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Introducing the LDPE degrading microbes of sedimentary systems: from dumpsite to laboratory†

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Plastic pollution is a growing environmental concern, necessitating sustainable degradation solutions. Microbial degradation offers an eco-friendly alternative to conventional disposal methods such as incineration and landfilling. This study investigates the biodegradation potential of a bacterial strain isolated from a plastic-rich dumpsite. Five bacterial isolates were screened, among which the BH-5 strain demonstrated the highest degradation efficiency of 10.5% within 30 days, as determined by weight loss measurements. The bacterial species was identified as *Bacillus paramycoides* through 16S rRNA gene sequencing. Fourier transform infrared (FTIR) spectroscopy confirmed the degradation process by revealing the appearance of hydroxyl and carbonyl groups (3329.50 cm^{-1} and 1650.47 cm^{-1}) in the treated low density polyethylene (LDPE) samples, absent in the control. Scanning Electron Microscopy (SEM) further revealed structural modifications, such as cracks and surface erosion, indicating bacterial degradation activity. Optimization of pH and temperature enhanced the biodegradation efficiency, with *Bacillus paramycoides* showing optimal growth at pH 7 and 30 °C, increasing degradation to 13.8% after 30 days. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified depolymerized byproducts, including alkanes, alcohols, and fatty acid esters, further validating microbial breakdown. This study highlights *Bacillus paramycoides* as a promising candidate for the biodegradation of LDPE, offering an environmentally sustainable approach for mitigating plastic pollution. Future research should focus on large-scale applications and enzymatic pathways involved in microbial plastic degradation.

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Environmental significance

Plastic pollution, particularly from low-density polyethylene (LDPE), poses a significant threat to ecosystems and human health due to its persistence and accumulation in the environment. Conventional disposal methods, such as landfilling and incineration, contribute to secondary pollution and greenhouse gas emissions. This study demonstrates that *Bacillus paramycoides*, a bacterium isolated from a plastic-rich dumpsite, can biodegrade LDPE, breaking it down into smaller, potentially less harmful compounds. The findings highlight microbial degradation as a viable, eco-friendly alternative for plastic waste management. Scaling up such bioremediation approaches could help mitigate plastic pollution in natural environments, reducing its impact on biodiversity and human well-being while contributing to sustainable waste management practices.

1 Introduction

Plastic is one of the major pollutants present in the environment. We humans are addicted to using plastic products in our day-to-day life.[1,2] Plastic being strong, durable, and resistant to degradation can persist in the environment for hundreds to thousands of years.[3] However, from the total produced plastics, only 21% of plastics get recycled, whereas the remaining get weathered and broken into small pieces.[4] These plastics pose significant environmental threats by impacting marine life and ecosystems through ingestion, causing physical harm and behavioural changes.[5] They accumulate in organisms, moving up the food chain and potentially affecting human health through bioaccumulation and biomagnification.[6,7]

Small size plastics are commonly found in the form of fragments from larger plastic items, fibers from synthetic textiles, microbeads from personal care products, thin plastic films from agricultural and packaging materials, foams from polystyrene products, and pellets used in plastic manufacturing.[8] LDPE is one such major plastic found in agricultural land and inland water bodies. LDPE is polymerised ethylene, extensively used in packing industries, protective coats on paper and making plastic bags for food and non-food items.[9–11]

Biodegradable plastics derived from natural polymers such as cellulose and starch are feasibly degraded by microorganisms,[12] while the synthetic plastics derived from petrochemicals are hard to degrade by microbes.[13] The conventional techniques used for disposal of plastic waste are burning and dumping in

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landfills and in the oceans, which causes secondary pollution.[14] Plastic degradation in the environment follows 4 mechanisms, *i.e.*, hydrolytic, photo, thermo-oxidative and biodegradation. Researchers worldwide are exploring methods to lower the plastic pollution by combining conventional and innovative ways of plastic disposal, and it was found there are some microbes which are capable of degrading synthetic plastics.[15,16]

Biodegradation plays a crucial role in the environment, where microbes utilize plastic polymers as a source of carbon and energy, breaking them down through diverse metabolic pathways to fuel essential cellular functions.[17] Plastic provides an ecological niche to the microbes by providing support for growth and colonization and also serves as a carbon source.[18] Plastics can be degraded by microorganisms under aerobic and anaerobic conditions. In aerobic biodegradation, bacteria utilize oxygen to metabolize plastics, breaking them down into carbon dioxide, water, and biomass.[19] Anaerobic biodegradation, on the other hand, occurs in the absence of oxygen, where bacteria decompose plastics into methane, carbon dioxide, water, and biomass. This process is comparatively slower and takes place in oxygen-deprived environments like landfills and deep sediments.[20] Anaerobic bacteria utilize alternative electron acceptors such as nitrate, iron, sulfate, manganese, and carbon dioxide (CO_2) instead of oxygen to degrade large organic compounds into smaller molecules. Additionally, there are numerous factors that direct biodegradation, like features of a polymer, type of pre-treatment and class of microorganism. Features of a polymer include molecular weight, mobility, elasticity, type of additive and functional groups present in the structure, which influences the degradation process.[21]

Various studies examined the biodegradation of various types of plastics by different microorganisms, highlighting their efficiency and degradation times. *Pseudomonas fluorescens* was found to degrade polyethylene (PE) over 270 days with a biodegradation efficiency of 18%.[22] *Bacillus vallismortis* (bt-dsce01) degraded LDPE in 120 days, achieving an efficiency of 75%.[23] *Klebsiella pneumoniae* (CH001) degraded high-density polyethylene (HDPE) in 60 days with an efficiency of 18.4%.[24] *Aspergillus oryzae* (A5) and *Bacillus cereus* (A5) both degraded LDPE in 112 days, with efficiencies of 36.4% and 35.72%, respectively.[25] *Trichoderma viride* (RH03) and *Aspergillus nomius* (RH06) degraded LDPE in 45 days, with efficiencies of 5.13% and 6.63%, respectively.[26] *Bacillus* sp. and *Paenibacillus* sp. were found to degrade PE in 60 days with a 14.7% efficiency.[27] *Aspergillus flavus* degraded HDPE in 100 days with an efficiency of 5.5%,[28] and *Bacillus siamensis* degraded LDPE in 90 days with an efficiency of 8.46%.[29] *Streptomyces* sp. B2 degraded bio- and petroleum-based polyurethane by 5.69% and 5.25% in 28 days.[30] *Sphingobacterium* sp. AS8 and *Bacillus* sp. AS3 strains isolated from a garbage site showed efficiencies of 2.01% and 3.06% within 28 days.[31] These findings demonstrate the varying capabilities of different microorganisms in degrading various types of plastics.

