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Formulation of a Chitosan-Laccase-Cutinase Composite for Bio-Coagulation and Enzymatic Degradation of Microplastics

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ABSTRACT

The widespread presence of microplastics in aquatic environments has emerged as a global issue because of their persistence and detrimental impacts on ecosystems and human health. Conventional methods for eliminating microplastics are frequently ineffective, expensive, and harmful to the environment. This research investigates the development of a sustainable bio-coagulant and biodegradation method for microplastics using chitosan-cutinase and chitosan-laccase based system. Chitosan, a natural biopolymer extracted from shrimp shells, acts as a biodegradable coagulant to enhance enzyme immobilization and aid in the aggregation of microplastics. Cutinase enzymes extracted from Fusarium oxysporum and laccase enzymes extracted from cabbage were utilized for their catalytic activity in breaking down the polymeric bonds present in microplastic particles. The formulated bio-coagulant's degradation performance was assessed through system testing. Findings indicated that the chitosan–cutinase and chitosan-laccase composite improved microplastic coagulation and showed notable degradation activity, validated by alterations in particle morphology using the UV–visible spectral analysis. Overall, the formulation offers a hopeful environmentally friendly option for reducing microplastic pollution by utilizing both bio-coagulation and enzymatic-degradation processes concurrently.

Keywords: Bio-coagulation, Chitosan, Enzymatic-degradation, Microplastics

INTRODUCTION

In recent decades, plastics have been used in almost every sector of the modern world. These synthetic polymers like polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) have been used due to their desirable properties such as low cost, light weight and high durability. However, the same properties also result in environmental persistence.[1] It is estimated that only 10% of plastic waste is recycled, 14% is incinerated, and the rest is dumped into landfills, ultimately entering the natural environment.[2] Nowadays, scientists have been formulating biodegradable plastics as a solution for plastic pollution and environmental sustainability. However, high amounts of conventional plastics are still accumulating in the environment for several years.

Microplastics, or known as plastic particles <5mm, have emerged as a contaminant of global concern.[1,2] Plastic is ubiquitous in all compartments of the environment,

including air, water, and soil. Nevertheless, microplastics have been one of contaminants that have generated intense public concern since its discovery in 2004.[1] An investigation conducted by Kosuth et al. (2018) recently shows the contamination of 81% of tap water with microplastics and synthetic fibers from 14 countries sampled around the globe.[3] Moreover, research by Yakovenko et al. (2025) has shown that human exposure to PM10 PE microplastics in indoor apartments has reached 76%.[4] These tiny particles are often small enough to pass through water filtration systems, flowing through the air, and we can then unknowingly ingest them. Microplastics interfere the production, release, transport, metabolism, and elimination of hormones, which can cause endocrine disruption and lead to various endocrine disorders, including metabolic disorders, developmental disorders, and even reproductive disorders (i.e., infertility, miscarriage, and congenital malformations).[5]

Among biological approaches for mitigation, the enzyme PETase, first identified in Ideonella sakaiensis, has shown a promising ability to hydrolyze PET into its monomers, terephthalic acid (TPA) and ethylene glycol.[6] However, organisms producing PETase are not only rare in natural environments, but they are also faced with many difficult challenges, such as their limitation to certain conditions. Large-scale production also remains technically and economically challenging.[7] Although engineered variants such as "FAST-PETase" exhibits improved stability and activity,[8] accessibility and deployment in real oceanic conditions remains limited.

Therefore, this study proposes the use of cutinase and laccase enzymes as alternatives to PETase. Cutinase, a versatile polyester hydrolase obtained from *Fusarium oxysporum*, can efficiently depolymerize PET and aliphatic and aromatic polyesters. Meanwhile laccase is a multicopper proteins that catalyze the oxidation of various phenolic and non-phenolic compounds via a radical-catalyzed reaction mechanism by the reduction of molecular oxygen, mainly found in fungi and *Brassica oleracea* (cabbage).[9,10] By combining these enzymes with chitosan, a natural biopolymer with strong coagulation capacity,[11] we aim to formulate a chitosan–laccase and chitosan-cutinase composite. We hypothesize that combining chitosan's coagulation ability with laccase's oxidative surface modification and cutinase's hydrolytic depolymerization will significantly accelerate microplastic aggregation and degradation in both freshwater and simulated seawater conditions.

