Isolation and screening of low-density polyethylene degrading bacteria

A thesis Submitted in partial fulfillment of the requirements for the award of the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

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DECLARATION

I, **Ms. Kashish**, hereby declare that the project entitled "**Isolation and screening of low-density polyethylene degrading bacteria.**" is an authentic record of my work during the period of six months from January 2024 to June 2024 carried out under the guidance and supervision of Prof. (Dr.) Dinesh Goyal. This dissertation is submitted in partial fulfilment of the requirements for the Degree of "**Master of Science in Biotechnology"** of Thapar Institute of Engineering and Technology, Patiala, Punjab. The information derived from the literature has been duly acknowledged in the text, and a list of references is provided. No part of this dissertation is submitted for the reward of any other degree or certificate in this or any other university or institute.

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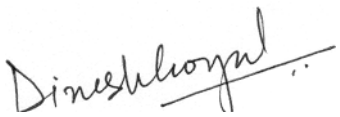
CERTIFICATE

This is to certify that the dissertation report entitled "**Isolation and screening of low-density polyethylene degrading bacteria.**" submitted by **Kashish** (302201005) in the partial fulfilment of the requirement for the award of the degree of the "**Master of Science in Biotechnology**" Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab is an authentic record of student's work carried out during the period of six months, i.e., from January 2024 to June 2024 under my supervision and guidance. This work has not been submitted for the award of any other degree or certificate in this or any other university or institute.



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Place: Patiala Kashish

In dedication to my parents for

making me who I am and my brother

who supported me all the way!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

C: N	Carbon-to-Nitrogen Ratio
C-H	Carbon-hydrogen
CHN content	Carbon, Hydrogen and Nitrogen content
CO_2	Carbon dioxide
CPCB	Central pollution control board
EPS	Extracellular polymeric substances
FE-SEM	Field emission scanning electron microscopy
FMCG	Fast-moving consumer goods
FT-IR	Fourier-transform infrared spectroscopy
GC-MS	Gas chromatography mass spectrometry
H_2O_2	Hydrogen peroxide
HDPE	High-density polyethylene
HNO_3	Nitric acid
ITS	Internal transcribed spacer
LDPE	Low-density polyethylene
LMWPE	Low-molecular-weight polyethylene
N:P	Nitrogen-to-Phosphorus Ratio
NMR	Nuclear magnetic resonance spectroscopy
OD	Optical density
PE	Polyethylene
PEG	Polyethylene glycol

PET	Polyethylene terephthalate
pH	Potential of Hydrogen
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
SBP	Starch-based plastics
SDBS	Sodium dodecyl benzene sulfonate
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
Sp.	Species
Spp.	Species
TGA	Thermogravimetric analysis
T-LDPE	Heat-treated low-density polyethylene
TPS	Thermoplastic starch
UV	Ultraviolent
UV-B	Ultraviolent-B
VC	Vermicompost
VCB	Vermicompost with bacterial consortium
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

LIST OF SYMBOLS

%	Percentage
°C	Degree celsius
µ	Micron
µg	Microgram
µL	Microlitre
µm	Micrometer
g	Gram
g/L	Gram / litre
L	Litre
mg	Milligram
mL	Millilitre
mm	Millimetre
nm	Nanometer
psi	Pound per square inch
rpm	Revolutions per minute
w/w	Weight per weight