Some of the common techniques to evaluate the degradation of plastics include visual changes like the formation of cracks and holes, changes in colour, biofilm formation, dry weight loss measurement, CO_2 evolution, O_2 consumption, clear-zone formation, *etc.*[18,21] According to Bhardwaj *et al.*, biodegradation of the plastic can be ensured through the following points: changes in physical and mechanical properties, changes on the surface, and accumulation of biomass showing microbes use the carbon source of plastic for the growth of microorganisms.[32,33]

The study addresses the growing environmental concern of plastic pollution and the need for sustainable degradation solutions, highlighting microbial degradation as an eco-friendly alternative, while previous research has focused on microbial consortia or individual strains with slow degradation rates without systematically optimizing environmental conditions to enhance biodegradation efficiency. This study addresses these limitations by investigating the biodegradation potential of selected bacterial strains isolated from a plastic-rich dumpsite and optimizing key parameters such as pH and temperature to maximize degradation within a shorter period (30 days). Additionally, we employed a multi-analytical approach (FTIR, SEM, and GC-MS) to track structural and chemical modifications in LDPE, providing a more comprehensive understanding of microbial degradation mechanisms.

2 Methodology

2.1 LDPE sample preparation

LDPE pellets were procured from Sigma-Aldrich (428043, Lot no. MKCK8003). LDPE polybag sheets purchased from the local market were cut into pieces of 2 × 2 cm, each weighing 100 mg with a thickness of 51 μm. LDPE pellets and sheets were dipped in 70% ethanol solution for about 30 minutes and dried in a hot air oven at 60 °C to eradicate surface impurities and contaminations.[34] This was followed by treatment under UV light (<200 nm) for 15 minutes for sterilization. These sterile 2 × 2 cm LDPE sheets were used for further screening of plastic degrading bacterial species among the bacterial strains isolated from collected soil samples.

2.2 Soil sample collection, isolation of bacterial strains and screening of LDPE degrading bacteria

Soil samples were collected from the hostel dump yard (denoted as Hostel-H) rich in plastic content, of NIT Raipur campus, Chhattisgarh, India, at a depth of about 01 feet in zipper plastic bags. The collected soil sample was diluted to 10^{-8} and was inoculated on a nutrient agar plate and incubated for 24 hours at 35 °C.[35] The resulting bacterial colonies were then subjected to pure culturing and screening for their LDPE degradation ability by inoculating them in modified nutrient agar plates (peptone = 5 g, NaCl = 5 g, Agar–Agar = 15 g and LDPE pellets = 3 g) by the streak plate method and incubation at 35 °C for 24 hours.

2.3 LDPE degradation assay and change in the weight of the LDPE sample

LDPE degradation assay was performed in Bushnell-Haas (BH) broth media (composition KH_2PO_4 (1 g L^{-1}), $FeCl_3$ (0.05 g L^{-1}), NH_4NO_3 (1 g L^{-1}), $CaCl_2$ (0.02 g L^{-1}), K_2HPO_4 (1 g L^{-1}), $MgSO_4$ (0.20 g L^{-1}), pH (7 ± 0.2)).[36] 100 μL of the broth culture of the

positive LDPE degrading bacterial isolate obtained in the screening was inoculated in 100 mL of BH media containing a single pre-weighed sheet of LDPE (2 × 2 cm) and incubated at 35 °C for a period of 30 days. The 30-day incubation period for the biodegradation assay was chosen, as it allows adequate time for bacterial colonisation, biofilm development, enzymatic activity essential for initiating plastic degradation, and quantifiable polymer disintegration while also being feasible for experimental implementation and data collection. Numerous studies have shown microbes degrading plastic in this timeframe.

An LDPE sheet in BH broth without bacterial inoculation was used as the control group. All the experiments were performed in triplicate. On completion of the 30 day incubation period, the LDPE sheet was withdrawn from the culture media and treated with 1% SDS solution to remove adhered bacterial cells, if any, followed by washing in distilled water thrice and subjected to overnight drying in an oven at 50 °C.[37] The dried LDPE sheet was weighed on an electronic balance (Sartorius analytical balance, model BCE224). The weight measurement was performed with a precision of 0.001 grams. Finally, the percent degradation was calculated in terms of weight loss (%) in the 2 × 2 cm LDPE sheet using the formula given below:

$$\text{Degree of degradation}(\%) = \frac{\text{Control weight} - \text{Test sample weight}}{\text{Control weight}} \times 100$$

2.4 Microbial molecular sequencing and identification

The bacterial isolate with the highest LDPE degrading potential was subjected to 16S rRNA sequencing for its taxonomical identification at National Collection of Industrial Microorganisms (NCIM) in Pune, India. 16S rRNA for the bacteria isolates was determined by extracting genomic DNA from a 24 hour cultured bacteria strain with the help of an HiPurA Bacterial DNA purification spin column kit (MB505-250PR, HiMedia, India), and the same was verified by agarose gel electrophoresis. This was followed by the amplification of the bacterial-specific 16S rRNA gene (1500 bp) *via* the PCR technique (Applied Biosystems Veriti Thermal Cycler), employing the primers F27 (5′AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ GGTTACCTTGTTACGACTT 3′) as specified by Jill E. Clarridge.[38] The PCR product was purified (Shrimp Alkaline Phosphatase Purification Kit (New England Biolabs, Inc)) and verified by using GelRed Nucleic Acid Gel Stain on a 1% agarose electrophoresis gel in (1×) TBE buffer and visualized under a UV transilluminator (Protein Simple Red Imager SA-1000). The purified product was subjected to sequencing using a Cycle Sequencing Kit (Applied Biosystems, USA) and the sequencing reactions were executed on a 3500xL Genetic Analyzer (Applied Biosystems, USA).

The sequencing files (.abl) were edited using CHROMASLITE (version 1.5) and subsequently subjected to analysis through the Basic Local Alignment Search Tool (BLAST), which utilizes the closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database.[39] The BLAST algorithms employed not only facilitate the inference of functional and evolutionary relationships between sequences but also aid in the identification of members within gene families. Gene sequence data collected from NCIM, Pune, India, for isolates were evaluated for sequence similarity by comparing them with sequences from the NCBI database using BLAST, and a phylogenetic tree was constructed using MEGA (version 11.0.11) software.

