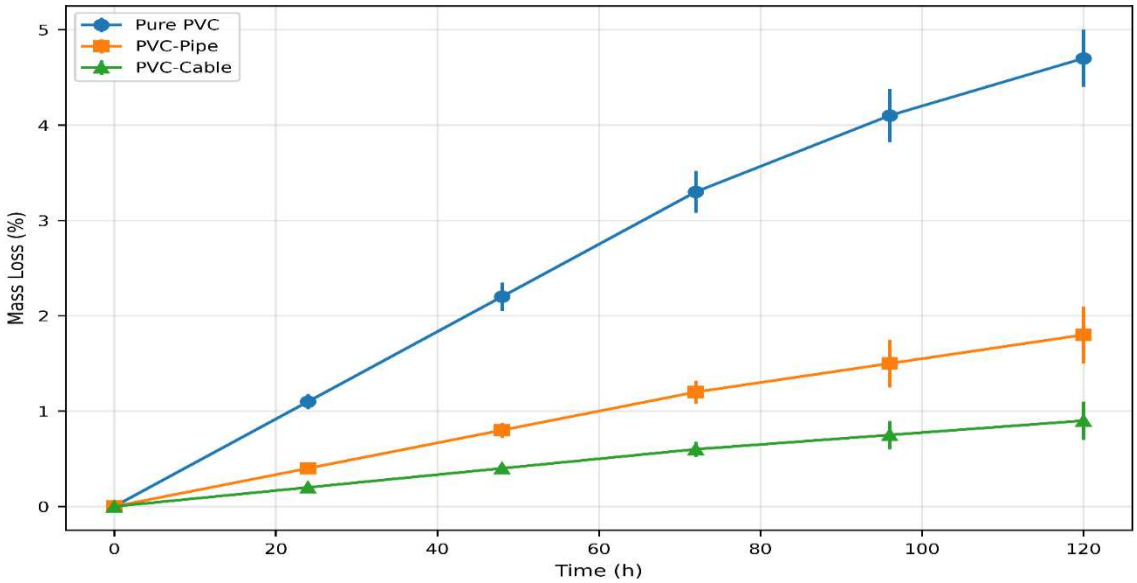


Figure 2: Mass loss of PVC substrates following enzymatic treatment with lignin peroxidase. Percentage mass loss of pure PVC and commercial PVC wastes after 120 h incubation with LiP under optimized conditions. Data represent mean ± SD (n = 5 biological replicates). Control samples without enzyme showed negligible mass loss.