MATERIALS & METHODS

The experiment was carried out on 14/9/2025 – 9/10/2025, at the Chemistry and Biology Laboratory of Immanuel Christian School, Pontianak. This study used some tools which consist of analytical balance, watch glass, petri dish, spatula, mortar and pestle, wire gauze, tripod, test tube, blender, pipette, micro-pipette, graduated cylinder, syringe, beaker, Erlenmeyer flask, filter funnel, spirit burner, thermometer, pH meter, laboratory oven, centrifuge machine, autoclave, magnetic stirrer, blender, UV flashlight, and incubator. In addition, the materials used in this study include Fusarium oxysporum, cabbage, shrimp shells, distilled water, acetic acid (CH_3COOH), sodium-alginate ($(C6H7NaO6)n$), sodium acetate ($NaCH_3COO$), activated charcoal, chloroform (CHCl3), methanol (CH3OH), pectinase powder, cellulase powder, potato dextrose broth (PDB), potassium chloride (KCl), Iron (II) sulfate ($FeSO_4$), glucose, 2-N hydrochloric acid (HCl), and calcium chloride ($CaCl_2$).

In addition, the reference used for this study in the literature review and data sources were acquired from former relevant research that were cited from research journals.

Chitosan Extraction

Shrimp shell waste was collected from the market and washed with distilled water.

Then, it is dried for 12 hours, and further dried in the oven at a temperature of 80˚C. The dried samples were later blended and sieved to 80-mesh sizes. The shrimp powders were then added with 3.5% Sodium hydroxide (NaOH) 1:5 (w/v), followed by stirring with heat for over 1 hour at 90˚C to separate protein from the shells. The residue was collected and washed to neutral pH to be then re-dried again at 60˚C for 4 hours in oven, resulting in chitin powder. The chitin powder was reacted with 100 mL 2-N hydrochloric acid with stirring at 90˚C to remove minerals. The residue was collected and washed to neutral pH and dried at 60˚C for 4 hours in oven. 5 grams of chitin powder were reacted with 50mL of 50% sodium hydroxide, followed by heating at a temperature of 100˚C for 80 minutes, and then were filtered and washed until neutral pH, and lastly to be then re-dried again with a temperature of 60˚C for 4 hours.

Laccase Enzyme Extraction

100 grams worth of cabbage was bought from the store, and it was prepared by cutting the outer and middle leaves into small pieces and rinsing briefly to remove dirt. The leaves were then blended with 200 mL of 0.05 M acetate buffer (pH 5.0) until liquified, followed by the addition of 0.1-0.2 g activated charcoal and further blending for 30-60 seconds to form a green slurry. The slurry was then filtered through a coffee filter into a chilled beaker (4°C) and centrifuged at 10 000 rpm for 15 minutes (4°C). If the extract appeared dark or brown, an additional 0.1-0.5 g activated charcoal was added, stirred on ice for 5-10 minutes and centrifuged again under the same conditions. The resulting supernatant, representing the crude laccase extract, was collected and used immediately stored at -20°C.

Cutinase Enzyme Extraction

A total of 50 g of apple peels is boiled in 150 mL acetate buffer pH 3.5 for 1 hour. Then, it is filtered on a filter paper, washed with distilled water, and dried in the oven at 60°C for 6 hours. The dried apple peels are then cut into small pieces using a blender and placed in a 200 mL beaker. The apple peels were extracted overnight in 100 mL chloroform-methanol (2:1) at room temperature. The layer containing cutin (a plastic-like powder on top of the mixture) was then separated from the solvent and other impurities. Cutin was treated with cellulase (5 g/L) and pectinase (1 g/L) in 150 mL 50mM acetate buffer pH 4.5 at 50°C for 3 h. A waxy like layer, cutin is scraped using a spatula, washed with distilled water, and filtered.