2.5 Parameter optimization to enhance the degradation efficiency

To optimize the growth conditions for the bacterial strain, two critical parameters, pH and temperature, were systematically varied.[40] For this study, *Bacillus paramycoides* identified as the most potential strain for degrading LDPE was used. The growth of the culture was measured by using a UV-spectrophotometer at 600 nm on every alternate day for 10 days.

(1) pH of the media plays a significant role in LDPE degradation as well as microbial growth in the test media; therefore the pH condition for maximum growth and LDPE degradation was standardized. For the experiment, 2 × 2 cm of LDPE was added as the sole carbon source to 20 mL of BH media, and 100 μL of *Bacillus paramycoides* culture was inoculated into the media. The effect of pH was assessed by adjusting the pH of the media from 1 to 12, with increments of 1 unit. The pH adjustments were made using 1 M HCl and 1 M NaOH to create the desired acidic to basic conditions. The culture tubes were incubated at 35 °C with continuous shaking at 200 rpm.

(2) The temperature of the media is a critical factor influencing both LDPE degradation and microbial growth. For the experiment, 2 × 2 cm of LDPE was added as the sole carbon source to 20 mL of BH media, and 100 μL of *Bacillus paramycoides* culture was inoculated into the media. The cultures were incubated at various temperatures, ranging from 15 °C to 45 °C, with increments of 5 °C. All culture tubes were maintained at a pH of 7, which was previously identified as the optimal pH, and incubated with continuous shaking at 200 rpm. Growth was monitored over a 10-day period to identify the temperature that supported maximum bacterial growth and LDPE degradation.

2.6 Biodegradation of LDPE by *Bacillus paramycoides* under optimized conditions

100 μL of *Bacillus paramycoides* broth culture was inoculated in 100 mL of BH media containing 2 × 2 cm of LDPE as a source of carbon. The pH of the media was adjusted at 7 and was incubated at 30 °C under shaking conditions (200 rpm) for 30 days. The stated experiment was performed in parallel in triplicate to reduce the experimental errors, and a control without LDPE was also kept under similar conditions.

After 30 days of incubation, the LDPE sheet was recovered from the flask processed as outlined in Section 2.3 to determine weight loss.

2.7 Degraded LDPE constitutional analysis

The infrared (IR) spectra of the 30 day incubated LDPE plastic sheets were captured using a Fourier Transform Infrared (FTIR)

spectroscopy instrument (Bruker, ALPHA II ATR-FTIR), covering a spectral scan range of 600–4000 cm^{-1}.[41] Bacterial co-incubation with an LDPE sheet is hypothesized to cause chemical bond changes in polyethylene. Finally, control LDPE sheets were compared with the test LDPE sheet.

2.8 LDPE surficial scanning

Bacterial growth may cause surficial abrasion on LDPE sheets. Magnified scanning by using a scanning electron microscope (Zeiss EVO 18) from 500–5000× was performed for individual LDPE sheets. LDPE sheets were taken out of the incubation flasks aseptically and treated with 2% SDS followed by drying at 50 °C for 30 minutes.[34] Lastly, gold sputter coating was performed and microscopic examination was conducted.

2.9 GCMS analysis of the degraded LDPE sheet

The degradation products of the LDPE sample resulting from bacterial degradation were analyzed using GC-MS. The degraded LDPE sample was treated with 5 mL of chloroform in a culture tube, followed by ultrasonication for 1 hour in a water bath maintained at 55 °C. The extract was concentrated by allowing the solvent to evaporate at room temperature. Afterward, 2 mL of chloroform was added, and the mixture was filtered using a 0.22 μm Whatman grade 2 qualitative cellulose filter paper. During the analysis, the oven temperature was held at 40 °C for 3 minutes, then increased up to 280 °C at a rate of 10 °C per minute and held at 280 °C for 4 minutes.[41] Helium was used as the carrier gas, and the degradation products were identified by comparing their mass spectra with the NIST database.

3 Results and discussion

3.1 Screened LDPE degrading bacterial species

Fig. 1a shows the master culture plate for the soil sample (dump area of the boys hostel) on nutrient agar media containing LDPE, as it can be seen that 5 dominating LDPE utilizing colonies were observed on the plate. An individual colony was subjected to the pure culturing technique. A total of five distinct bacterial colonies, showing the ability to thrive on LDPE containing modified nutrient agar media as their sole source of carbon were cultured. The isolates were marked and denoted as BH1, BH2, BH3, BH4, and BH5 (Fig. 1b) independently.

3.2 LDPE degradation assay and change in the weight of LDPE

After LDPE degradation assay, a weight measurement of all LDPE sheets was performed. Fig. 2 shows the degree of degradation in % for each strain tested along with the control. The maximum degradation of 10.5% exhibited by strain BH5 was recorded. Though the degree of degradation is not very appreciable, it is in accordance with the other strains tested and reported by other research groups. Various bacterial and fungal species were tested for degradation of plastic for their degradation rate: *Trichoderma viride* (5.13%), *Aspergillus nomius* (6.63%), *Bacillus siamensis* (8.46%), *Bacillus cereus* (6.33%), *Bacillus wiedmannii* (5.39%), *Bacillus subtilis* (3.75%) and *Aspergillus flavus* (5.5%).[26,28,29] According to Wu *et al.*, side chains of PE are anticipated to serve as the primary site of interaction for bacterial-secreted enzymes, resulting in the polymer undergoing partial degradation. The removal of low molar mass molecules from the surface of the polymer occurs without inducing any scission in the polymer backbone. Consequently, the primary factor contributing to the initial weight reduction observed in the degradation of PE is the enzymatic hydrolysis of its easily accessible side chains.[34]

3.3 Microbial molecular identification

The potential isolate BH 5 with the highest degree of LDPE degradation was subjected to 16S rRNA sequencing. An NCBI BLAST run indicated that the isolate shows 99.79 and 99.72% similarity with respect to *Bacillus paramycoides* (Sequence ID:

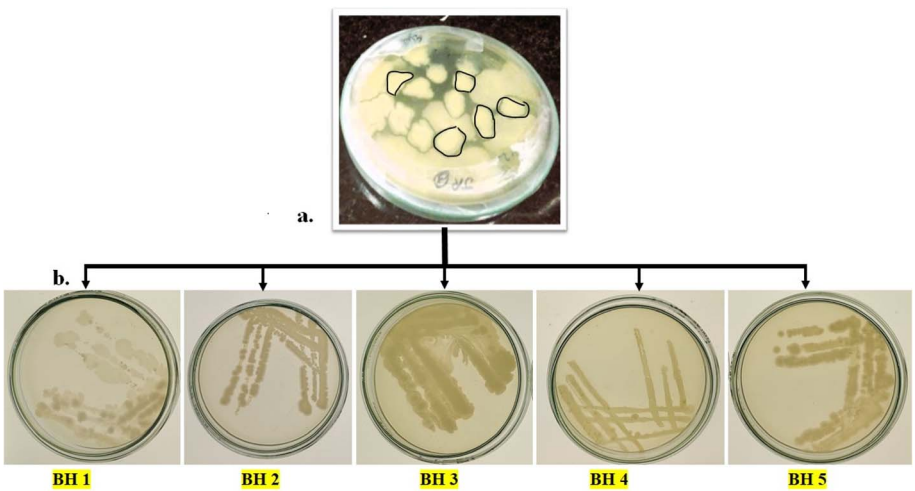

Fig. 1 Microbial colonies found in sediment soil: the (a) master plate and (b) microbial agar plate of five potential LDPE degrading bacterial strains, BH1, BH2, BH3, BH4, and BH5.

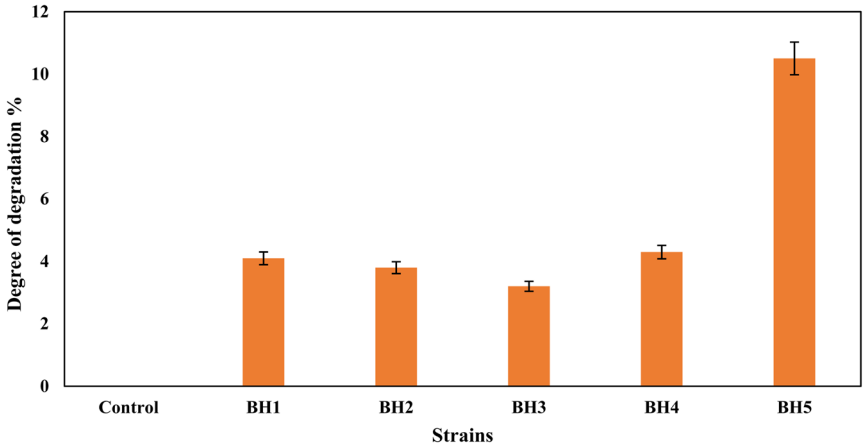

Fig. 2 Degree of degradation (%) of LDPE sheets by individual isolates.

NR 157734) and *Bacillus tropicus* (Sequence ID: NR157736) respectively. A phylogenetic tree was prepared using MEGA software and is illustrated in Fig. 3, constructed by the neighbor joining method. Thus, the strain was identified as *Bacillus paramycoides*.

3.4 Optimum conditions for biodegradation of LDPE

The biodegradation of polyethylene by microorganisms is significantly influenced by various environmental factors. The complexity of the surrounding environment plays a pivotal role in regulating the kinetics of enzymes involved in the degradation process.

(i) Effect of pH: Fig. 4 shows the effect of the pH range from 1 to 12 on the growth of the *Bacillus paramycoides* strain with respect to incubation time. The results revealed that the strain exhibited optimal growth at pH 7, where the absorbance was highest, indicating that neutral conditions are most conducive to growth; a similar trend was observed in the degree of degradation, showing maximum degradation at pH 7 (Fig. S1†). Moderate growth was observed at pH 6, 8 and 9, demonstrating

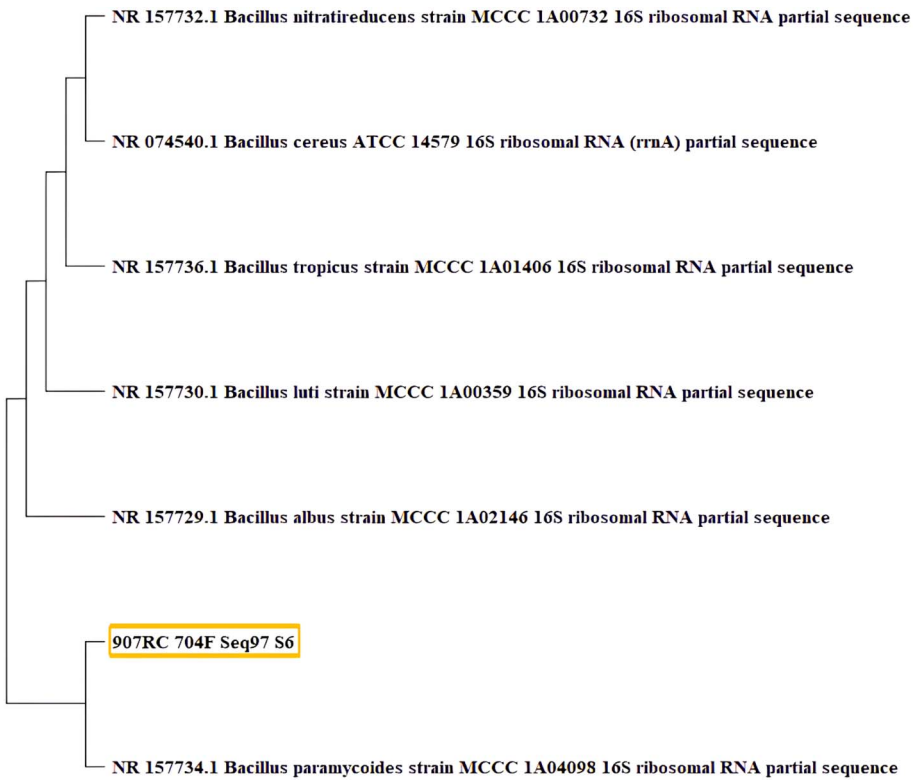

Fig. 3 A phylogenetic analysis was performed on strain BH-5 using 16S rDNA, and a phylogenetic tree was constructed using the neighbor-joining method through software MEGA X.

