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Article

Optimized biochemical depolymerization of plastics from surgical face masks

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SUMMARY

The plastic component of surgical masks containing polypropylene is posing an environmental threat owing to the overuse of masks with non-biodegradable layers all over the world. The non-biodegradable layer of masks can pose serious environmental problems. There are a few reported chemical, enzymatic, and microbial-based methods known to depolymerize plastics; however, these individual processes may be slow. There is a possibility that chemicals may work together with enzymes and microbes and act as catalysts for depolymerization. Hence, we hypothesized that chemicals, enzymes, and microbes may act synergistically to degrade plastic at a higher speed. To evaluate the main and interactive effects of processes meant for the degradation of plastics, we tested the effects of enzymes, microbes, and zinc oxide on the degradation of plastics in a 1.5 L bioreactor and studied the synergy between these three processes using a two-level, three-variable factorial design. The results indicated a significant interactive and synergistic effect between ZnO and the microbial mix, which could accelerate the depolymerization reaction. These experiments helped us to conclude that depolymerization of polypropylene can be done prior to its disposal to the environment using a combination of chemical and microbial mix.

INTRODUCTION

The COVID-19 pandemic has affected roughly 420 million people worldwide directly and billions indirectly (1). In addition to vaccines, wearing masks and social distancing are the two best practices that can protect people from COVID-19. The use of masks has led to an increased demand for single-use plastic-containing surgical masks and thus has contributed to the prevalence of plastic waste (2). The World Health Organization (WHO) has indicated that 89 million surgical masks per year are needed in the United States, and these numbers can be higher for other parts of the world (1, 2). Surgical masks have three layers: the outer nonwoven fabric layer, the middle plastic polymer layer, and the inner soft nonwoven fabric layer (3). Masks get littered after use, and approximately eight million tons of such COVID-19 pandemic-related plastic waste have been generated annually (4). The number of N95 masks with plastic layering required in the United States would be about 7.4 billion, for 6.4 billion US dollars per year (5). This would lead to 84 million kilograms of waste. The plastic generated from this waste will take more than 400 years to degrade (6).

Authorities have been under huge pressure to come up with solutions for waste management to address this issue, as we might be sitting on a potential environmental pandemic that can affect a great deal of terrestrial and marine environment, and thus, we need to proactively act to find scientific solutions (7-10). Plastics break down in the environment through four main processes: photodegradation, thermo-oxidative degradation, hydrolytic degradation, and biodegradation by microorganisms (11). It is important to note that the depolymerization of plastic is the first step in the degradation of plastics. The purpose of this project is to evaluate the effects of chemical, enzymatic, and microbial processes of depolymerization of the plastic from the surgical masks (11-13).

It has been reported that one can use zinc oxide (ZnO)-based photocatalytic reactions to carry out chemical degradation due to the ability of ZnO to absorb photons from light sources and become photoexcited, which in turn results in the generation of radical molecules such as hydroxyl and superoxide. These reactive oxygen species can damage polymer chains, thus causing depolymerization (12-13). A promising strategy for plastic degradation involves microbial and/or enzymatic methods. This process generally includes microbes attaching to plastics and secreting enzymes that break down the plastic into monomers or convert it into carbon dioxide, water, and new biomass. Mohanan et al. reviewed recent advancements in the microbial degradation of synthetic plastics and provided an overview of the enzymes involved in this biodegradation process. (14). Several *Bacillus*, *Pseudomonas*, and fungal species have been reported to participate in plastic degradation (15,16). Among enzymes, esterases such as cutinase first degrade polymers into monomers or short chains, which are then transported to cells for complete oxidation and get used up as substrate for catabolic degradation (17). Many enzymes are produced by microbes, so by saving the time needed for production, the direct use of enzymes may accelerate the depolymerization process.