Production of Inoculum

A total of 150 mL of liquid medium containing 3.6 g potato dextrose broth powder (PDB), 75 mg KCl (Potassium chloride) 1.5 mg $FeSO_4$ (Iron (II) sulfate), and 3 g glucose was prepared in a 200 mL beaker. The liquid medium is transferred to 5 Erlenmeyer flasks, containing 30 mL each. The flask was then stoppered with cotton, covered with aluminum foil and then sterilized by autoclaving at 121°C and 15 psi for 15 minutes. After sterilization, the flask was cooled down until room temperature and once cooled, 3 mL of *Fusarium oxysporum* culture was aseptically added into the flasks using a sterile pipette, bringing the total working volume to 33 mL. The flasks are then incubated for 3 days at room temperature to allow adaptation. After 3 days, the inoculum is inoculated into a different batch of liquid medium, containing 0.6 g of apple peel cutin extract, replacing glucose. The inoculated flask was incubated for 96 hours at room temperature to allow cutinase enyzme production. After 4 days of incubation, the inoculum was added to 10 mL of acetate buffer at pH 5. Centrifugation is carried out 10,000 rpm for 15 minutes at 4°C. The crude enzyme will then appear as a light-yellow supernatant then stored in a sterile container.

Enzyme Immobilization

Enzyme immobilization was performed by attaching or entrapping enzymes within a

solid support or matrix. In this case, laccase and cutinase were immobilized in chitosan-alginate hydrogel beads. Immobilization was done to improve the overall enzyme stability, protect against denaturation, and prevent loss into the medium. It also allows repeated use of the same beads, making the process more cost-effective and sustainable compared to free enzymes. physically cross-linked chitosan hydrogel granules are loaded with either chitosan-laccase (product A; pH 4-6) or chitosan-cutinase (product B; pH 7-9). A 2% (w/v) chitosan solution was prepared by dissolving chitosan in acetic acid. The mixture was stirred using a magnetic stirrer at 40–50 °C for 30–60 minutes until a homogeneous solution was obtained. Subsequently, a 2% (w/v) sodium alginate solution was prepared by dissolving sodium alginate in distilled water with continuous stirring until fully dissolved. Finally, a 4% (w/v) $CaCl_2$ solution was prepared in distilled water and mixed thoroughly to ensure complete dissolution. These prepared solutions were then used for the hydrogel bead formation process.

Product A: In a 100 mL beaker, mix the 2% (w/v) sodium alginate solution and the 2% (w/v) chitosan solution at a ratio of 3:1. Separately, prepare 3 mL of acetate buffer (pH 5) and add 3 mL of laccase enzyme solution to it. Once combined, gradually mix this enzyme-buffer solution with the alginate–chitosan mixture while stirring gently to ensure uniform distribution of the enzyme throughout the polymer matrix.

Product B: In a 100 mL beaker, mix 2% (w/v) sodium alginate solution and 2% (w/v) chitosan solution at a 3:1 ratio. Then, prepare 3 mL of cutinase solution and mix it with 2.5 mL of Tris-HCl buffer (pH 8.5) for 5–10 minutes. Finally, combine the chitosan–alginate mixture with the enzyme solution and stir gently until a homogeneous mixture is formed.

Hydrogel Beads Formation

Draw the prepared solution into a syringe. Place the 2% (w/v) $CaCl_2$ solution on a magnetic stirrer and maintain gentle stirring. Hold the syringe approximately 1–2 cm above the beaker and slowly press the plunger to extrude the mixture drop by drop into the $CaCl_2$ bath. After all the mixture has been added, allow the formed beads to cure in the $CaCl_2$ solution for 30 minutes while stirring gently. This step facilitates ionic crosslinking, ensuring that the beads formed become firm, uniform, and mechanically stable. The crosslinked hydrogel beads were subjected to three consecutive washing steps to remove residual chemicals and impurities. In the first step, Product A beads were washed with acetate buffer (pH 5), while Product B beads were washed with Tris-HCl buffer (pH 8.5) at 4 °C to effectively remove any excess $CaCl_2$ remaining from the crosslinking process.

Sample Preparation

PE and PET plastic bottles were collected and cut into small film pieces (1 cm x 1 cm). For PE, 0.0213 g and 0.0205 g of plastic film was prepared in 2 different beakers and labelled PA1 (control) and PA2 (treatment). Similarly, for PET, 0.0237 g and 0.0260 g of plastic film were prepared in 2 different beakers and labelled PB1 (control) and PB2 (treatment). Each sample was heated in distilled water for 20 minutes to promote the release of microplastic fibers and then allowed to cool to room temperature. After cooling, the mass of plastic films was weighed and noted to observe microplastic presence. Afterwards, the plastic films were placed back into their respective beakers. Then, the microplastic particles were examined under a UV flashlight to observe the distribution.