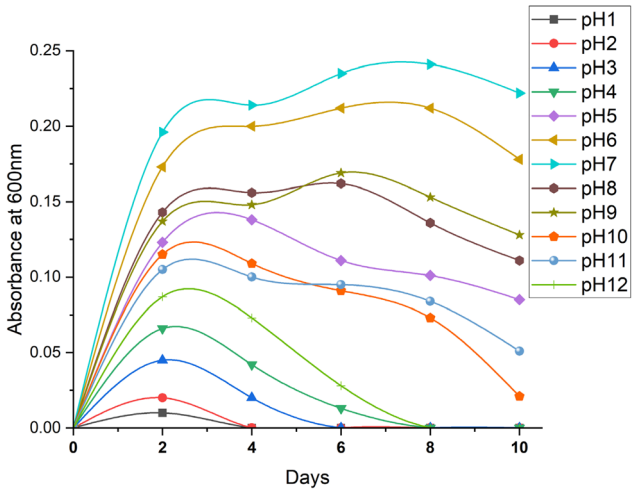

Fig. 4 Absorbance of the culture media was measured at 600 nm to determine the optimal pH for maximum growth of *Bacillus paramycoides*.

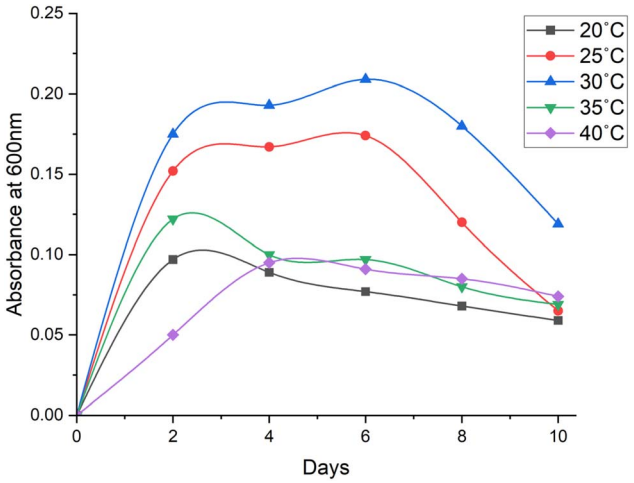

Fig. 5 Absorbance of the culture media was measured at 600 nm to determine the optimal temperature for maximum growth of *Bacillus paramycoides*.

some tolerance to slightly acidic and alkaline conditions. In contrast, minimal growth was recorded at pH values of 1, 2, 3, 4, 5 and 12, suggesting the strain's inability to thrive in highly acidic or highly alkaline environments. In 2023, Wu *et al.* reported that the best pH condition for the growth of *Bacillus* sp. was 7 (neutral), and at high acidic or alkaline pH, the growth of bacteria was inhibited.[34] In 2022, Yao *et al.* reported that pH is a crucial factor influencing enzyme activity, as each enzyme has a specific optimal pH range. Deviation from this range can result in partial or complete inhibition of enzyme activity. For instance, certain enzymes involved in polyethylene degradation function effectively within a neutral pH range.[42] Hydroxylase extracted from *Acinetobacter* strain AQ5NOL1 exhibited peak activity at pH 7–7.5.[43]

(ii) Effect of temperature: the effect of temperature on the growth of *Bacillus paramycoides* was assessed at temperatures ranging from 20 °C to 40 °C over a 10-day period. The strain displayed optimal growth at 30 °C, with the highest absorbance observed at around day 6, followed by a gradual decline as displayed in Fig. 5. Moderate growth was noted at 25 °C, while minimal growth occurred at extreme temperatures of 20 °C, 35 °C and 40 °C, indicating suboptimal conditions for bacterial metabolism. Additionally, maximum LDPE degradation was observed at 30 °C, further confirming that this temperature provides the most favorable conditions for both bacterial growth and biodegradation efficiency (Fig. S2†). These findings suggest that *Bacillus paramycoides* thrives best in a mesophilic range, with 30 °C providing the most favorable conditions for growth and plastic degradation potential. Yao *et al.* stated that temperature plays a vital role in the degradation of polyethylene in natural environments, as it directly impacts the activity of microbial enzymes. As proteins, enzymes are highly sensitive to temperature changes. Low temperatures can suppress their activity, while high temperatures may alter the protein's structure, potentially leading to a complete loss of enzymatic function.[42]

Fig. 4, 5, S1 and S2† clearly demonstrate that bacterial growth was optimal at pH 7 and 30 °C, conditions that also correlated with the maximum weight reduction of LDPE. This finding underscores the importance of maintaining optimal environmental conditions to support bacterial proliferation, which is directly linked to the efficiency of plastic degradation.[43]

3.5 Biodegradation of LDPE *via Bacillus paramycoide* under optimal conditions

After incubation, the weight of the LDPE sample reduced, reflecting a degradation improvement of 3.3% under optimized conditions. The results indicate that the optimized growth conditions for *Bacillus* significantly enhanced the degradation efficiency of the LDPE sample. This enhancement highlights the critical role of tailored environmental factors, such as nutrient availability, pH, temperature, and incubation time, in facilitating microbial activity. The overall degradation of 13.8% demonstrates the potential of *Bacillus* as an effective agent for breaking down LDPE, a typically recalcitrant polymer. These findings underscore the importance of optimizing bacterial growth conditions to maximize the biodegradation of plastics and contribute to sustainable waste management solutions. To support our findings, Wu *et al.* conducted a similar experiment involving the degradation of an LDPE film using *Bacillus paramycoides*. They optimized the growth conditions to a pH of 7 and a temperature of 35 °C, achieving 12% degradation in 45 days.[34] In contrast, our study demonstrated 13.8% degradation within just 30 days, indicating improved efficiency under the optimized conditions utilized in this work. Similar results have also been reported in previous studies.[44]

Biofilm forming microorganisms have better proximity with the surface of plastic; thus, plastic degrading enzyme local concentration on the plastic surface increases, resulting into an overall enhancement in the rate of biodegradation.[45] *Bacillus paramycoides* is a biofilm forming bacterial strain. Fig. 6 shows

Fig. 6 Presence of black colonies on the Congo red agar plate confirms that *Bacillus paramycoides* is capable of forming a biofilm.

the positive Congo red test for a pure culture of *Bacillus paramycoides*.

