Research Article

Bridging Abiotic Pretreatment and Microbial Action: *Bacillus cereus*-Mediated Biodegradation of Polypropylene for Sustainable Plastic Waste Management

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Abstract | Biodegradation was carried out in this work using *Bacillus cereus* (H23B00108), which was isolated from marine soil that was polluted with plastic and obtained from Tenneti Park in Visakhapatnam, India. To make commercial polypropylene (PP) beads more vulnerable to microbiological breakdown, they were pretreated with chemicals and radiation. Weight loss and physical structural characterisation using X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier Transform Infrared spectroscopy (FTIR) were used to track degradation. Over the course of six weeks, the *Bacillus cereus* isolate (H23B00108) degraded untreated PP by 3%, chemically pretreated PP by 17%, and combined UV and chemical pretreatment by 8.25%. Taken together, these results demonstrate that *Bacillus cereus* (H23B00108) has great promise as a PP degrader, lending credence to the idea that microbes can sustainably biodegrade plastic waste, which is great news for PP waste management.

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Introduction

The persistent nature of plastic waste has intensified its impact on environmental quality and aquatic ecological integrity. Plastic production was estimated to reach 390.7 million metric tons in 2021 (Statista, 2026) and is projected to virtually triple, reaching around 1124 million metric tons by 2050. Ubiquitous plastics and their variants are now an integral part of manufactured demand across all industries due to their benign impact on humans (Horton, 2022). With its exceptional packaging properties, polypropylene is quickly becoming one of the seven most used forms of plastic. The increasing use of single-use plastics, such as polypropylene, has exacerbated the worldwide plastic pollution situation. The non-biodegradability of PP materials and the 6.3% annual growth in PP production sales since 2013 raise serious environmental issues. Particularly during the COVID-19 pandemic, these PP polymers were

widely employed to make disposable items including packaging, rope, bottles, caps, fishing gear, carpet, strapping, drinking straws, and personal hygiene items like masks, gloves, and hairnets. Regrettably, the presence of polypropylene microplastics (MPs), which are shards of plastic smaller than 5 mm in diameter, can be caused by incorrect disposal and increased production of these PP plastics, which are worsened by natural processes. Microplastics can alter the distribution of energy and food, which in turn can reduce the generative productivity and physical fitness of marine organisms, and the widespread use of plastics in everyday life releases microscopic particles into the environment, which pose a greater threat to human health than environmental contaminants owing to direct contact. Microplastics have been found in a wide variety of environmental matrixes, such as soils, freshwater bodies, deep-sea sediments, and the air we breathe (Jeyavani *et al*., 2024).

New ways of dealing with plastic pollution are required because of its far-reaching effects. Not all solutions are without their flaws. Some plastics can't be recycled, burning plastics emits a cocktail of dangerous chemicals into the air, landfills take up valuable real estate that could be better used, and chemical treatments end up polluting our water supply (Ru *et al*., 2020). On the other hand, additional contamination issues may arise due to these technologies' inefficiency and limited treatment capacity. Because they are not biodegradable, PP materials in particular pose serious environmental risks. Research into novel approaches to plastic waste management is, hence, essential. A sustainable, eco-friendly, and long-term solution that doesn't hurt the environment is being considered thanks to new research that suggests microbes or enzymes may be able to biodegrade plastics (Milološa *et al*., 2022). Bacteria and fungi are just two examples of the microbes that engage in plastic biodegradation (Mohanan *et al*., 2020). Developing effective and sustainable solutions for plastic waste management may depend on our ability to understand and use microbial pathways for PP decomposition. Colonisation of the plastic surface by microbes initiates PP biodegradation by secreting enzymes that hydrolyse the ester linkages in the polymer (Sepperumal *et al*., 2014). The enzymatic mechanisms employed by these microbes are essential for overcoming the recalcitrant nature of polypropylene, characterised by its linear chain structure of propylene monomers that enhances its resistance to environmental degradation. The degradation process involves several steps, including microbial colonisation, enzyme-mediated breakdown, polymer fragmentation, and mineralisation.

This study set out to biodegrade PP beads purchased from a commercial source by treating them with a new strain of Bacillus cereus (H23B00108), which was found in Tenneti Park along the Visakhapatnam shoreline in India. Furthermore, the possibility of the strain forming a biofilm on a polypropylene surface was examined. Incorporating technical research such as FTIR, SEM, and XRD further confirmed that our indigenous strain could use PP as the only carbon source. In addition, pretreatment procedures such as UV irradiation, chemical oxidation, or a mix of the two to improve polypropylene degradation were also evaluated.