Outcomes Assessment

Twenty pieces of crosslinked hydrogel beads containing laccase (Product A) and twenty pieces containing cutinase (Product B) were added to treatment beaker (PA2 and PB2). The mixtures were then incubated for seven days to allow enzymatic activity and interaction with the microplastics. After the incubation period, the degree of microplastic coagulation and the results of enzymatic

degradation were examined under a UV flashlight to observe visible changes and potential breakdown of the plastic particles. To further evaluate the environmental compatibility of the developed hydrogel beads, they were placed in a fish aquarium for several days to observe any potential effects on the aquatic ecosystem, including water clarity, fish behaviour, and overall bio-friendly properties of the material.

RESULT

Mass Loss Analysis

The mass of each plastic film sample recorded was used to observe changes in weight before and after heating. The results are summarized in Table 1. Based on the results, all samples exhibited a slight decrease in mass after heating, suggesting the formation of microplastic particles due to partial degradation or the detachment of surface layers from the plastic films. For Product A, sample PA1 showed a mass loss of 2.82%, while PA2 had a mass loss of 3.41%. On the other hand, for Product B, sample PB1 experienced a 2.11% mass loss, and PB2 showed a 2.31% decrease. A higher percentage mass loss indicates greater amount of microplastic formed during heating.

Table 1: Change in mass between product A and B

Product A - Mass Change in Grams (mean ± SD)				**Product B - Mass Change in Grams (mean ± SD)**			
Before Heating		**After Heating**		**Before Heating**		**After Heating**	
0.02090 ± 0.000566		0.02025 ± 0.000636		0.02485 ± 0.001626		0.02430 ± 0.001556	
PA1	PA2	PA1	PA2	PB1	PB2	PB1	PB2
0.0213 g	0.0205 g	0.0207 g	0.0198 g	0.0237 g	0.0260 g	0.0232 g	0.0254 g

Remark: Abbreviations: PA1: Product A fist sample; PA2: Product A second sample; PB1: Product B first sample; PB2: Product B second sample

UV Light Analysis

Microplastic samples were examined under 365 nm UV illumination to assess enzymatic degradation and coagulation activity. Observations focused on changes in fluorescence intensity, particle reflectivity, and spatial distribution following treatment. A reduction in fluorescence brightness or the presence of aggregated particles was interpreted as evidence of polymer surface modification, enzymatic degradation, or coagulation.

In the PA1 beaker (**Figure 1a**), the microplastics appeared as bright, reflective fragments under UV light, consistent with intact polymer surfaces. After seven days, PA1 showed no appreciable reduction in particle abundance or fluorescence, indicating minimal degradation in the absence of enzymatic treatment. A similar pattern was observed in the PB1 beaker. The baseline image (**Figure 1b**) showed bright and clearly visible microplastic fragments. After seven days, PB1 likewise demonstrated no notable changes, confirming the absence of degradation activity in the control group.

In contrast, the PA2 beaker (**Figure 1c**) exhibited a marked decrease in fluorescence intensity following treatment. The microplastics appeared less reflective, and visible aggregation around the hydrogel beads was noted, suggesting effective coagulation and surface modification. With the enzyme-immobilized chitosan beads, both PA2 and PB2 samples (**Figure 1d**) demonstrated clear reductions in visible microplastic quantity. The particles appeared less bright and less abundant compared to their respective controls. Additionally, clusters of microplastic aggregates were observed on the water surface, consistent with chitosan-mediated coagulation. These findings indicate that cutinase contributed to polymer degradation while chitosan enhanced coagulation, confirming the functional effectiveness of the composite beads in promoting microplastic reduction through combined bio-coagulation and enzymatic degradation.