3.6 Degraded LDPE constitutional analysis

Surface functional group analysis can serve to assess the degradation of PE. The FTIR analysis serves as a valuable tool for confirming and understanding the microbial degradation of LDPE, contributing to a broader understanding of plastic biodegradation processes. As evident in Fig. 7, a peak at 1075.25 cm^{-1} was observed, indicating the presence of alcohol, ether, ester, and carboxylic acid groups through C–O stretching.[46] Another peak at 1538.86 cm^{-1}, indicating N–O stretching, has been observed. Enzyme nitric oxide synthase, found in *Bacillus* bacteria, is capable of producing nitric oxide gas through an enzymatic process. This enzymatic reaction is believed to contribute to the formation of nitro (NO) groups on LDPE.[47,48] Additionally, two peaks were observed at 1650.47 cm^{-1} and 3329.50 cm^{-1}, indicating the presence of C=O and –OH groups, respectively. These findings suggest that the LDPE film has undergone oxidation. Hydroxylation, an important process in the biodegradation of PE, involves the introduction of hydroxyl groups, which are crucial for the generation of carbonyl groups.[42] These carbonyl groups can subsequently be converted into esters, facilitating their eventual cleavage by lipase or esterase enzymes.[46] The observed changes in the FTIR spectra, such as alterations in peak intensities or shifts in peak positions, are indicative of the chemical modifications caused by the microbial degradation process. This finding confirms that the LDPE sheet has been biodegraded by the action of microorganisms, highlighting their capability to break down the polymer at a molecular level.

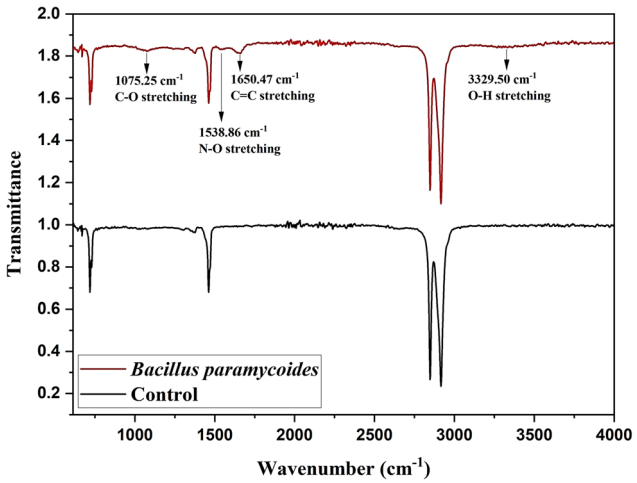

Fig. 7 Comparison of different absorption peaks of the LDPE sheet in the control (no microbial coincubation) and microbe mediated degradation of the LDPE sheet by *Bacillus paramycoides*.

3.7 SEM analysis of the degraded LDPE surface

A scanning electron microscope was used to observe the surficial changes obtained after incubation for 30 days with the microbial cultures. The SEM images of microbially degraded LDPE and untreated LDPE sheets are shown in Fig. 8. The images show a smooth surface of the sheet without any adhered microbial cells or cracks in the surface (Fig. 8a), while bacterial colonies of *Bacillus paramycoides* on the surface of the LDPE sheet can be observed in Fig. 8b, c and d. An increase in the incubation period from 10 days to 30 days increases the extent of degradation, as is evident from Fig. 8. Fig. 8b represents day 10, while Fig. 8c and d respectively show days 20 and 30. An increase in the break in the sheets with an increased incubation period can be seen. Similar results were observed in other studies, which indicate the primary release of debris due to microbial action.[49]

3.8 Depolymerized compounds identified by GCMS analysis

GC-MS analysis was conducted to identify the depolymerized compounds produced by *Bacillus* sp. after a four-week incubation, compared to a control set. The results revealed the presence of additives (bis(3-methylbutyl)fluorene-2,7-disulfonate and silane, trichlorodocosyl-) in the control, while in the bacteria degraded sample, alkane compounds ranging from C15 to C24 were found, as detailed in Table 1 and Fig. 9. The degradation of LDPE by *Bacillus paramycoides* produced a variety of byproducts, including alkanes (*e.g.*, heneicosane and eicosane), alcohols (*e.g.*, 1-hexadecanol), carboxylic acid and derivatives (*e.g.*, hexadecanoic acid methyl ester and heptadecanoic acid, 9-methyl-, methyl ester), and aldehydes (benzaldehyde, 2,4-dimethyl-). These compounds represent different stages of the biodegradation process and provide critical insights into the degradation pathway of LDPE.

The formation of alkanes indicates the fragmentation of the LDPE polymer chain into smaller, stable hydrocarbon fragments, as indicated by peaks at 2.39, 14.81, 15.96, 17.12, 21.75, 22.57 and 23.37, primarily through the scission of carbon–carbon bonds in the polymer backbone. These alkanes are often produced during the initial stages of degradation and may be serving as a source of carbon for microorganisms, as reported by Khampratueng *et al.*[31] The detection of 1-hexadecanol RT at

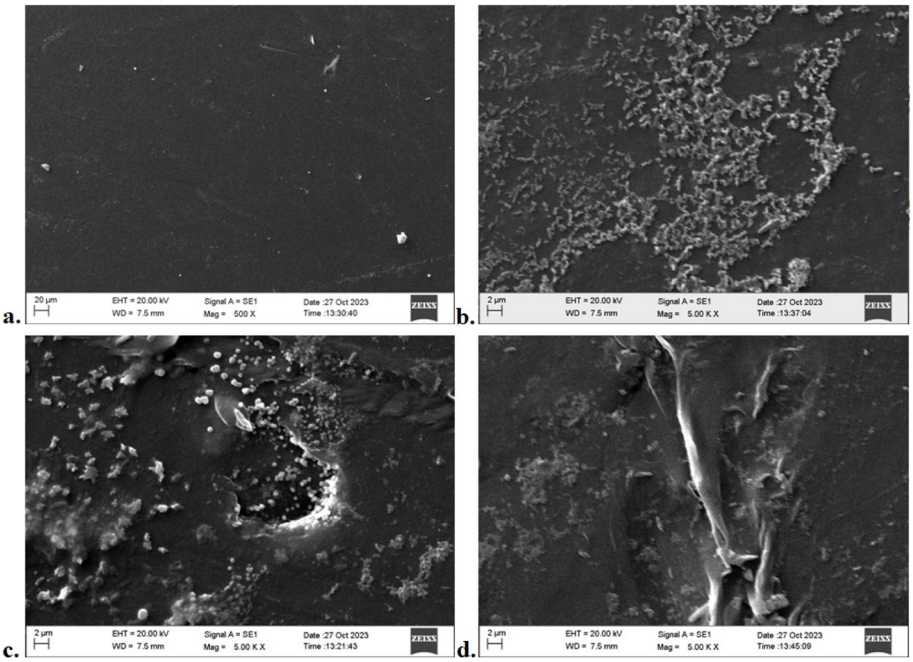

Fig. 8 SEM images: (a) the untreated LDPE sheet, (b) growth of bacteria on an LDPE sheet, and (c) and (d) bacteria consuming the LDPE sheet as a sole carbon source (heavy cracks and deep holes in the sheet were observed).