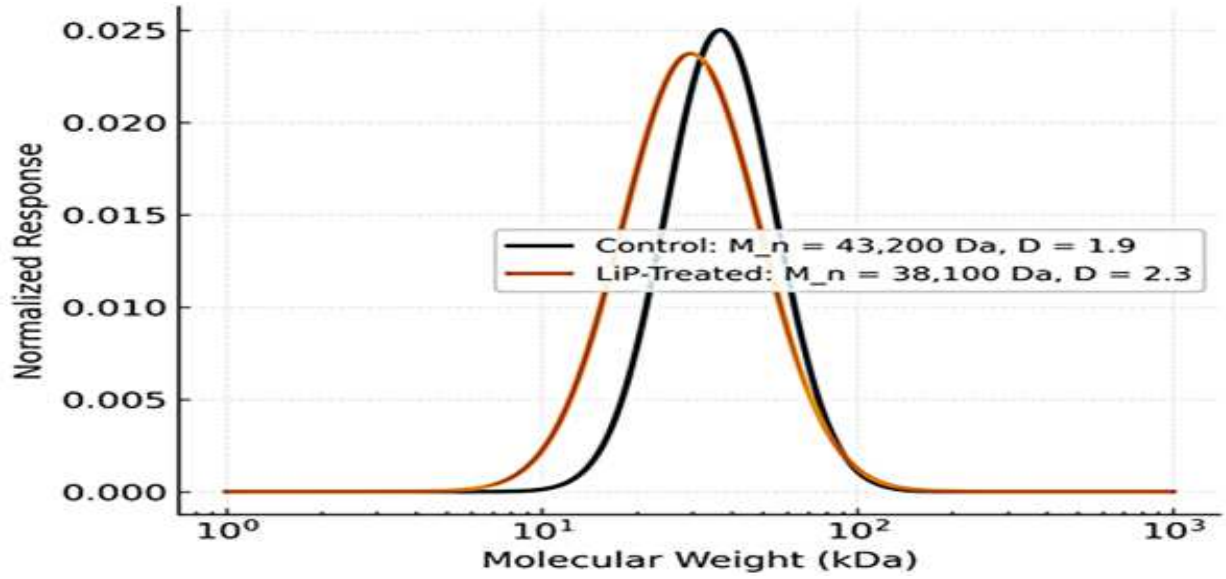
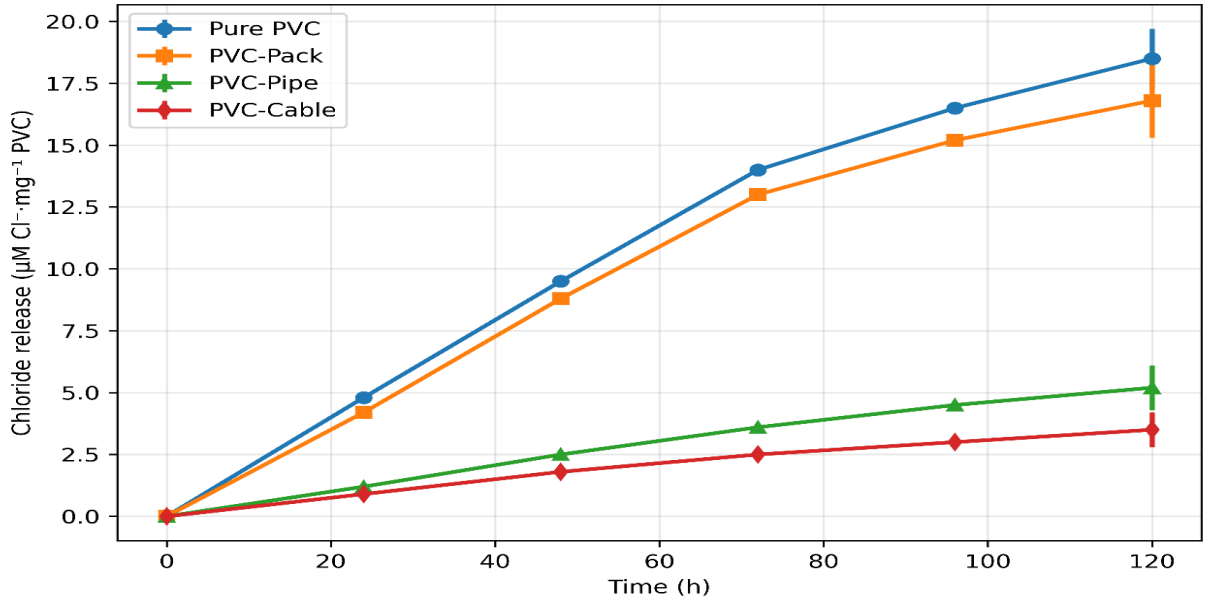
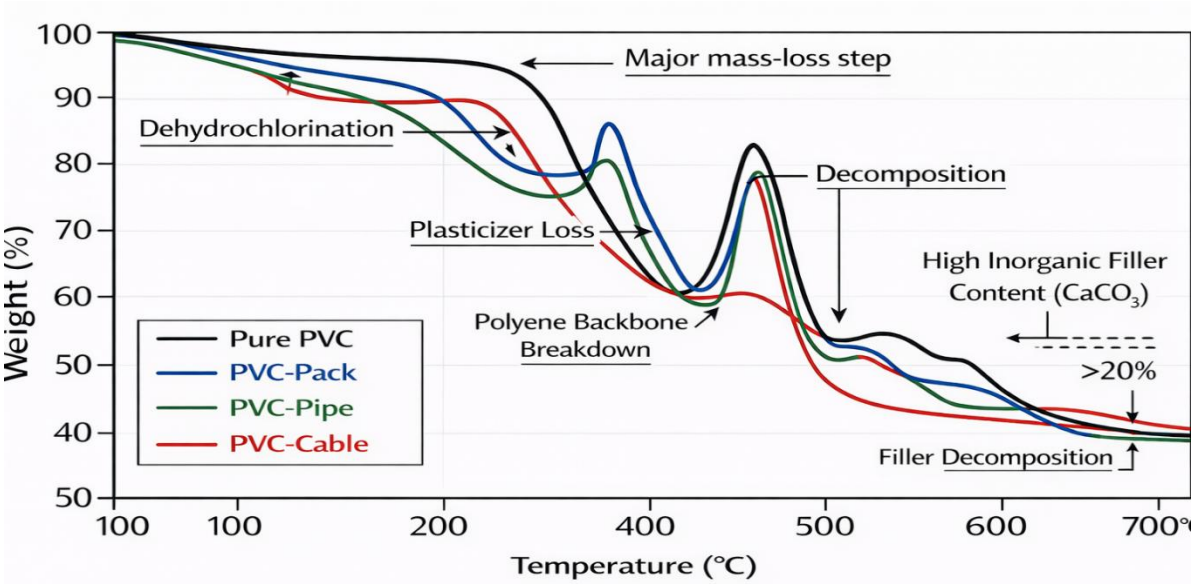