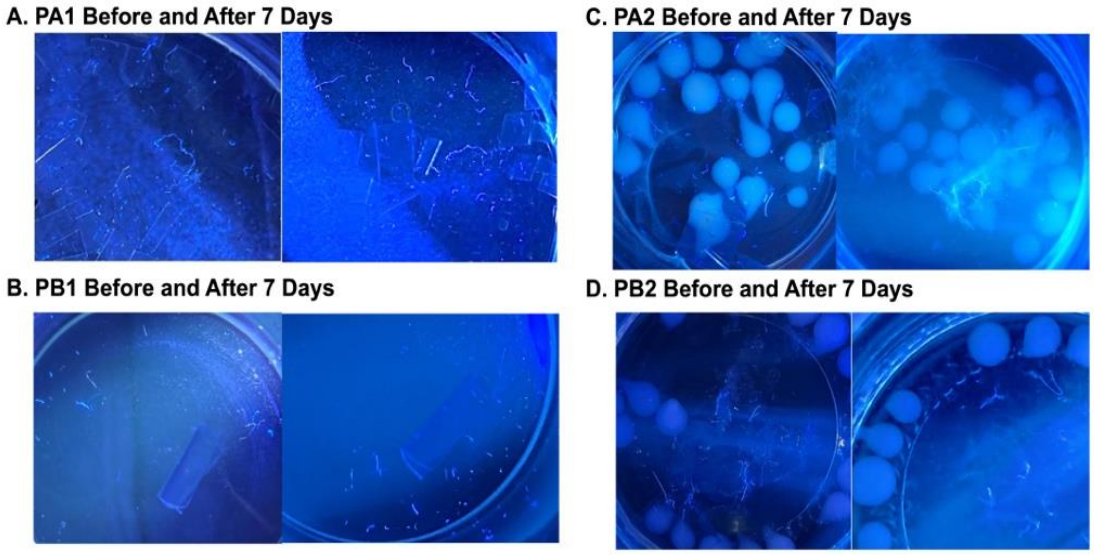

Figure 1. Ultraviolet light qualitative test results

Eco-Friendly Test

An eco-friendly test was performed in a fish aquarium for seven days to evaluate the environmental safety of the hydrogel beads (Figure 2). Acute toxicity tests on freshwater fish showed no mortality or behavioral changes after exposure to the hydrogel beads, indicating that the chitosan–enzyme hydrogel beads are environmentally safe and non-toxic to aquatic life.

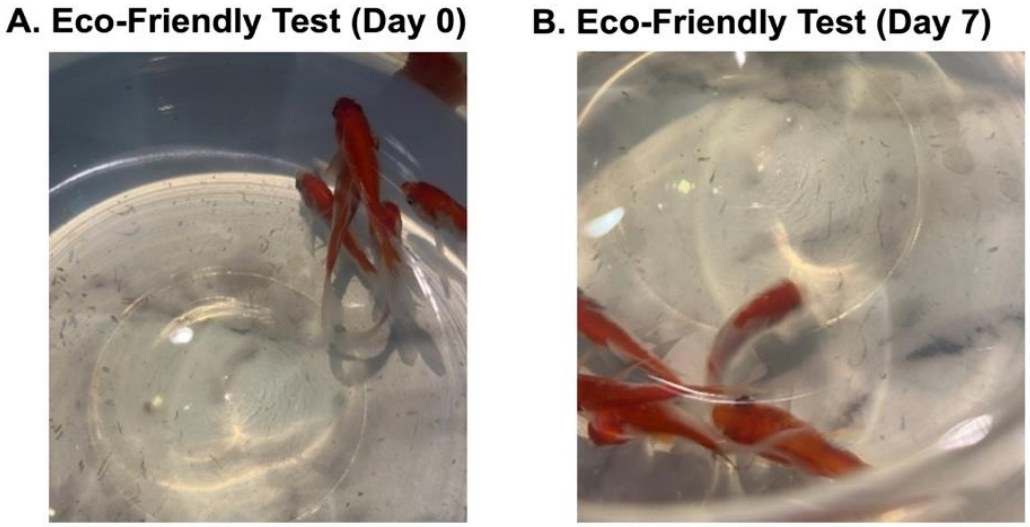

Figure 2. Eco-friendly test for acute toxicity

DISCUSSION

Microplastics have become pervasive in ecosystems and even human consumables, reflecting a global pollution crisis. Recent surveys found that 81% of tap water samples contain synthetic particles,[3] while indoor air can harbor extremely high levels (up to 68,000 inhalable microplastic particles per person per day).[4] PE is especially prevalent, constituting large amount of suspended microplastics in indoor environments.[12] Such exposure is not benign – micro- and nano-plastics are known to carry and leach endocrine-disrupting chemicals (e.g. bisphenols, phthalates), interfering with hormonal balance in mammals.[13] Chronic intake or inhalation of these particles has been linked

to oxidative stress and endocrine disruption, potentially leading to metabolic, developmental, and reproductive disorders.[14] This heightened awareness of microplastic ubiquity and toxicity underscores the urgent need for innovative, eco-friendly remediation strategies beyond conventional physical removal.