Materials and Methods

Collection of samples and isolation of microorganisms

The coastal soil at Tenneti Park along the Visakhapatnam shoreline was sampled at a depth of 15 cm, collected in glass vials, and kept in the laboratory for further examination. The coordinates of the park are 17.7486° N, 83.3424° E.

Characterisation of the isolated organism capable of degrading PP

Serial dilution and plating on Luria-Bertani agar were subsequent steps in isolating the enhanced cultures. The plates were kept at 30°C for 24-48 hours, and then colonies with different morphologies were selected and isolated by streaking them many times. The morphology of the bacteria was identified as Gram-positive using a staining process and a medical binocular microscope. The genotypic features of the potential bacteria were then identified. The most similar isolate was identified using the EzBioCloud 16S database, which compares partial *16S rRNA* gene sequences to previously described species.

Pretreatment of PP

The plastics supplier supplied the commercial-grade polypropylene (PP). Round beads with a particle size of 1 mm make up the PP. Prior to drying and weighing, the samples were disinfected with 70% ethanol. After being weighed, the PP was immersed in a 69% nitric acid solution for six days. To remove any possible contaminants, the beads were washed

with distilled water before being immersed in a 99.9% ethanol solution. They were then oven-dried at 70 °C for 30 minutes. Shorter exposure durations of 5, 15, and 30 minutes to 256 nm UV light were applied to a small number of PP samples that had been chemically pretreated in a UV chamber. Degradation is enhanced over 30 minutes because functional groups are formed to their fullest (Kong and Wang, 2015; Hamzah, 2025).

Biodegradation of PP by isolated bacterium

Based on KS M3100-1:2002 (Jeon and Kim, 2016), the biodegradability of PP was investigated. A rotary shaker incubator was used to cultivate pure bacterial strains in LB broth that contained tryptone, yeast extract, and sodium chloride at 30 °C for 24-48 hours until they reached the mid-log phase. The control group continued to use a flask containing LB broth inoculated with bacterial culture but did not contain PP beads. Afterwards, in conical flasks, 1% (v/v) of the culture was added to 500 mL of LB broth that already included 0.4 g of polypropylene (PP) beads. The beads were treated as follows: untreated, chemically pretreated, and UV + chemically pretreated. The incubation time was set at 30 minutes. A spectrometer (Genesys 50, Thermo Scientific, USA) was used to assess bacterial strain development using PP beads as a carbon source at an optical density of 540 nm (OD_{540}). The biodegradation experiments lasted for forty days at a temperature of 37 °C.

Weight loss determination and measurement of pH, total dissolved solids (TDS), and conductivity

After removing the residual PP microplastics from the medium using filtration, they were washed in a specific order with 70% ethanol to ensure that all cells and debris were removed. The samples were then oven-dried at 60 °C overnight, or until completely dry. Using an analytical balance with a readability of 0.0001 g (Sartorius ENTRIS 224-1S), the weight of the remaining polymer was measured to quantify the level of degradation (Mohan *et al.*, 2016). Using Equation 1, we calculated the percentage weight loss for PP microplastics.

$$\% \text{ Weight loss} = \frac{W_o - W}{W_o} \quad \ldots (1)$$

Where; W_0 is the initial weight of the polymer (g), and W is the residual weight of the polymer (g).

Characterisation studies of PP

Spectroscopic evaluation of PP beads using FTIR

Before and after a 40-day biodegradation period, FTIR spectra of PP films were collected (untreated, chemically pretreated, and UV+ chemically pretreated). The adherent bacterial biomass was then removed by washing the films sequentially three times with sterile distilled water and three times with 70% ethanol. The films were then oven-dried at 60 °C until they reached a constant weight according to the established procedure. The functional group analysis was carried out with the use of a Vertex 70v FT-IR spectrometer and a Hyperion 2000 microscope from Bruker in attenuated total reflectance (ATR) mode. The microscope had a field of view of 400 × 400 µm. FT-IR spectra were recorded by signal-averaging 32 scans across 4000–500 cm^{-1} at 4 cm^{-1} resolution; the quantified carbonyl index (CI) was calculated as $CI = A_{1710} / (A_{2918} + A_{2850})$.