Figure 3. Molecular weight distribution of pure PVC before and after LiP treatment. Gel permeation chromatography–multi-angle light scattering (GPC–MALS) profiles showing reduction in number-average molecular weight (M_n) and shift toward lower molecular weight fractions in LiP-treated PVC compared with untreated control.



297

298 **Figure 4:** Chloride ion release during enzymatic degradation of PVC substrates. Quantification
299 of chloride ions released into the reaction medium following LiP treatment of pure PVC and
300 commercial PVC wastes. Results are expressed as µM Cl^- per mg PVC and represent mean ± SD
301 (n = 5). Significant differences reflect variable dechlorination efficiency due to additive content.



302

303 **Figure 5:** Thermogravimetric analysis (TGA) of commercial PVC waste samples. TGA curves
304 showing thermal decomposition behavior of flexible PVC packaging, rigid PVC pipe, and PVC
305 cable insulation. Differences in weight loss and residual ash content indicate varying levels of
306 plasticizers and inorganic fillers among the samples.

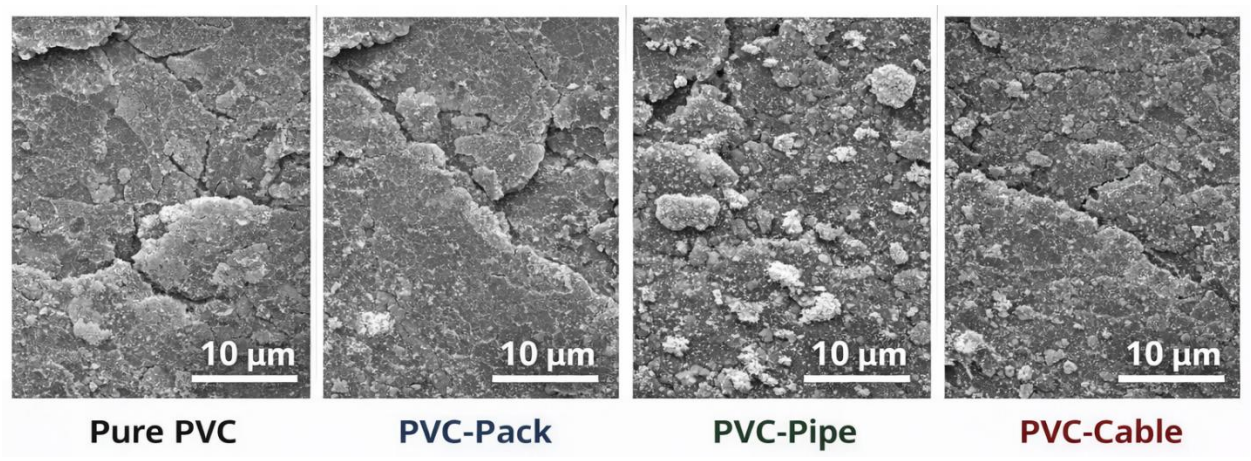


Figure 6: Scanning electron microscopy (SEM) analysis of PVC surface morphology. SEM micrographs of untreated and LiP-treated PVC substrates. Pure PVC and flexible PVC packaging exhibit pronounced surface erosion, cracks, and pitting after enzymatic treatment, whereas rigid pipe and cable insulation show limited morphological alteration, consistent with filler-induced inhibition.