Table 1 GC-MS analysis highlighting various products obtained in untreated and *Bacillus paramycoides* treated LDPE samples

				Compounds identified	
Group	Retention time	Compound	Molecular formula	Control	Degraded LDPE by *Bacillus paramycoide*
Additives	1.32	Bis(3-methylbutyl)fluorene-2,7-disulfonate	$C_{23}H_{30}O_6S_2$	✓	✗
	17.05	Silane, trichlorodocosyl-	$C_{22}H_{45}Cl_3Si$	✓	✗
Alkane	23.37	Eicosane	$C_{20}H_{42}$	✗	✓
	22.57	Tetracosane	$C_{24}H_{50}$	✗	✓
	17.12	Heptadecane	$C_{17}H_{36}$	✗	✓
	21.75	Heneicosane	$C_{21}H_{44}$	✗	✓
	15.96	Hexadecane	$C_{16}H_{34}$	✗	✓
	14.81	Pentadecane	$C_{15}H_{32}$	✗	
Alcohol	16.65	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	✗	✓
Fatty acid ester	19.43	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	✗	✓
	21.25	Heptadecanoic acid, 9-methyl-, methyl ester	$C_{19}H_{38}O_2$	✗	✓
Aldehyde	11.39	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	✗	✓

16.65 suggests that the hydroxylation of alkanes occurs as an early oxidative step, facilitated by alkane hydroxylase enzymes. The enzymatic incorporation of oxygen into the hydrocarbon chain leads to the formation of alcohols, which are subsequently oxidized into carboxylic acid esters, such as hexadecanoic acid methyl ester and heptadecanoic acid, 9-methyl-, methyl ester. These oxidized products, including fatty acid esters and alcohol, are commonly reported during the PE degradation process.[40]

Various researchers have reported similar byproducts identified from the degradation of LDPE sheets/films by microbes. Park and Kim reported the production of 2-dodecanol ($C_{13}H_{28}O$), 1,8-nonanediol ($C_{10}H_{22}O_2$), and 1-dodecene ($C_{11}H_{22}$) during the degradation of polyethylene by *Bacillus* and *Paenibacillus* isolated from landfill samples.[27] Similarly, Khandare *et al.* (2022) observed the degradation of LDPE sheets by marine bacteria into byproducts such as eicosane, heneicosane, and triacontane, and derivatives of siloxane and alkenes, along with carboxylic acid (octadecanoic acid and benzene dicarboxylic acid) esters such as 2-propenoic acid pentadecyl ester.[40] Jaiswal *et al.* (2022) also reported the presence of decanoic acid decyl ester and 2-methylhexadecan-1-ol, which were attributed to enzymatic action by a microbial consortium, leading to reduced hydrophobicity of LDPE sheets.[41] These findings collectively highlight the dynamic enzymatic processes involved in LDPE

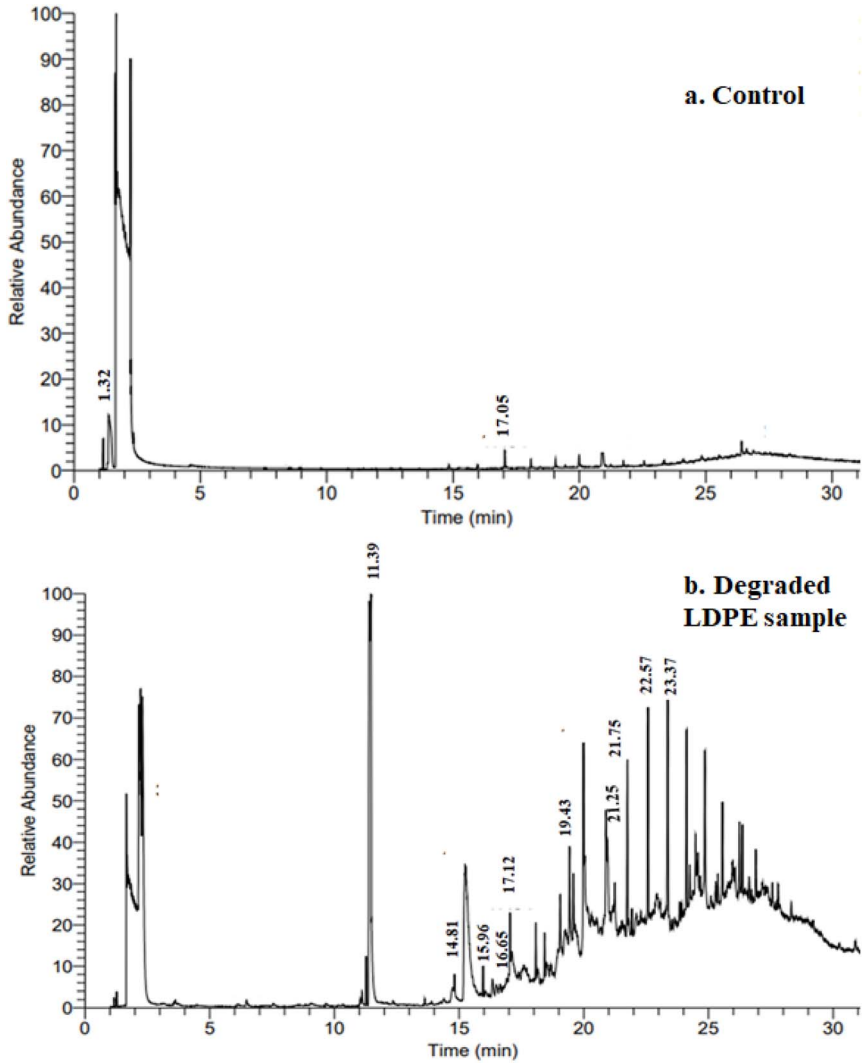

Fig. 9 GCMS chromatogram of compounds identified for the (a) control LDPE sample and (b) *Bacillus paramycoide* degraded LDPE sample.

degradation and the diverse range of compounds generated during the biodegradation pathway.