Scanning electron microscopy (SEM) of PP beads

After the experimental time (40 days), the morphology of the degraded PP microplastics was examined using SEM. Their preparation for CO_2 drying in critical-point ovens included soaking in 5% glutaraldehyde for an hour, washing in 100 mM phosphate-buffered saline (pH 7.0), and dehydrating with 30-100% graded ethanol for 15 minutes. With the help of a sputter coater (MSP-1S, SHINKKU VD, Japan), a thin layer of gold was sputtered onto dehydrated PLA films. Following this, SEM EDS (SEM MODEL) pictures were produced (Sekhar *et al.*, 2016; Hamzah *et al.*, 2026a; Akpan *et al.*, 2026).

X-Ray diffraction pattern of PP

The PP crystallinity was examined using the PANalytical-X 'Pert' Pro X-ray diffraction technique. Cu Kα radiation (1.54060 Å) was applied, and the procedure was conducted at 45 kV and 40 mA with a θ/θ geometry. The divergence slit size was set to 0.4785°. A scan rate of 10 m^{-1} and a step size of 0.0170 were used to capture the XRD patterns, which ranged from 10 to 60°, at 25 °C. Total crystallinity was calculated by dividing the integrated area of the diffraction peaks (the crystalline component) by the integrated area of the full diffractogram (Sudhakar *et al.*, 2008; Hamzah *et al.*, 2023, 2026).

Results and Discussion

Molecular identification and phylogenetic characterisation

By utilising UV-Visible spectroscopy at 540 nm, the

bacterial growth curve was quantified. When exposed to PP carbon, the strain grew swiftly, as seen by the growth curve. Noticeable progress was made in just two days. After reaching its peak within five days, the bacterial population began a slow but steady drop that persisted for the following week. The growth of the bacterial degrading strain with respective time. It was determined that this particular strain belonged to the genus *Bacillus cereus* (H23B00108) based on its morphological traits. Biotechnology relies heavily on *Bacillus cereus* (H23B00108), a rod-shaped, aerobic, gram-positive bacterium with a unipolar flagellum. *Bacillus cereus* (H23B00108) was shown to have the highest potential to biodegrade PP films in this investigation. Primers BF27 (AGA GTT TGA TCC TGG CTC AG) and B765R (CTG TTT GCT CCC CAC GCT TTC) were used to amplify and sequence the *16S rRNA* genes. By comparing the incomplete *16S rRNA* gene sequences to the EzBioCloud 16S database, which includes all validly described species, the most similar isolate could be found. The evolutionary history was inferred using the neighbour-joining method (Saito and Nei, 1987). The phylogenetic tree is displayed in Figure 1. The evolutionary distances (Tamura *et al.*, 2004) were calculated using the greatest composite likelihood technique, with each site representing the number of base substitutions. This analysis involved 11 nucleotide sequences. All first-, second-, third-, and noncoding codon locations were covered. The partial deletion option was considered present in any region where site coverage was less than 95%. Thus, no location could contain missing data, unresolved bases, or alignment gaps smaller than 5%. In the end, 711 locations made it into the dataset. The evolutionary analysis was performed using the MEGA11 software (Tamura *et al.*, 2021).

Weight loss determination

Bacillus cereus (H23B00108) was likely involved in the biodegradation process that sped up the breakdown of PP. Throughout the 10-to 40-day duration, the PP film's weight dropped dramatically. One possible way to determine PP biodegradation is to measure its weight loss. *Bacillus cereus* (H23B00108) inoculation resulted in weight loss after 32 days (Figure 2). Consumption of PP reduces carbon content, which leads to weight loss in pretreated PP infected with *Bacillus cereus* (H23B00108). After 30 days of incubation, untreated polypropylene (PP) lost about 3% of its weight, demonstrating relatively little degradation. However, after being chemically pretreated, the breakdown of PP increased dramatically, reaching around 17% at 30-32 days. This suggests that the pretreatment greatly increased PP's susceptibility to degradation. A maximum weight loss of around 8.25% was noted around 30 days of incubation after combining chemical pretreatment with UV (30 min) exposure, demonstrating significant deterioration. In particular, in the chemically treated samples, Bacillus cereus (H23B00108) persisted on the surfaces of the PP beads and aided their breakdown (Andrady, 2017).