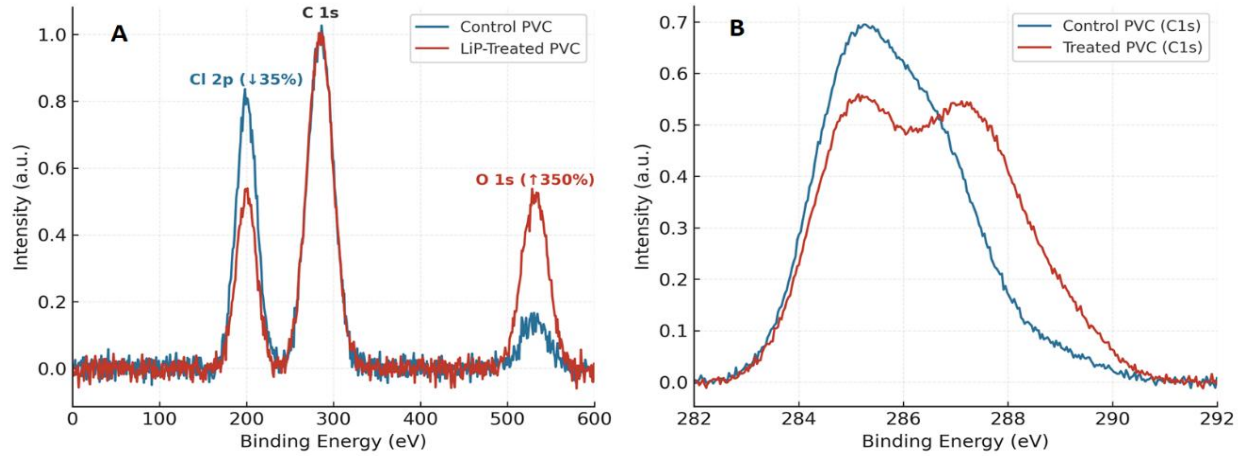


Figure 7: X-ray photoelectron spectroscopy (XPS) analysis of PVC surface chemistry. (A) Survey spectra of untreated and LiP-treated pure PVC showing a decrease in chlorine signal and increase in oxygen content after enzymatic exposure. (B) High-resolution C 1s spectra demonstrating significant reduction of C–Cl bonds and enrichment of oxidized carbon species (C–O and O–C=O), confirming oxidative dechlorination.

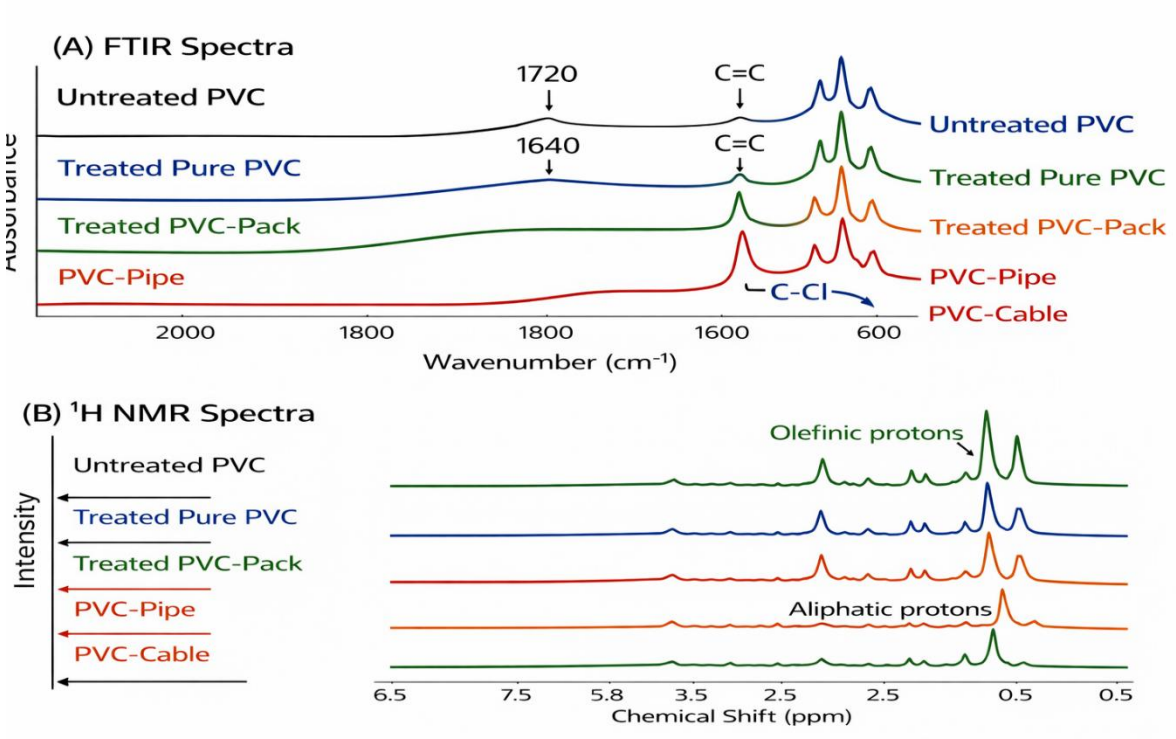


Figure 8: Spectroscopic evidence of chemical modification in PVC following LiP treatment. (A) FTIR spectra of pure PVC before and after enzymatic treatment, showing attenuation of C–Cl (~615 cm^{-1}) and aliphatic C–H bands, with emergence of carbonyl (~1720 cm^{-1}) and unsaturated C=C (~1640 cm^{-1}) signals. (B) Comparative FTIR spectra of commercial PVC wastes, where similar changes are observed but with reduced intensity, reflecting the influence of additives and fillers.