Degradation rates of plastics, characterized by degradation rates and estimated half-lives of plastics types, depend a lot on environmental factors such as heat, moisture, light, microbial action, and oxygen level (18). Three processes studied here (chemical, microbial, and enzymatic) for polypropylene depolymerization also depend a lot on experimental conditions and exposure of enzymes and microbes. The natural conditions for enzymes and microbes are not always favorable, and this might lead the process of depolymerization to be very slow (18). Hence, it is important to study the synergy between these processes, which might help to accelerate the degradation. Previous research has focused on either chemical or micro-

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enzymatic degradation of waste plastics using microbes such as *Pseudomonas* and enzymes such as cutinase. Studies on the synergy of plastic degradation strategies, particularly at the depolymerization stage, are limited. There are reports of the use of microbes and enzymes in combination to get synergistic degradation (19). However, there are no reports on coupling chemical mechanisms with microbial and/or enzymatic mechanisms.

It is more likely that one independent mechanism might not give us the desired results of rapid depolymerization. We hypothesized that if we deploy micro-enzymatic treatment before chemical photo-oxidation, it will make polymers more prone to accelerated degradation, as well as their eventual degradation owing to reactive radicals. In this project, we tested chemical (ZnO), microbial, and enzymatic methods of degradation individually and then in combination using a statistical two-level, three-variable factorial design. Eight variables were identified as major ones that influence depolymerization. Five variables (aeration, light, agitation, temperature, and incubation time) were kept fixed, and three, namely ZnO concentrations, microbes, and enzyme dose, were studied for interactive effects. The results gave us the optimum combination of three factors, which works faster than one factor or method at a time. This can help us to deal with potential environmental pandemics associated with the overuse of plastic-containing masks in a better manner.

Results

In this study, the depolymerization reactions were carried out in a bioreactor under continuous stirring and aeration (**Figure 1**). To monitor the depolymerization process, samples were collected from the reactor for microscopic and near-infrared (NIR) evaluation every 12 hours for the first 48 hours and then every 24 hours until the end, as we observed slow degradation. The NIR evaluation was performed using a self-built NIR spectroscope (**Figure 2**). Near-infrared spectroscopy (NIRS) is a method that uses the near-infrared region of the electromagnetic spectrum (from 780 nm to 2500 nm). Polymers used in plastics have unique NIR spectral fingerprints related to the presence of functional groups. These characteristic spectral peaks can be used for quantitative measurements and qualitative assessment of plastics.

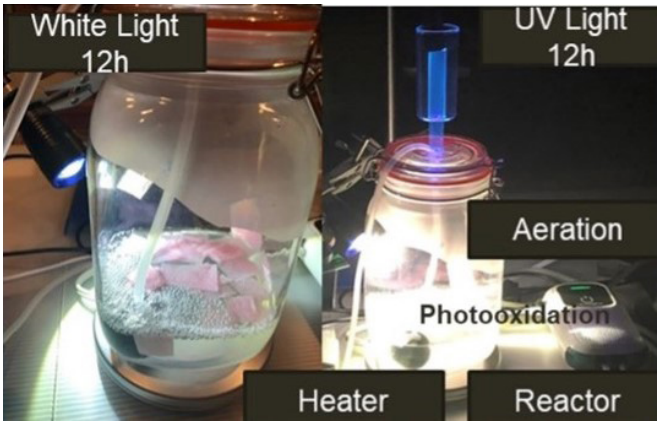

Figure 1: Bioreactor set up to study depolymerization reaction. The bioreactor consists of Jillmo's 1.5 L fermentation jar with fermenting weights and airlocks coupled with an air pump, a warming plate, a magnetic stirrer plate, and a battery aquarium air pump.

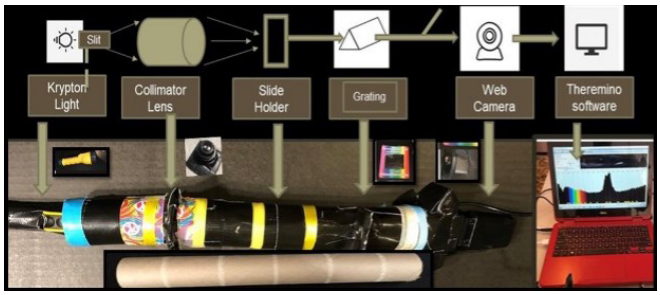

Figure 2: Homemade NIR spectroscope used for experiments. NIR spectroscope was built using a bathroom tissue roll, a krypton flashlight as a light source, a collimator lens, a grating prism, and a web camera coupled with the open-source Theremino software.