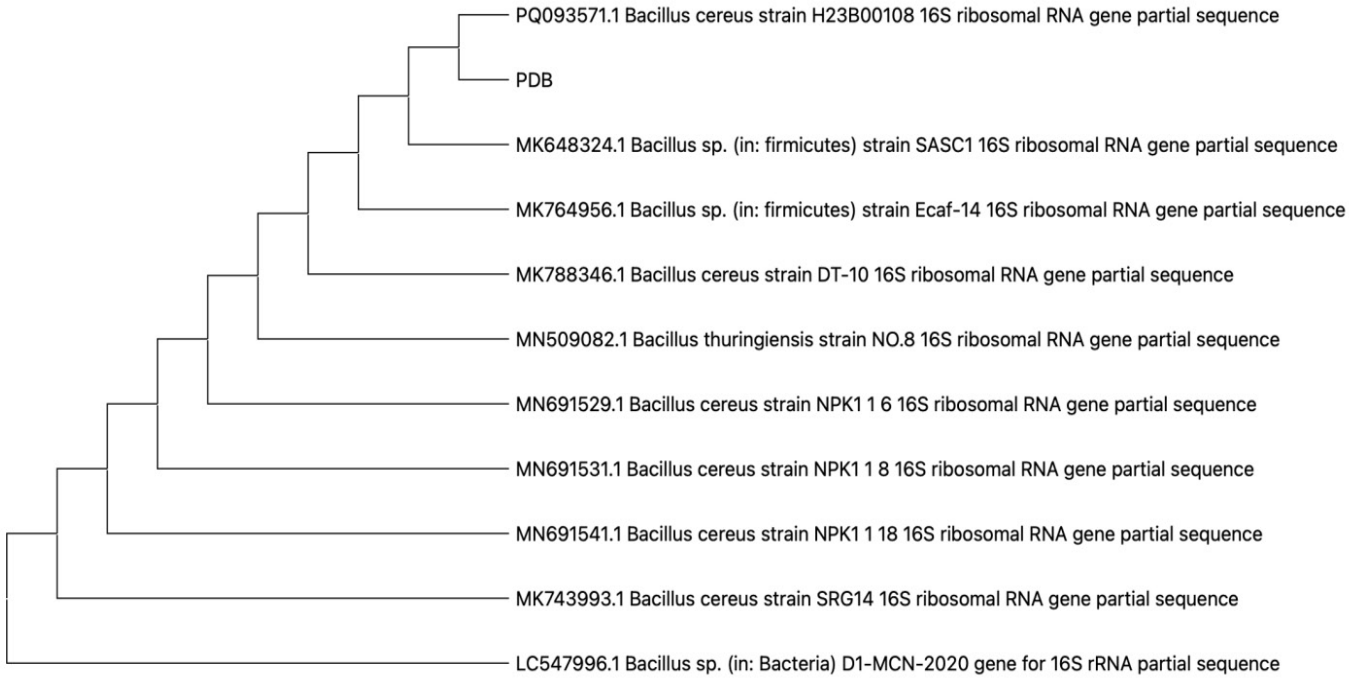

Figure 1: *Identification of PP-degrading bacteria through Phylogenetic tree.*

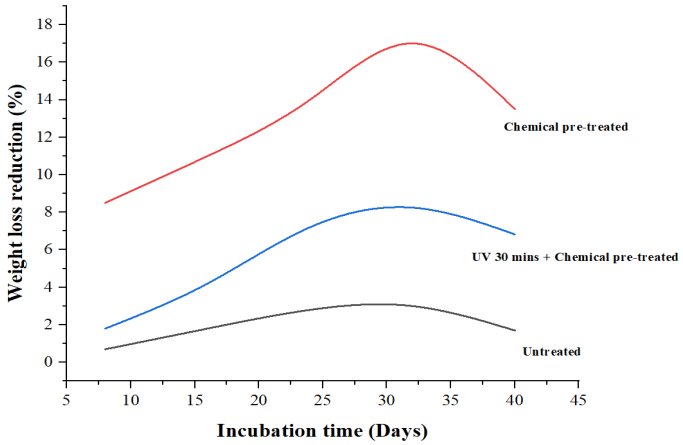

Figure 2: *Weight loss reduction % of PP that has been incubated with Bacillus cereus (H23B00108).*