One promising approach is enzymatic biodegradation, inspired by the discovery of PETase from *Ideonella sakaiensis* that can depolymerize PET plastics.[15] Engineered PETase variants such as FAST-PETase exhibit dramatically improved activity (up to 38-fold higher at 50 °C) and can nearly completely depolymerize post-consumer PET in hours.[16] However, PETase-producing organisms are rare and require specific growth conditions, and scaling up enzyme production remains challenging.[17] As alternatives, cutinases from fungi have drawn attention for polyester degradation. For example, cutinases from *Fusarium* species can hydrolyze PET. *F. oxysporum* cutinase in particular shows measurable activity against low-crystallinity PET.[18] In laboratory tests, a cutinase from *Fusarium solani* achieved 5% weight loss of PET film over 4 days at 40 °C,[18] and with protein engineering it reached 90% conversion of PET to monomers in 10 hours.[19] These findings confirm that fungal cutinases can cleave the ester bonds of PET into soluble products like terephthalic acid and ethylene glycol.[18] On the other hand, laccase enzymes (multi-copper oxidases) have shown capability to attack the surface of durable plastics. Wood-degrading fungi such as *Pleurotus ostreatus* secrete laccase that can oxidize polymers like PE, PS, PVC, and PET, breaking long chains into shorter, less harmful fragments.[20] A novel thermophilic laccase (LfLAC3) was even demonstrated to degrade low-density polyethylene (LDPE) film over an 8-week incubation, evidenced by new carbonyl and oxidized groups in the plastic detected via Fourier-transform infrared spectroscopy (FTIR)/XPS and visible pitting under SEM.[21] Laccases typically work by generating reactive radical species that can oxidatively cleave C–C or aromatic bonds in polymers.[21] Given their complementary modes of action, hydrolases like cutinase targeting ester linkages in polyesters, and laccases introducing oxidative cuts in polyolefins. These enzymes present a compelling toolkit for biologically attacking diverse microplastic pollutants.

Our results demonstrate that combining these enzymes with chitosan, a natural biopolymer coagulant, can simultaneously aggregate microplastics and initiate their breakdown. Chitosan (derived from shrimp shell waste) is well-known as an effective, eco-friendly flocculant for microplastic remediation.[22] It carries abundant amino groups that become positively charged in acidic conditions, allowing it to bind and neutralize negatively charged microplastic particles, leading to agglomeration (polymer bridging) and settling or skimming of the debris.[11] In fact, prior studies have achieved over 68.3% removal of microplastics from water using optimized chitosan dosing.[11,23] Unlike conventional chemical coagulants (e.g. alum or polyaluminum chloride), natural biocoagulants like chitosan are non-toxic and biodegradable, causing minimal secondary pollution.[24] In our system, the chitosan–alginate hydrogel beads provided a solid support to immobilize the laccase or cutinase, preventing enzyme loss and allowing reuse. Enzyme immobilization is a known strategy to enhance stability and cost-effectiveness in bioremediation.[20] The beads not only localize enzyme activity around trapped microplastic clusters, but also act as macro-flocculants themselves. This dual function led to visible coagulation of microplastic fragments in the treated samples: within 7 days, fine plastic particles that were initially dispersed became clumped together, often adhering to or surrounding the hydrogel beads. We observed floating aggregates on the water surface in treated beakers (PA2, PB2), whereas untreated controls retained evenly distributed, free-floating micro-debris. This

outcome is consistent with the coagulative removal mechanism reported for chitosan and other natural coagulants, which form large flocs that can be readily separated from water.[11,23] By gathering countless tiny particles into a few larger masses, the system facilitates downstream removal (e.g. filtration or skimming) and reduces the risk of microplastics escaping into the environment.