The effects of ZnO depolymerization were first investigated (**Figure 3**). We observed a peak at 1212 nm for untreated polypropylene, so the peak height at 1212 nm was used to qualitatively compare the samples for the degree of depolymerization (**Figure 3A**: upper panel)**.** Nine days into the ZnO-assisted depolymerization, new peaks emerged in the NIR spectrum, suggesting the formation of free hydrocarbon molecules (**Figure 3A**: middle panel). The nature of these molecules may need further investigation. In parallel, microscopic images indicate that from day 8 onwards, the morphology of polypropylene changed: the threads appeared more melted and not as intact as can be seen at the beginning of the experiments.

Then, we examined the changes during the subsequent microbial and enzymatic degradations (**Figures 4-5**). During microbial and enzymatic degradation, mineralization of the monomers occurs and releases end products such as CO_2, H2O, CH4, N2, and various other metabolic products (20). The emergence of a new peak in NIR spectra after 72 hours at 957 nm and 1151 nm suggests the formation of water and free hydrocarbon molecules, respectively (**Figures 4-5**).

We observed the emergence of a peak at 1157 nm, which was concomitant with the disappearance of peaks at 1212/1232/1253 nm, which are also typical NIR peaks for intact polypropylene. The peak at 1157 nm more distinctly displayed an upward trend with each passing day and was thus used for quantitative measurement of depolymerization. We used percent absorbance as a measure for depolymerization (**Figure 4**). The new peaks detected may need further investigation with more sensitive tools like Fourier transform infrared spectroscopy (FTIR). Microscopic pictures indicate that from day 8 onwards, the morphology of polypropylene changed.

The individual main effect, interactive effect, and three-way interactive effect between three factors were evaluated based on numerical numbers (effect) calculated in the factorial experiment (**Table 1**). The results of the main effects indicate that microbial and enzymatic processes can depolymerize polypropylene faster than chemical processes, as their effects were both higher than ZnO at day 9. All three variables had noticeable main effects, with microbial treatment being the most effective of all at day 25. It is most likely that these three processes may come into action simultaneously. Hence, the interactive action was further studied. Results show a significant interactive effect and synergy between ZnO and microbial treatment, especially at higher concentrations of

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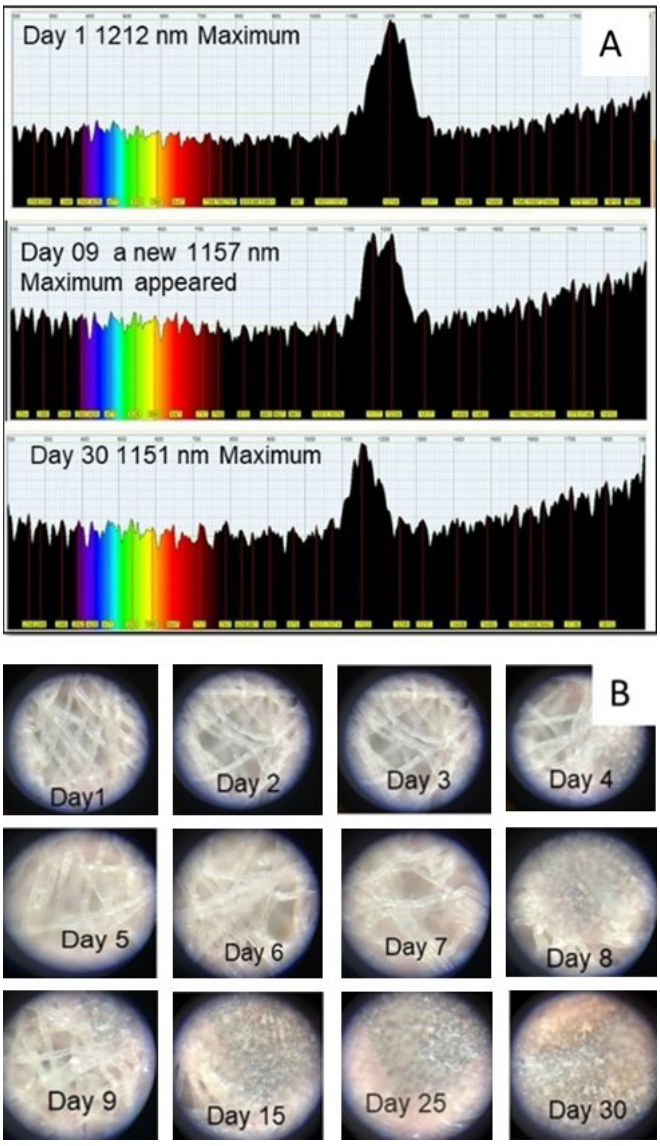