Abstract

Low-density polyethylene (LDPE) is a thermoplastic made from monomer ethylene and its extensive usage and persistent nature, has contributed significantly to several environmental issues concerning its indiscriminate disposal and inadequate recycling. This thesis is aimed to identify and characterize bacterial isolates with potential LDPE biodegradation capabilities. Six partially degraded plastic samples were collected from dump sites near M Hostel at Thapar Institute of Engineering and Technology. From these, 24 bacterial isolates were screened using various assays, including zone of clearance, Polyethylene glycol-4000 (PEG) utilization in Bushnell Haas medium (1% and 5% PEG), and hydrophobicity assays. The zone of clearance assay indicated that isolates DGK1, DGK2, DGK4, DGK5, DGK7, DGK8, DGK9, DGK20, DGK21, and DGK24 exhibited plastic-degrading potential, with DGK4 and DGK7 showing the most significant zones of clearance. PEG utilization data revealed that DGK4 and DGK7 demonstrated high efficiency in degrading PEG, with utilization rates of 43.47 ± 0.96% and 43.87 ± 0.39% for 1% PEG, and 39.11 ± 1.20% and 40.13 ± 0.32% for 5% PEG, respectively. Hydrophobicity assays showed that DGK7 had the highest hydrophobicity (50.14 ± 0.28%), suggesting a strong affinity for hydrophobic materials like LDPE. Consequently, DGK4 (Gram-negative) and DGK7 (Gram-positive) were selected for LDPE biodegradation studies. Gravimetric weight loss measurements confirmed substantial degradation of LDPE by DGK4 and DGK7, with DGK7 exhibiting the highest degradation rates. For DGK4, weight loss percentages were 0.46 ± 0.09% for Plastic Sample 1 (44 µm) UV and 5.19 ± 0.17% for Plastic Sample 2 (22 µm) UV. For DGK7, weight loss percentages were 4.95 ± 0.21% for Plastic Sample 1 (44 µm) UV and 12.13 ± 0.56% for Plastic Sample 2 (22 µm) UV, highlighting DGK7 as the most effective strain in degrading LDPE. SEM analysis of DGK7 revealed significant surface erosion and morphological changes in LDPE samples, underscoring its effective degradation capabilities. These findings underscore the potential of DGK4 and particularly DGK7 as effective candidates for bioremediation of LDPE, contributing to the ongoing efforts to address plastic pollution.

Keywords: LDPE biodegradation, bacterial isolates, zone of clearance, PEG utilization, hydrophobicity, gravimetric weight loss, SEM analysis

Introduction

Plastics, derived from the Greek term "plastikos" meaning "able to be shaped or molded," are integral to modern economies, including agriculture, telecommunications, construction, consumer products, and healthcare (Strong, 2006). These synthetic polymers are primarily made from non-renewable petrochemicals and consist of long chains of organic subunits linked by covalent bonds, making them durable, lightweight, and resistant to environmental degradation (Xanthos and Walker, 2017; Chamas et al., 2020). Common types include Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), and Polyvinyl Chloride (PVC), which are especially prevalent in packaging due to their adaptability and cost-effectiveness. LDPE was introduced in 1933 and is the most widely used plastic (Britannica., 2024). However, their durability causes significant environmental issues, contributing to global plastic waste (Kibria et al., 2023). Plastic wastes are a major component of global solid waste, consisting of polyethylene, polypropylene, polystyrene and PVC (Ncube et al., 2021). Plastic pollution, categorized by size into macro (above 20mm), meso (5-20mm), and micro (under 5mm) plastics, adversely affects all habitats, wildlife, and human health (Shimao et al., 2001). Plastic production surged from 1.5 million tonnes in the 1950s to over 359 million tonnes in 2018, leading to widespread environmental problems (Dris et al., 2020).

Conventional plastic waste management methods, like landfilling or burning, pose environmental risks, affecting soil fertility and crop yields. Efforts are underway to develop biodegradable alternatives and improve waste management to mitigate the ecological impact of plastic pollution. There is a pressing requirement for sustainable and environmentally friendly measures to alleviate plastic pollution. Biodegradation, a sustainable method involving microorganisms, could help alleviate plastic pollution (Wojnowska-Baryła et al., 2022). Microorganisms such as bacteria and fungi can break down complex polymers like LDPE, releasing extracellular enzymes for easier processing (Bonhomme et al., 2003). Recent research has shown that several types of microorganisms have the ability to break down LDPE, emphasizing the significance of microbial communities that combine the enzymatic capabilities of multiple species to improve the effectiveness of degradation (Restrepo-Flórez et al., 2014). In the present investigation, LDPE-degrading bacteria were isolated from plastic dump sites and screened with the following objectives:

1. Isolation and screening of LDPE degrading bacteria from plastisphere
2. Degradation of LDPE by bacterial isolates

Review of literature

Plastics, a versatile and cost-effective material, have become a significant part of modern life due to their durability and cost-effectiveness. Common types include polyethylene, polypropylene, polystyrene, PVC, and PET (Geyer et al., 2017). These plastics can take hundreds to thousands of years to degrade, leading to environmental hazards like wildlife entanglement, toxic additive leaching, and food chain microplastics (Thompson et al., 2009). Conventional waste management practices like landfilling and incineration are seen as unsustainable due to their environmental impact (Siddiqua et al., 2022). Biodegradation, a promising alternative, involves microorganisms breaking down plastics and converting them into harmless end-products (Shah et al., 2008). Understanding the mechanisms and effectiveness of biodegradation is crucial for advancing this field and developing practical applications for mitigating plastic waste.

Plastic waste production in India

Plastic waste has become a major environmental problem in India due to its widespread use and insufficient treatment procedures. Based on the report from the Central Pollution Control Board (CPCB, 2021), India produces around 4,126,997 tonnes of plastic garbage annually, making it one of the leading contributors globally (CPCB, 2021). India's plastic usage has been consistently rising, especially in packaging, agriculture, and construction industries. The packaging sector, in particular, contributes significantly to the overall plastic waste produced, primarily due to the growth of e-commerce and fast-moving consumer goods (FMCG) industries (CPCB, 2021). Notwithstanding endeavours to control plastic consumption and enhance trash management, a substantial proportion of plastic waste in India remains uncollected and inadequately managed. The informal recycling industries, which have a significant impact, frequently function in inadequate conditions, leading to environmental contamination and health risks (CPCB, 2021). The improper disposal and incineration of plastic waste result in environmental degradation, soil contamination, and air pollution. This poses long-term risks to ecosystems and wildlife due to the slow disintegration of plastics and the release of harmful compounds throughout this process (CPCB, 2021).

Harmful effects of plastic waste

The extensive utilization and persistent buildup of synthetic polymers in the environment have led to plastic pollution, which is now a critical global environmental concern. Plastics are essential in contemporary civilization and utilized in several sectors such as packaging,

building, agriculture, and consumer products. Nevertheless, the long-lasting nature and ability to withstand deterioration provide notable obstacles to achieving environmental sustainability. Low-density polyethylene (LDPE) stands out among the many types of plastics due to its extensive usage and ability to withstand microbial degradation. The indiscriminate disposal and inadequate recycling of plastic waste in India lead to numerous environmental issues. The manufacturing of plastics releases harmful emissions, and improper disposal renders soil unproductive due to its impermeability. Burning plastics emits toxic substances like microplastics, bisphenols, and phthalates (Cosier, S., 2022). Burning plastic can affect air quality and public health. Plastic additives pose health risks by leaching out. Non-recyclable plastics, such as multilayer pouches and thin plastic bags, complicate waste management and recycling efforts. Scattered plastic waste diminishes city aesthetics, clogs drainage systems, and worsens flooding during monsoons. Unregulated recycling industries pose environmental hazards (Sugata et al., 2022).

Plastics in landfills persist for long periods, harming soil fertility and crop yields by obstructing water flow and nutrient absorption. Recycling, though necessary, is complex and costly, often degrading the quality of the final product (Teuten et al., 2009). LDPE can be recycled into items like plastic bags but releases harmful volatile compounds. Recycled plastics may contain harmful additives, reducing utility (Goodship, 2007). Incineration produces hazardous gases and chemicals, contributing to air pollution and health risks, including respiratory issues and cancer (Sharma et al., 2013). Plastics also adversely affect terrestrial and aquatic ecosystems. Marine organisms mistakenly consume plastics, leading to suffocation, reduced food intake, and damage to internal organs and reproductive systems (Teuten et al., 2009; Browne et al., 2011).