FTIR spectrum of PP beads

The FTIR study presented strong molecular evidence that *Bacillus cereus* (H23B00108) microbes are causing chemical changes in polypropylene (PP) films.In order to analyse the chemical and structural changes in polypropylene (PP) films subjected to various treatments including untreated, chemically pretreated, and chemically pretreated combined with UV irradiation a series of important spectral shifts and intensity changes were observed in the films before and after biodegradation. These changes reflect the underlying biochemical alterations that occur during degradation. Several changes in peak intensity and band locations were noticed in the spectra, which were in the range of 4000-500 cm^{-1} (Figure 3). The presence of prominent peaks in the 2950-2850 cm^{-1} region of the FTIR spectrum of untreated polypropylene prior to biodegradation was ascribed to the symmetric and asymmetric C-H stretching vibrations of the methyl ($-CH_3$) and methylene ($-CH_2-$) groups, respectively. Polypropylene is characterised by CH_3 bending and deformation vibrations, which were detected at peaks at 1450-1375 cm^{-1}. These vibrations indicate the presence of a stable hydrocarbon backbone (Abbas-Abadi *et al.*, 2014; Hamzah et al., 2023).

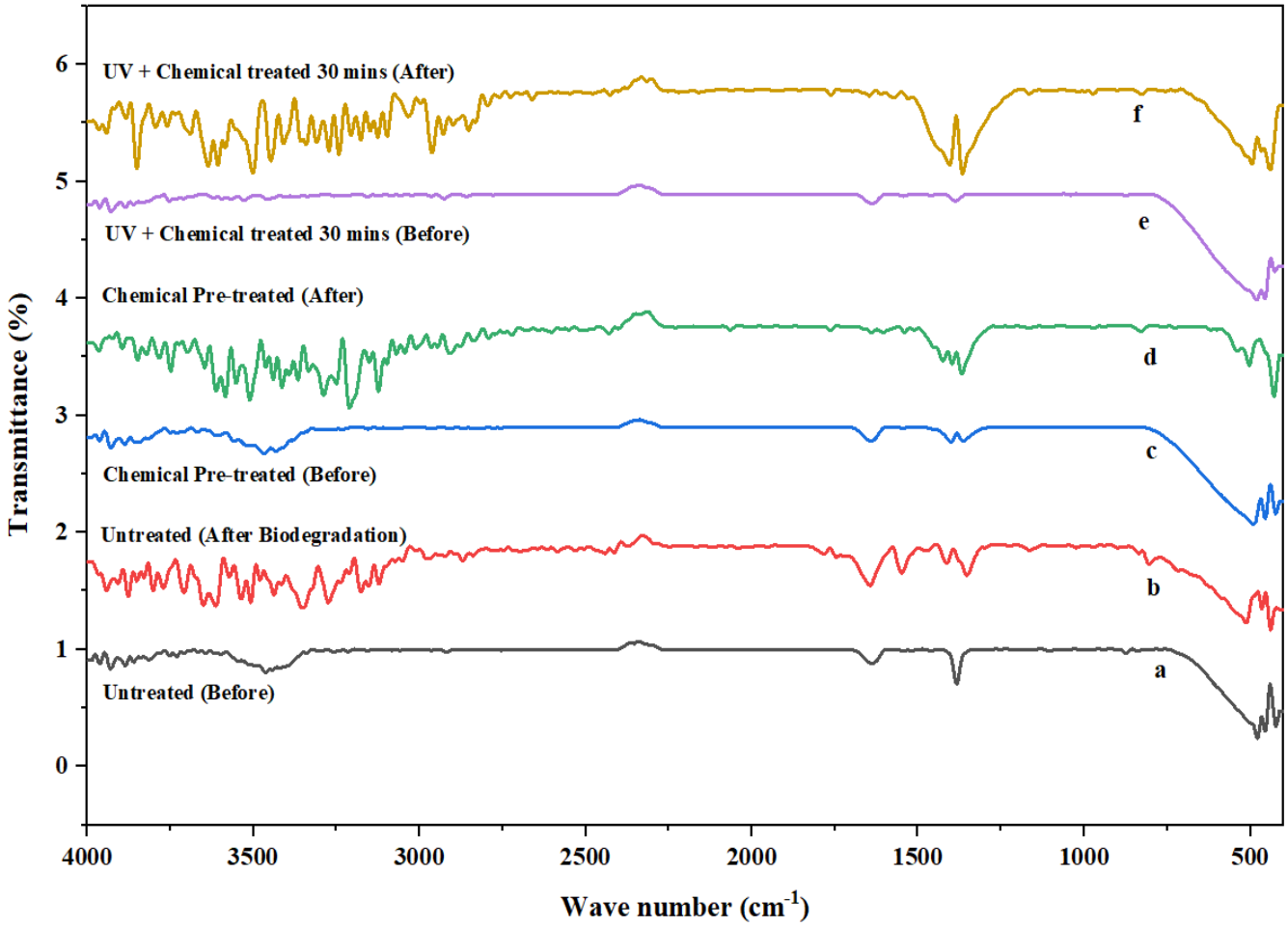

Figure 3: *FTIR spectrum of PP beads: (a) Untreated PP beads before biodegradation, (b) Untreated PP after biodegradation, (c) Chemically pretreated PP before biodegradation, (d) Chemically pretreated PP after biodegradation, (e) UV 30 mins and chemically pretreated PP before biodegradation, (f) UV 30 mins and chemically pretreated PP before biodegradation.*