Figure 3: Tracking of depolymerization reaction from sample set taken out from chemical depolymerization reaction. A) Changes in NIR spectra of polypropylene depolymerized using zinc oxide for one month in the bioreactor. **B)** Microscopic images of depolymerized polypropylene.

both factors. The effect was visible both on day 9 and day 25. Interestingly, an antagonistic effect, as represented by the negative E value of the three-way interactive effect (EZnO.Enz. Mic) between the three factors, was also observed (**Table 1**). Further, NIR data suggest that on both day 9 and day 25, there was an improvement in depolymerization with both enzyme and microbial groups, wherein ZnO was used in the bioreactor prior to the addition of enzyme and microbial mix. We observed improved depolymerization with microbial and enzymic mix on day 25 (**Figure 6**). These data allow us to conclude that a combination of chemical and microbial/enzymatic reactions will significantly improve depolymerization reactions.

Discussion

NIR spectroscopy is a common tool that is used to monitor microplastics in various matrices. In this study, an NIR spectroscope was prepared at home and was used to monitor the depolymerization reaction. Literature reports suggest that the NIR spectra of polypropylene show classic peaks at 1200, 1400, and 1700 nm (21-26). The appearance of new peaks upon depolymerization and the concomitant decrease of the peaks at 1200 nm suggests that some changes happening at a molecular level, which is beyond the scope of this work, led to depolymerization. The absorption peaks can be attributed to the methylene group (1700 nm), water (957 nm), C-H stretching overtones (1151 and 1157 nm), C–H second overtone, the C–H combination, and the C–H stretch first overtone (1200, 1212, 1232, 1253, and 1400 nm) (22-28). In our study, with the increase in depolymerization, it was observed that 1157 nm intensity increased with time.

Previous studies on microplastic degradation using NIR spectroscopy have shown similar trends in the appearance and disappearance of characteristic peaks, with variations in the position and intensity of specific peaks. These studies have