Degradation of plastics

Mechanical degradation

The process of mechanical degradation of plastics entails the physical fragmentation of the material into smaller fragments or microplastics while maintaining the integrity of its chemical composition. The main driving forces behind this process include UV radiation, wind abrasion, wave action, and mechanical stresses (Andrady, 2011). Therefore, mechanical deterioration plays a vital role in the buildup of microplastics in the environment. Microplastics are long-lasting in ecosystems and can be consumed by marine and terrestrial creatures. This poses a threat to biodiversity and has the potential to enter the food chain (Roy et al., 2022).

Chemical degradation

The chemical degradation process of plastics entails the disintegration of polymer chains by chemical reactions, resulting in alterations in molecular weight and physical characteristics. The deterioration process is expedited by sunlight, heat, oxygen, and pollutants. Additionally, the additives and impurities found in plastics can impact the degradation processes (Thompson et al., 2009; Jambeck et al., 2015). Chemical degradation leads to the release of detrimental chemicals and microplastics into the environment, potentially contaminating soil, water, and creatures. Plastic pollution has significant implications for human health and ecosystems, underscoring its environmental consequences (Rochman et al., 2013; Geyer et al., 2017).

Biodegradation

The biological degradation of plastics involves the enzymatic decomposition of polymer chains by microbes, transforming polymers into less complex molecules. This process depends on microorganisms that can break down plastic, as well as the proper environmental parameters, including temperature and humidity and the availability of plastics for microbial activity (Ghatge et al., 2020). These parameters are essential in determining the speed and degree of biological breakdown, emphasizing its promise as a sustainable method for managing plastic trash.

i. Bacterial biodegradation

The impact of *Arthrobacter paraffines* on the degradation of low-density polyethylene (LDPE) was studied by Albertsson *et al*. (1995) revealing that abiotic degradation involves thermo-oxidation, while biotic degradation involves exposure to the bacteria. Morphological changes, such as decreased lamellar thickness and crystallinity, were observed in biotically degraded samples, while abiotically aged samples showed constant or increased values. This differentiation can help fingerprint synthetic polymer degradation processes.

The degradation of low-density polyethylene (LDPE) film containing starch, using a soil microbe identified as *Pseudomonas* sp, was studied by Jana *et al*. (1999), revealing that the rate of degradation depends on the accessibility of starch and external carbon sources. Maltose was found to be the most effective external carbon source, causing a 50% loss in tensile strength in 58 days.

Bacterial strains from waste disposal sites in Uttaranchal, India, and soil beds with polyethylene pieces were screened by Satlewal *et al*. (2008). A consortium of potential strains was developed for biodegradation. Results showed significant degradation of HDPE and

LDPE, with consortium-treated HDPE showing a 22.41% weight loss compared to LDPE's 21.70%. Untreated LDPE and HDPE showed lower weight losses, 4.5%, and 2.5%, respectively, suggesting microbial consortia can accelerate degradation in natural environments.

The degradation of LDPE films containing trace amounts of cobalt stearate using enriched microbial strains (*Bacillus pumilus*, *Bacillus halodenitrificans*, and *Bacillus cereus*) in a Basal salt medium was investigated by Roy *et al*. (2008). The films underwent UV-B irradiation and were inoculated with bacterial strains. The bacterial consortium degraded the polymer, resulting in a mass loss of 8.4 ± 1.37% and increased bacterial count. This suggests that trace cobalt stearate accelerates degradation under UV exposure and supports bacterial growth.

The biodegradation of low- and high-density polyethylene (LDPE and HDPE) using marine microorganisms, *Bacillus sphericus* GC subgroup IV and *Bacillus cereus* subgroup A, was investigated by Sudhakar *et al*. (2008). Thermal pretreatment was found to enhance biodegradation rates and higher biodegradability of starch-blended LDPE, suggesting a synergistic relationship between abiotic and biotic degradation processes. This highlights the importance of thermal pretreatment in enhancing the biodegradability of LDPE.