Observations of changes in the fingerprint region (1500-500 cm^{-1}) following biodegradation suggest structural modifications and the potential formation of oxygen-containing functional groups, like carbonyl and hydroxyl groups, due to oxidative degradation processes (Sivan, 2011). When comparing spectral changes in untreated PP to chemically pretreatment samples prior to biodegradation, very small alterations were noted. Nevertheless, upon biodegradation, chemically prepared PP samples exhibit additional spectrum alterations, such as a notable diminution in the strength of the C-H stretching bands (2950-2850 cm^{-1}) and methyl deformation bands (1450-1375 cm^{-1}). In addition, after biodegradation, the most noticeable spectral changes were observed in polypropylene samples pretreated with both ultraviolet light and chemicals. The presence of additional bands (about 1700 cm^{-1} and 3200-3500 cm^{-1}) implies the establishment of carbonyl and hydroxyl groups. The FTIR spectra indicate accelerated oxidation and significant breakdown of long polymer chains, as evidenced by substantial distortion and reduced peak intensities (Hamzah et al., 2026; 2026a). There were minimal spectral changes in untreated PP, moderate structural modification in chemically pretreated PP, and the highest degree of polymer degradation and alteration in characteristic absorption bands in UV combined with chemical pretreatment. Microplastics made of polypropylene can be more easily broken down by microbes when subjected to certain pretreatment techniques, such as a mix of chemical treatment and ultraviolet light (Shah *et al.*, 2008). Russell *et al.* (2011) found similar findings, such as the absence or loss of peaks linked to ester bonds in polymer degradation.

SEM analysis of polypropylene

Using scanning electron microscopy, we observed bacterial colonisation and the subsequent changes in the surface micromorphology of PP films. A ZEISS scanning electron microscope was used to analyse all of the materials. Polypropylene (PP) surface morphological changes were examined using Scanning Electron Microscopy (SEM) before and after deterioration in untreated, chemically pretreated (HNO_3), and UV-chemically treated samples. Prior to degradation, the SEM micrograph of untreated polypropylene (Figure 4a) showed a consistent, smooth, and compact surface morphology with few surface flaws. The structural deformations that occur during degradation are usually caused by microorganisms that inhabit the polymer surface. These deformations include surface modifications such as small fractures and shallow pits (Figure 4b). Figure 4c shows that chemically prepared polypropylene had microcracks and increased surface roughness after being treated with HNO_3 before deterioration. More morphological abnormalities, including deep fissures, holes, pores, and broken areas, were seen in the chemically treated PP during degradation (Figure 4d). The combination of ultraviolet light and chemical pretreatment of PP samples resulted in more noticeable morphological alterations (Figure 4e).