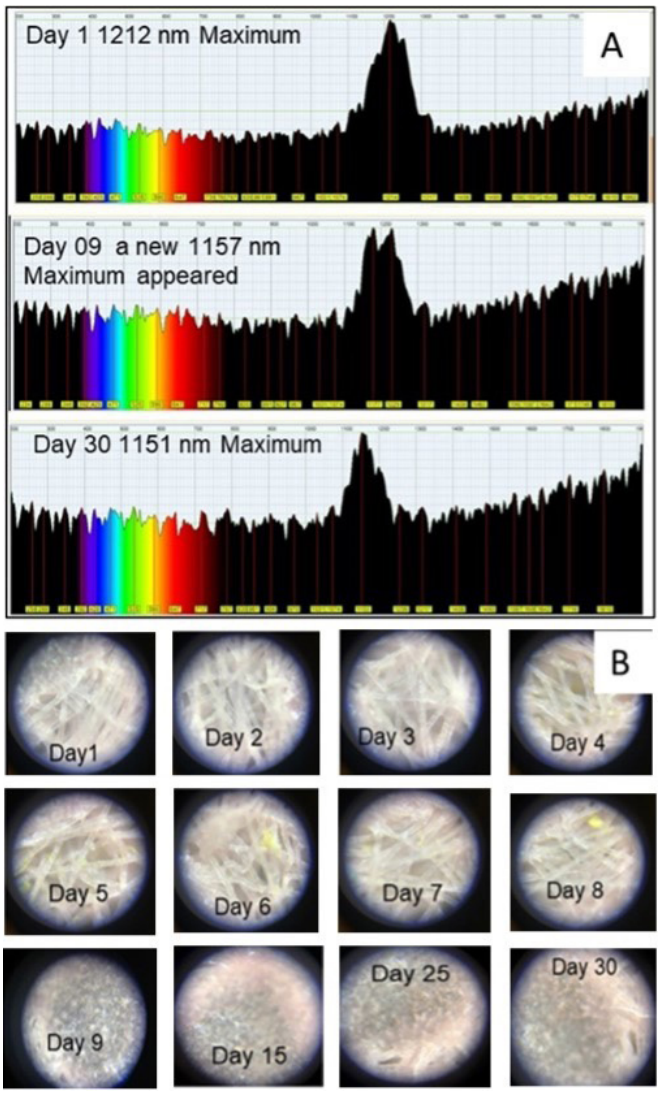

Figure 4: Tracking of depolymerization reaction from sample set taken out from enzymatic depolymerization reaction. A) Changes in NIR spectra of polypropylene undergoing enzymatic depolymerization reactions in bioreactor and the concomitant. **B)** Microscopic images of depolymerized polypropylene.

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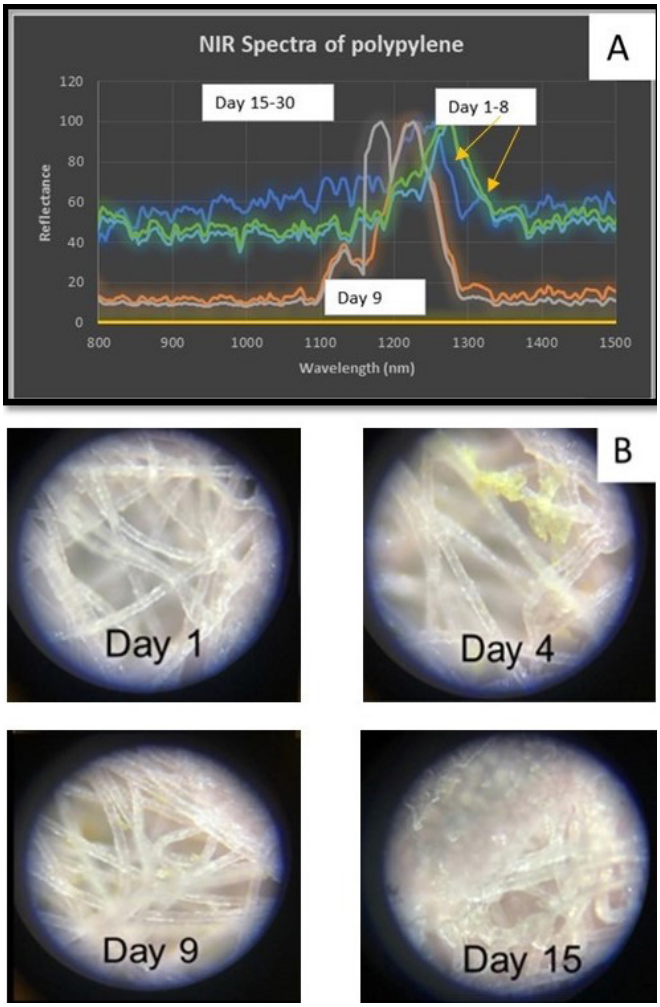

Figure 5: Tracking of depolymerization reaction from sample set taken out from microbial depolymerization. A) Changes in NIR spectra of samples taken on days 1, 4, 8, 9, and 15-30 from the and the microbial depolymerization reactions in the bioreactor. **B)** Microscopic images as observed on days 1, 4, 9, and 15.

observed similar peak changes in the degradation of plastic waste materials, validating the use of NIR spectroscopy for monitoring polymer degradation (28). Additionally, consistent peak shifts in long-term studies have supported the reliability of NIR spectroscopy for predicting the age of plastics (29). Chen et al. reported variations in peak positions and intensities due to the presence of bioplastics, demonstrating the versatility of NIR spectroscopy in polymer analysis (30). However, these studies were conducted over extended periods, often up to 30 years, and under non-laboratory conditions such as landfills (28-30). In contrast, the current study focuses on laboratory conditions and specifically on the depolymerization of polypropylene.