In tandem with biocoagulation, the enzymatic degradation activity in our composite produced qualitative signs of polymer breakdown. Under 365 nm UV illumination, control microplastic samples fluoresced as bright, intact fragments, whereas enzyme-treated samples showed markedly dimmer and fewer fluorescent specks. This diminished UV reflectivity suggests that the enzyme-laden beads modified the plastic surfaces or partially degraded the polymers, reducing their ability to scatter or emit under UV. In the PE sample treated with the laccase–chitosan beads (Product A), the once-shiny fragments appeared dulled and clumped, indicating oxidative surface modification by laccase along with chitosan-induced flocculation. Oxidative enzymes like laccases can introduce carbonyl or hydroxyl groups into polyethylene chains,[21] which not only make the polymer less hydrophobic but also quench fluorescence of certain additives or dyes present in the plastic. Similar observations have been made in prior work where laccase action on polyethylene generated new oxygen-containing functional groups (detected by FTIR) and a loss of the polymer's original gloss. In the PET sample treated with the cutinase–chitosan beads (Product B), UV imaging likewise revealed a reduction in bright microplastic fragments compared to the untreated PET control. Cutinases cleave the ester bonds in PET, potentially producing soluble oligomers or monomers[18]; although these small molecules are not directly visible under UV, their release would correspond to a loss of solid particle mass. While the mass change in our samples was small (on the order of 2–3% after the 7-day treatment), this is in line with other short-term enzymatic degradation experiments. For instance, Ronkvist et al. reported only about 5% weight loss of low-crystallinity PET film after 96 hours of cutinase treatment at 40 °C.[25] The partial degradation observed in our study is thus realistic given the limited time and moderate conditions. Importantly, even a modest degree of polymer chain scission can roughen the microplastic surfaces and produce polar fragments, which tends to weaken the plastic's structural integrity and could accelerate subsequent biodegradation by environmental microbes. It was also noted that many of the enzyme-treated microplastic bits became attached to the hydrogel matrix or to each other, forming composite aggregates. This likely reflects the synergy between chitosan's physical binding and the enzymes' chemical alteration of the particles. As the enzymes erode the polymer surfaces (creating functional groups or tiny cracks), the chitosan can more easily adhere to and bridge the particles, enhancing floc formation. In turn, by holding microplastics in close proximity, the chitosan matrix may increase the local enzyme concentration around the particles, potentially improving the catalytic efficiency compared to free, dispersed enzymes. This synergistic interplay is advantageous for tackling microplastics, which often require both concentration (to remove dilute pollutants from water) and depolymerization (to truly eliminate the plastic). Our findings validate that a combined bio-coagulation and enzymatic degradation strategy is feasible: the chitosan–enzyme composite not only gathered microplastics into removable flocs, but also initiated their breakdown at the molecular level. Overall, the chitosan–laccase and chitosan–cutinase composites developed in this study demonstrate a multi-pronged solution that leverages biopolymer chemistry for coagulation and enzymatic catalysis for degradation. This integrated approach could be particularly useful for water treatment applications, where

coagulation-flocculation is already a standard step by infusing it with biodegradative capability, microplastics might not only be removed from water but also begin to be detoxified in the same process. The fact that our hydrogel beads remained intact and effective throughout the tests and showed no acute toxicity to aquatic life is a promising indication of real-world applicability. They could potentially be deployed in filtration units, settling tanks, or even in natural water bodies (e.g. as slow-release bioremediation capsules) to trap and degrade microplastics in situ.

While our findings are positive, the degradation achieved was partial and primarily surface-level. A significant fraction of the microplastics remained undegraded after the 7-day treatment, which is not unexpected given the complexity of crystalline polymers and the short duration. Future studies should incorporate more quantitative analytics, for instance, FTIR or gas chromatography–mass spectrometry (GC–MS), to track chemical changes in the plastics and detect any released monomers. Additionally, optimizing the enzyme loading and improving enzyme–substrate contact will be crucial. However, co-immobilization must account for each enzyme's optimal pH and conditions. One possible solution is a sequential reactor or layered hydrogel that compartmentalizes the enzymes but still yields a one-pot treatment. Testing the composite beads in different environmental conditions, such as real wastewater or seawater, would provide insight into their performance amidst potential inhibitors like salts, organic matter, or biofilms. By iteratively improving these aspects, the bio-coagulation and enzymatic degradation approach introduced here can be refined into a potent, green solution for mitigating microplastic pollution on a larger scale.

CONCLUSION

This study successfully formulated chitosan-laccase and chitosan-cutinase hydrogel beads composites that exhibit potential for microplastic degradation and bio-coagulation. It is indicated by the decreased fluorescence intensity and particle distribution after treatment. The laccase composite (Product A) promoted oxidative surface modification and aggregation, while the cutinase composite (Product B) initiated a hydrolytic cleavage of polymer chains. Although the degradation was partial, the combined biopolymer-enzyme system proved to be an eco-friendly and sustainable strategy for mitigating microplastic pollution.

Declaration by Authors

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