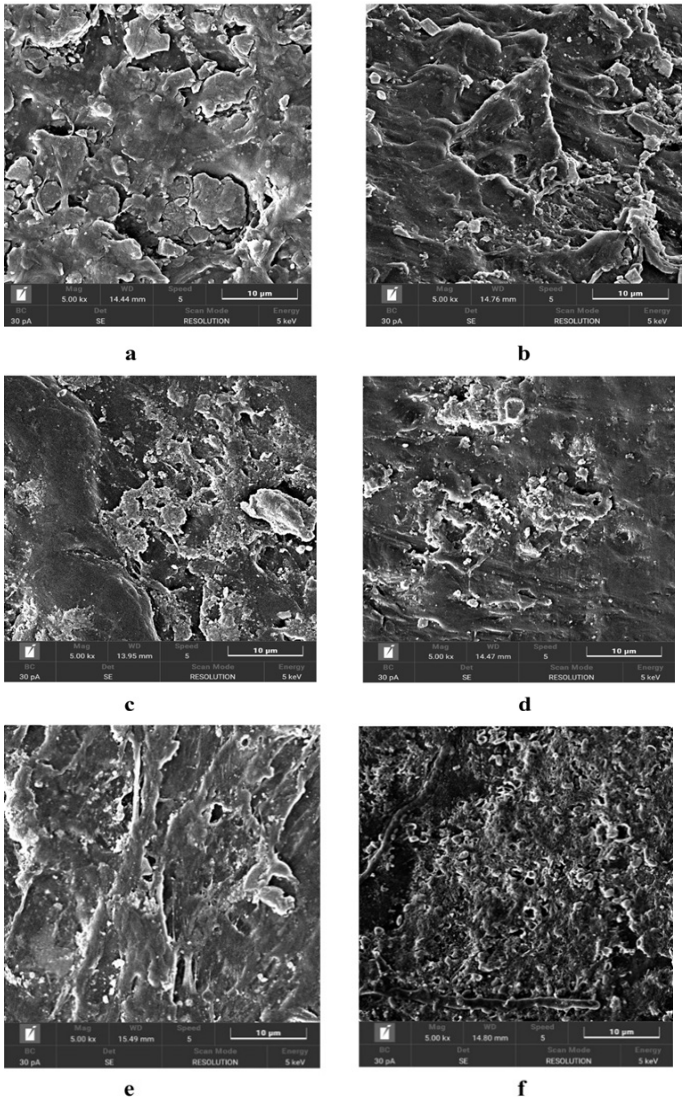

Figure 4: *SEM images depicting the colonisation, biofilm formation, and biodegradation of polypropylene by bacterial strain Bacillus cereus (H23B00108), (a-b) Untreated PP before degradation and after 40 days of degradation, (c-d) chemically pretreated PP before degradation and after degradation, (e-f) UV 30 mins and chemically pretreated PP before degradation and after 40 days of degradation.*

Surface imperfections and grooves were clearly visible in the scanning electron micrograph of the pre-degradation sample. Figure 4f shows that after biodegradation, the surface micrographs revealed significant erosion, big holes, deep fissures, and cavities, all of which pointed to advanced polymer degradation and strong microbial activity. Previous reports have shown that surface erosion, oxidation, and molecular fragmentation of polymers occur during microbial colonisation, which is consistent with the modifications observed (Wróbel *et al.*, 2023; Jeon *et al.*, 2021; Basak *et al.*, 2924).

XRD of polypropylene

Figure 5 shows the X-ray diffraction (XRD) patterns of three different types of polypropylene (PP): untreated, chemically pretreated, and UV + chemically pretreated PP, following incubation with the *Bacillus cereus* (H23B00108) bacterial strain. Figure 5a shows that the untreated polypropylene had diffraction peaks at 2θ = 9.58°, 14.23°, 17.09°, 18.77°, 21.79°, 25.60°, and 29.70°, which are indicative of the polypropylene's semi-crystalline structure. The diffraction peaks of the untreated sample are at 9.51°, 11.80°, 14.21°, 17.05°, 18.65°, 21.79°, 24.09°, 25.55°, and 29.63°, indicating that the chemical treatment of the polymer surface has somewhat disrupted the crystalline areas. The PP that was pretreated with chemicals and exposed to UV light for 30 minutes also showed peaks at 9.60°, 14.18°, 17.04°, 18.66°, 21.71°, 28.75°, and 29.64°. Compared with the control sample, the treated sample showed a slight shift in the diffraction peaks and a weakening of their intensity. This decrease in crystallinity demonstrated that the combined actions of chemical preparation, UV irradiation, and bacterial activity disturbed the polymer chains' orderly crystalline arrangement (Varma *et al.*, 2024). According to Bragg's law, the space between the reflection sites (d) decreases as the sinθ value increases. Additionally, there was a little change in the interplanar spacing (d-spacing) determined from the XRD peaks following treatment. While the d-spacing values of the untreated PP ranged from 9.22 Å to 1.60 Å, the samples that were chemically treated and those that were UV + chemically treated revealed values ranging from 9.29-1.66 Å and 9.20-2.08 Å, respectively. Disruption of the crystalline lattice occurs during biodegradation, as indicated by the modest increase in d-spacing (Sridevi *et al.*, 2024, 2026).