We used ZnO for the chemical treatment of plastics. ZnO facilitates the cleavage of molecules into smaller fragments containing hydrophilic oxygenated groups that can be easily degraded by microorganisms in the environment (12-13). This reaction can produce byproducts like formaldehyde, acetaldehydes, acetone, and butanal, which are useful for chemical industries. (27, 31). The use of stand-alone chemical reactions might need additional steps to dispose of offside products. Hence, it is important to couple chemical reactions with enzymatic and microbial processes so that byproducts can be used as energy sources. Enzymatic degradation is also a promising strategy for depolymerization of propylene. Cutinase (α/β-hydrolase) is the main enzyme known to degrade polypropylene (32). To study the effect of enzymes of polypropylene, we added a food-grade multi-enzyme complex to polypropylene. Some of these enzymes are expected to have sequence homology with enzymes such as cutinase. We observed depolymerization phenomena with this enzyme mix.

In this study, the simultaneous use of chemical, enzymatic, and microbial processes was evaluated for their feasibility at the pilot and scaled-up fermenter levels. Using a factorial design of the experiment, we demonstrated the synergy between chemical and microbial depolymerization. The factorial design provided several advantages over the traditional one-factor-at-a-time approach. It was efficient and capable of identifying both main and interactive effects. This method had never been applied to study the depolymerization of polypropylene by previous researchers.

The underlying mechanism of synergy is beyond the scope of this study. Still, we speculated that with chemical treatment under photooxidative conditions, reactive oxygen species might make polymer chains a bit more unstable and, thus, more prone to microbial and enzymatic attack (33). It is more likely that the enzymatic mechanism may work better than the microbial process as reactive species may also kill microbes, and that might be the reason for the antagonistic effect observed during three-way interaction (32). It will thus be critical to optimize the timing at which microbes and enzymes should be added to the reaction mixture. This, however, needs some detailed investigation. In addition to that, further study should be expanded to anaerobic microbial degradation as sometimes the environmental conditions are anaerobic. Also, despite sterile conditions at the beginning of the experiment, the sterile conditions cannot be guaranteed in home settings and thus can be considered as one of the limitations of this experiment. Although we used a microscope to ensure that there was no visible contaminant growing in the reactor, it would be better to perform the experiments in laboratory conditions.

In this study, using a bioreactor and NIR spectroscope, we demonstrated that depolymerization of polypropylene can be done prior to its disposal to the environment using chemical, enzymatic, and microbial methods. The depolymerization can be synergistically accelerated by using a combination of chemical, enzymatic, and microbial methods. We envision a process where microbes can colonize plastic surfaces and secrete enzymes that can then catalyze the depolymerization of polypropylene into smaller molecules that microbes can further metabolize. Chemical agents can enhance this process

Experiment	Effect	**Response** (ABS at 1157 nm Day 9)	**Effect Day 9**	**Response** (ABS at 1157 nm Day 25)	**Effect Day 25**
1	E_{mean}	0.11±0.09	4.34	0.13±0.09	5.83
2	E_{ZnO}	0.14±0.10	-0.98	0.40±0.08	-0.55
3	E_{Enz}	0.47±0.13	-0.32	0.62±0.08	-0.49
4	$E_{ZnO,Enz,}$	0.34±0.12	0.68	0.45±0.06	0.53
5	E_{Mic}	0.58±0.19	-0.17	0.85±0.08	0.17
6	$E_{Mic:ZnO}$	0.61±0.12	1.00	0.91±0.11	0.99
7	$E_{Enz:Mic}$	0.61±0.09	0.34	0.72±0.12	0.17
8	$E_{ZnO,Enz,Mic}$	0.59±0.07	-0.78	0.88±0.01	-0.33

Table 1: Results of the factorial experiment effect were calculated on day 9 and day 25.