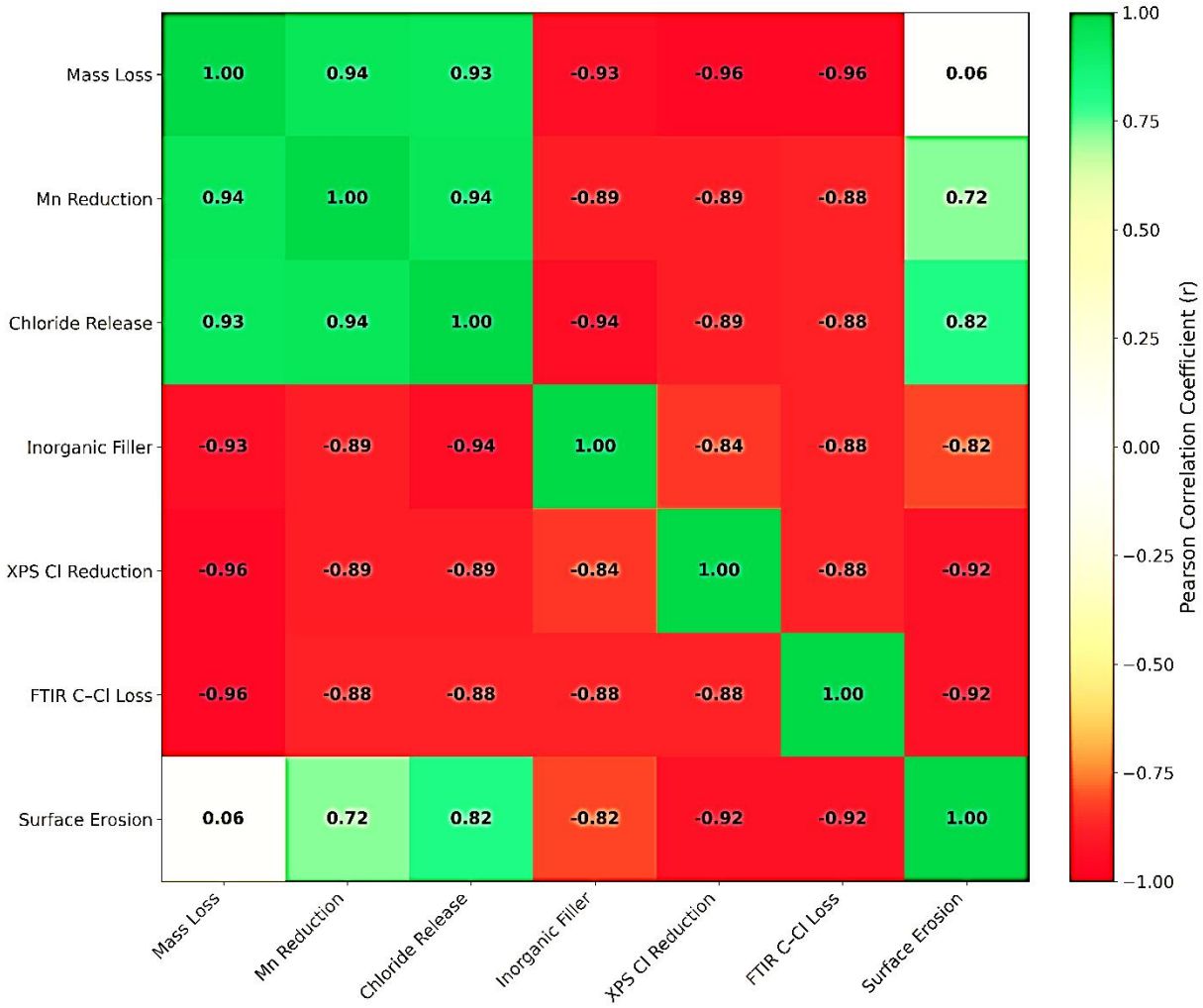


Figure 9: Pearson correlation heat map of measured parameters, with colors indicating correlation strength from −1 (red, negative) to +1 (green, positive). Numbers in each cell represent Pearson's r values, highlighting strong positive and negative interdependencies among variables.

Supplementary Files

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