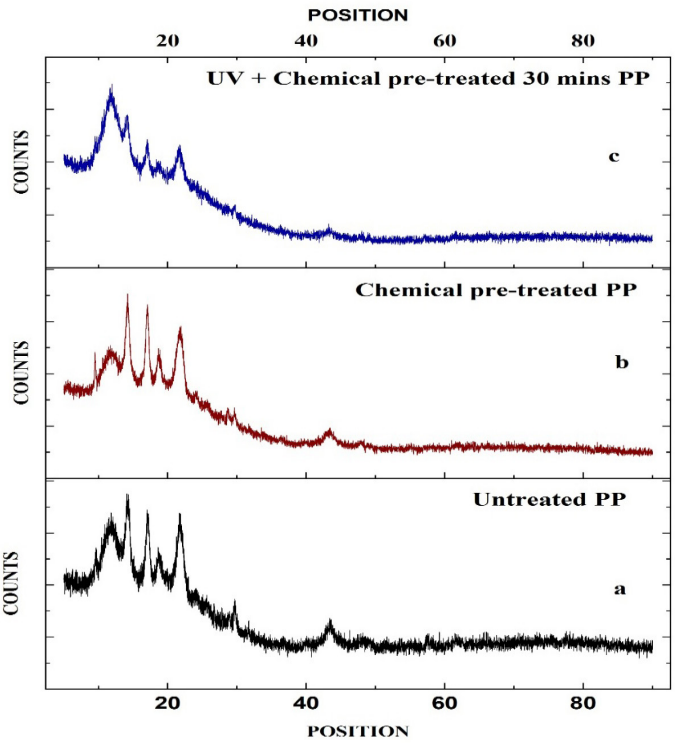

Figure 5: *XRD patterns of: (a) Untreated PP, (b) Chemical Pretreated PP, (c) UV 30 mins + chemical pretreated PP (after biodegradation).*

Conclusion

Bacillus cereus (H23B00108) proved to be highly effective in breaking down chemically processed polypropylene (PP) after 40 days of incubation under ideal growth circumstances. Weight loss of 17% was found to be the most efficient degradation rate. Subsequently, an 8.25% reduction in weight was achieved by a mix of ultraviolet light and chemical treatments. The untreated PP, on the other hand, exhibited minimal deterioration, losing a mere 3% of its weight. Pure *Bacillus cereus* (H23B00108) used PP beads as a carbon source, according to characterisation tests using FT-IR, SEM, and XRD. Chemical and photo-oxidative pretreatments enhance microbial colonisation and enzymatic destruction of the polymer structure, according to these investigations' findings. Taken as a whole, this research shows that increasing the biodegradability of polymers like polypropylene (PP) through a combination of pretreatment methods and microbial breakdown is a significant step toward more sustainable plastic waste management.

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affiliated universities for sample collection, microbial culturing, and instrumental characterisation (FTIR, SEM, XRD). This research received no specific external grant from funding agencies in the public, commercial, or not-for-profit sectors.

Novelty Statement

This study reports, for the first time, the biodegradation potential of an indigenous marine-sediment-derived Bacillus cereus strain (H23B00108), isolated from plastic-contaminated soil at Tenneti Park, Visakhapatnam, against commercial polypropylene beads. Unlike prior work that typically evaluates untreated PP alone, this investigation systematically compares three pretreatment regimes untreated, chemical (HNO_3) pretreated, and combined UV–chemical pretreated under identical biodegradation conditions, and couples weight-loss kinetics with FTIR carbonyl-index tracking, SEM surface-morphology mapping, and XRD crystallinity analysis to build a mechanistically integrated picture of pretreatment-enhanced microbial attack. The finding that chemical pretreatment alone (17% weight loss) outperformed combined UV–chemical pretreatment (8.25%) is a notable and underreported result that challenges the common assumption that UV pre-oxidation is always additive, offering new insight for optimising pretreatment protocols in PP bioremediation strategies.

Authors' Contribution

Geethika Gudapati: Sample collection, bacterial isolation and identification, experimental design and execution of biodegradation assays, weight-loss and characterisation data acquisition, writing original draft. Almuntadher Alwhelat: conceptualisation of the pretreatment strategy, FTIR/SEM/XRD data analysis and interpretation, methodology validation, writing review and editing. Veluru Sridevi: supervision, project administration, resources, critical revision of the manuscript for intellectual content. All authors read, critically reviewed, and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

During the preparation of this manuscript, the authors used AI-assisted language tools solely to improve grammatical accuracy, sentence clarity, and readability of the English text. No AI tool was used to generate, analyse, or interpret experimental data, figures, or scientific conclusions. All intellectual content, data interpretation, and conclusions presented in this work are the sole responsibility of the authors, who reviewed and edited the output of any such tool and take full accountability for the accuracy and integrity of the final manuscript.

Conflict of interest

The authors have declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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