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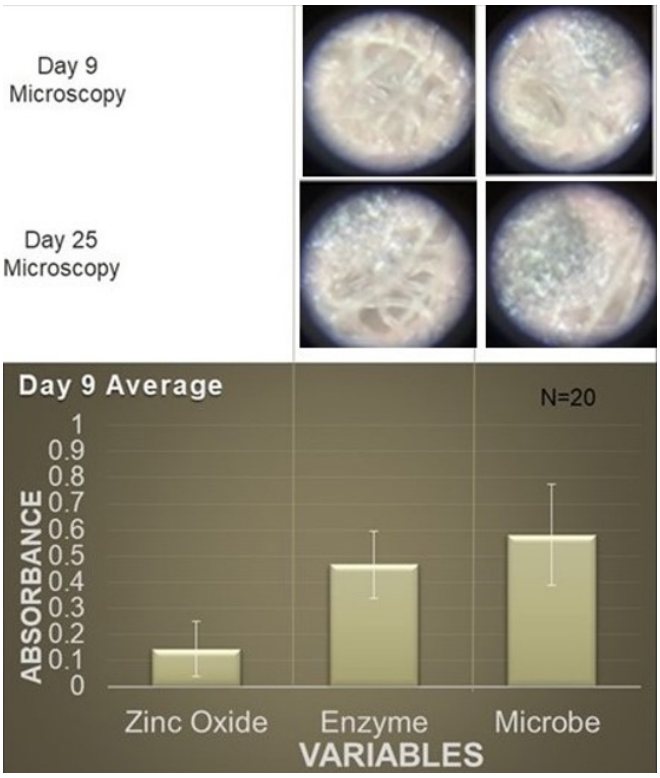

Figure 6: Changes in the polypropylene sample observed during the factorial experiment. A) Microscopic images of samples from days 9 and 25. **B)** NIR spectroscopy data measured at 1157 nm with ZnO was compared with groups with added enzyme and microbes after factorial experiments.

by creating reactive oxygen species that help break down the plastic structure, making it easier for enzymes to act. Our findings suggest that enzymes, microbes, and zinc oxide can work together to expedite the breakdown of polypropylene, offering a promising approach to mitigate plastic waste. Future research should focus on optimizing the timing and conditions for the addition of microbes and enzymes to maximize the efficiency of the depolymerization process. Additionally, expanding the study to include anaerobic microbial degradation and improving the sensitivity of the NIR spectroscope will further enhance our understanding and application of these methods for other plastic contaminants.

MATERIALS AND METHODS

NIR spectroscope and microscopy

An NIR spectroscope was built at home using a bathroom tissue roll, a krypton flashlight as a light source in combination with a collimator lens, a grating prism, and a web camera coupled with the open-source software called Theremino (**Figure 2**). Polypropylene gave a distinct fingerprint spectrum with peaks at 1200, 1400, and 1700 nm, which were used for monitoring depolymerization reaction qualitatively (12-14). Raw spectra were processed to generate relative quantitation data based on the area under the curve. Microscopic digital pictures were also simultaneously taken by light microscope (Maxlapter microscope for Kids) with 10x and 40x objective lenses to qualitatively assess the depolymerizations. For all experiments and measurements, 12 time points were measured, and 4-12 data points were presented here.

Depolymerization experiments

The depolymerization experiments were done in multiple steps: 1) Pilot experiments with ZnO, enzyme, and microbes; 2) Chemical depolymerization: photooxidation of polypropylene by zinc oxide in the reactor; 3) Enzymatic degradation of polypropylene in the bioreactor; 4) Microbial degradation of polypropylene in the fermenter; 5) Design of experiment and factorial design for statistical synergy testing. Reactions were replicated a minimum of three times to ensure repeatability. Factorial experiments were the only experiments done in duplicates.

All depolymerization experiments were done with a Jillmo fermentation kit and a 1.5 L fermentation jar with fermenting weights and airlocks (**Figure 1**). The reactor was coupled with an air pump, a warming plate, and a magnetic stirrer plate (Anzeser with a max stirring capacity of 3000 RPM) as needed. The bioreactor was aerated using a Kedsum battery aquarium air pump. A few 1-inch square pieces of plastic portions of the face masks (BYD care single disposable 3-ply masks from BYD care store) were put in the reactor for different depolymerization reactions. The sterilized distilled water was used for the reaction, and the reactor was sterilized using an instant-pressure cooker.