3.9 Degradation mechanism

Several species of *Bacillus* have been previously documented for their ability to biodegrade plastics. Fig. 10 shows the possible LDPE degradation mechanism by *Bacillus* species in this study. Yao *et al.* classified the LDPE degradation mechanism into four distinct stages, namely (1) colonization, (2) depolymerization, (3) assimilation, and (4) mineralization. At the initial stage, microbial cells and their extracellular polymers (EPS) attach to the plastic surface and form a colony of cells, as observed in Fig. 8b in the current study.[50] *Bacillus* sp., by secreting a variety of extracellular enzymes like lipases and hydrolases, tapers the hydrophobicity and chemical inertness of a polymer's surface (Fig. 7). *Bacillus* extracellular enzymes reduce the molecular weight of plastic by hydrolyzing its hydrolysable chemical bonds, resulting in the release of few monomeric or dimeric plastic materials. The rate of degradation is simultaneously also supported by the oxidation process, resulting in the formation of hydroxyl and carbonyl groups that serve as the site of enzymatic degradation.[42] According to Van Bailin *et al.* there are further enhancements in the degradation due to the uptake of released oligomeric fragments of plastic by the microbial cell and its intracellular degradation by co metabolic phenomena. These oligomers might also be serving as an inducer for the release of the plastic degrading enzyme system of the cell, leading to further enhancement in the degradation process. *Bacillus* enzymes like lipases, esterases and oxidoreductases are found to be facilitated by the intermediate taken up by the cell. The intermediates undergo additional catalysis by terminal oxidase enzymes, resulting in the formation of alcohol. The resultant alcohols are further oxidized by the action of enzyme alcohol dehydrogenase. The oligomeric intermediate may be also converted to an acidic derivative and is degraded by the fatty acid-based oxidation cycle.[51]

Wu *et al.* and Yao *et al.* reported the degradation of polyethylene by monooxygenase mediated oxidation of polyethylene

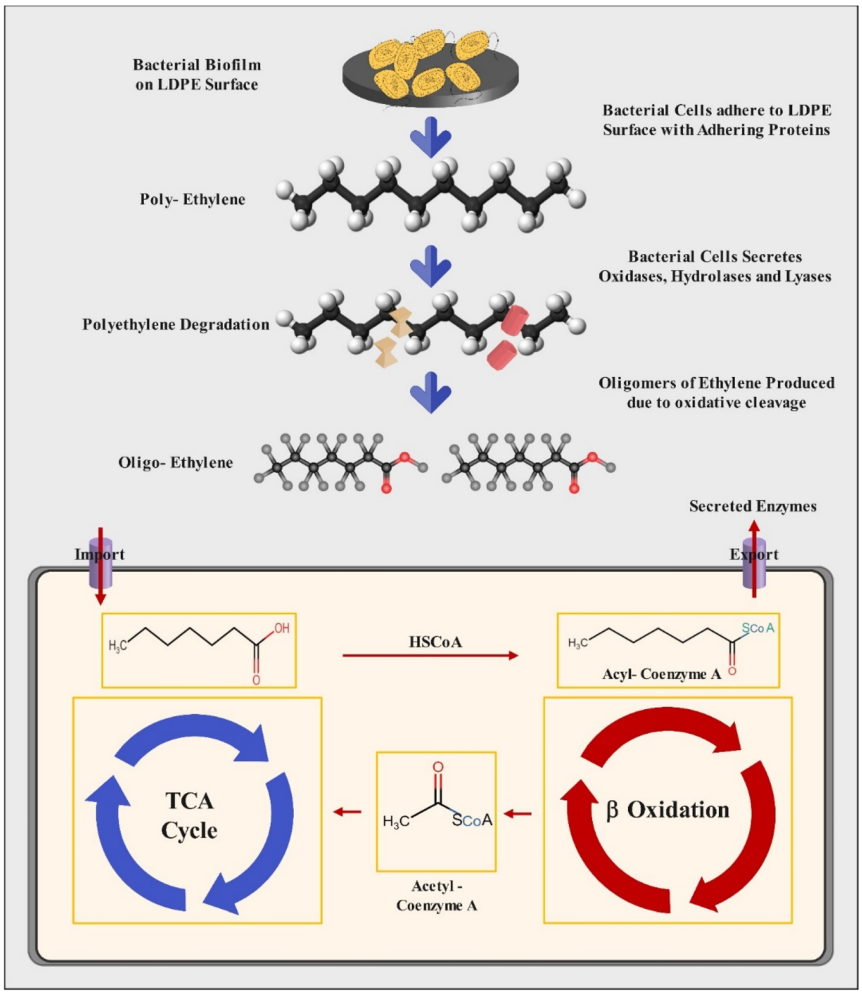

Fig. 10 Possible degradation mechanism of low density polyethylene by *Bacillus* species.

from the subterminal region and the formation of secondary alcohols which are further converted to their ketonic derivative by the action of enzyme aldehyde dehydrogenase. The degraded products of polyethylene are chemically similar to alkanes and hence are metabolized by similar metabolic pathways. Some of the intermediates of polyethylene are further degraded by the fatty acid metabolizing pathway. Depolymerization of polyethylene result in smaller fragments which are comparatively hydrophilic in nature with short chain lengths which can be easily recognized by the *Bacillus* surface receptors and internalized to be utilized in the microbial metabolic activity promoting cellular growth. Energy, CO_2, and H_2O are the final products generated due to cellular assimilation of depolymerized intermediate plastics and their utilization as cellular metabolites.[34,36]

4 Conclusion

This study demonstrates the potential of *Bacillus paramycoides* in the biodegradation of LDPE, a persistent environmental pollutant. The bacterial strain was isolated from a plastic-rich dumpsite and exhibited a degradation efficiency of 10.5% within 30 days, which improved to 13.8% under optimized conditions (pH 7, 30 °C). Characterization using FTIR spectroscopy confirmed the presence of hydroxyl and carbonyl functional groups, indicative of oxidative degradation. SEM analysis revealed surface cracks and erosion, further supporting microbial degradation. Additionally, GC-MS analysis identified depolymerized byproducts, such as alkanes, alcohols, and fatty acid esters, confirming the breakdown of LDPE into smaller, more biodegradable compounds.

These findings highlight *Bacillus paramycoides* as a promising candidate for microbial plastic degradation, providing an eco-friendly and sustainable alternative to conventional plastic waste management strategies. However, further studies are needed to understand the enzymatic pathways involved in polymer degradation and to enhance degradation rates through metabolic engineering or microbial consortia. Scaling up these findings to real-world applications, such as in wastewater treatment or landfill bioremediation, could significantly contribute to mitigating plastic pollution.

Data availability

The data will be made available on request to the corresponding author.

Author contributions

Anita Tirkey: conceptualization, investigation, methodology, writing – original draft. Lata Sheo Bachan Upadhyay: supervision, validation, writing – review and editing.

Conflicts of interest

The authors declare no competing interest.

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