Chemical depolymerization

Generally regarded as safe (GRAS) grade prooxidants of zinc oxide (10 mM, ZnO dissolved in ethanol) were added to the 1-inch square piece of polypropylene portions of the mask suspended in distilled water. The final concentration of ethanol in the mixture was 0.01%, which should not cause any protein aggregation (34). The mixture was exposed to visible and ultraviolet (UV) light each for 12-hour intervals for over a month. The reactor was placed on a candle warmer and maintained between 60-70°C. Day 1 was used as control, and samples were collected every 12 hours initially and then every 24 hours and were monitored using an NIR spectroscope and optical microscope.

Enzymatic depolymerization

Cutinase (α/β-hydrolase) is the main enzyme that is known to degrade polypropylene (32). An 18-in-1 food-grade multi-enzyme complex was added (1 capsule, nutracraft digest ezy #1) to the polypropylene portion of the mask suspended in distilled water. Some of these enzymes are expected to have sequence homology with cutinase. The mixture was stirred and aerated continuously. Day 1 was used as the control, and samples were collected every 12 hours initially and then every 24 hours and were monitored using an NIR spectroscope and light microscope.

Experiment	ZnO concentration	Enzyme load	Microbial load
1	Low (10 mM)	Low (1 capsule)	Low (1 capsule)
2	High (100 mM)	Low (1 capsule)	Low (1 capsule)
3	Low (10 mM)	High (2 capsules)	Low (1 capsule)
4	High (100 mM)	High (2 capsules)	Low (1 capsule)
5	Low (10 mM)	Low (1 capsule)	High (2 capsules)
6	High (100 mM)	Low (1 capsule)	High (2 capsules)
7	Low (10 mM)	High (2 capsules)	High (2 capsules)
8	High (100 mM)	High (2 capsules)	High (2 capsules)

Table 2: Experiment design for two-level three-variable factorial experiments for statistically testing main and interactive effect between variables (zinc oxide, enzyme load, and microbial load). Each experiment was done in duplicate.

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Microbial depolymerization

Zenwise digestive enzymes (Zenwise health store) which had a good mix of probiotic and prebiotic microbes (namely *Bacillus Subtilis, Bifidobacterium longum, Lactobacillus acidophilus, Biidobacterium animalis, Biidobacterium bifidum, Biidobacterium breve, Lactobacillus casei, Lactobacillus salivarius,* and *Lactobacillus plantarum)* was used as a source of microbes (1 capsule). The mixture was stirred and aerated continuously. Day 1 was used as the control. Samples were collected every 12 hours for 48 hours and then for every 24 hours and were monitored using an NIR spectroscope and light microscope.

Factorial design

Two-level, three-variable factorial experiments were designed using the approach described previously (35, 36). Eight variables were identified as major ones to influence depolymerization. Five variables (aeration, light, agitation, temperature, and incubation time) were kept fixed, and three variables, namely, ZnO concentrations, microbes, and enzyme dose were studied for synergy (**Tables 1-2**). The depolymerization started with the chemical process, and on Day 5 it was switched over to enzymatic or microbial processes (N=2). The synergy was studied using classical factorial design experiments at two levels.

The response for each experiment was assessed based on quantitative depolymerization of plastics as observed by an NIR spectroscope. Changes in spectral behavior and peak intensity at 1157 nm were used as an indicator of depolymerization, and raw spectra were processed to generate quantitative data. The change in the response from the 'minus' to 'plus' level was calculated as effect (E) of the variable under study. Eight experiments were designed (**Table 2**), and readings were taken at two time points: day 9 and day 25. Data from the factorial experiments were analyzed using a Yates algorithm to assess the relative significance of each variable, as described by Box et al. (35). This method was used to calculate both main and interaction effects.

The main effect in this design is how the independent variable affects the dependent variable (depolymerization parameters in this case). The main effect ignores the effects of all other independent variables. The effect of other variables is considered an interactive effect. The main effects of the quantitative variables (E_{ZnO}, E_{Enz}, E_{Mic}), the interactive effects of two variables ($E_{ZnO.Enz}$, $E_{Enz.Mic}$, *and* $E_{Mic.ZnO}$), and the three-factor interaction ($E_{ZnO.Enz.Mic}$). E_0 represents the average effect of eight experiments carried out in the 23 factorial design matrices.

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