Seventeen bacterial isolates were screened by Soni *et al*. (2009) for their ability to use LDPE as a carbon source. Five strains formed a consortium, and in vitro biodegradation experiments showed superior degradation of poronized LDPE. This suggests the consortium's potential for plastic waste management strategies, highlighting the influence of LDPE poronization.

Pseudomonas aeruginosa was found to be a key factor in the biodegradation of oxo-biodegradable polyethylene films by Reddy *et al*. (2009). The study involved abiotic oxidation, where *P. aeruginosa* was inoculated onto the polyethylene film and monitored for molecular weight and oxidation product concentrations. The biodegradation progress was observed, with *P. aeruginosa* forming biofilms on the polymer film.

The microbial degradation of low-density polyethylene (LDPE) film under natural conditions using bacterial consortia was studied by Negi *et al*. (2011). The results showed significant surface degradation, hydroxyl functionality, and shifts in the film's fingerprint region. Environmental factors like sunlight, temperature, and rainfall enhanced the degradation

rate, suggesting bacterial consortia could accelerate LDPE degradation in soil for large-scale applications.

The development of a talc-based formulation for long-term storage and viability of bacterial consortia capable of degrading polymers like LDPE and PVC was the focus of Sah *et al*. (2011). The consortia, including *Microbacterium* sp., *Pseudomonas putida*, and *Bacillus aerius,* showed significant surface disruption and retained their biodegradation properties even after 70 days of storage. Further in situ trials are underway.

Microorganisms' adaptive ability to degrade oxidized polymers using Fourier Transform Infrared coupled Attenuated Total Reflectance (FT-IR -ATR) spectroscopy was demonstrated by Rajandas *et al*. (2012). Two bacterial strains, *Microbacterium paraoxydans* and *Pseudomonas aeruginosa,* degraded 61.0% and 50.5% of low-density polyethylene (LDPE) in two months, respectively. The technique was effective, sensitive, and reproducible, simplifying the process.

The biodegradation of low-density polyethylene (LDPE) by bacteria isolated from a landfill in Chennai, South India, was explored by Pramila *et al*. (2012). The bacteria, identified through 16S rRNA gene sequencing, were tested for growth in LDPE-incorporated media. Results showed that LDPE can act as a substrate for biofilm formation, and these isolates could grow using LDPE as the sole carbon source, demonstrating the potential of isolating specific microorganisms for sustainable plastic waste management.

A Gram-positive bacterium, *Bacillus megaterium* (T6), was found to effectively degrade low-density polyethylene (LDPE) films in a study by Song *et al*. (2012). The strain, isolated from the Indian meal moth *Plodia interpunctella*, showed a 5.8% decrease in LDPE tensile strength after 28 days, indicating its potential for solid pollution reduction.

Aspergillus sp. and *Lysinibacillus* sp. were isolated from Tehran landfill soils by Esmaeili *et al*. (2013), demonstrating their ability to degrade low-density polyethylene (LDPE). The biodegradation process was conducted over 126 days using pure LDPE films without prooxidant additives, with and without UV irradiation. The microorganisms showed higher efficiency, with biodegradation rates of 29.5% and 15.8% for UV and non-UV-irradiated films, respectively. This highlights the potential of these isolates for LDPE biodegradation in natural soil conditions.

Sixty marine bacteria from pelagic waters were evaluated by Harshvardhan *et al*. (2013) for their ability to degrade low-density polyethylene (LDPE). Three positive strains, *Kocuria palustris* M16, *Bacillus pumilus* M27, and *Bacillus subtilis* H1584, showed growth and weight loss after 30 days. The bacteria's viability was confirmed by triphenyltetrazolium chloride reduction tests, and their biodegradation process was supported by FT-IR spectra.

The impact of Iron, Cobalt, and Manganese stearates on the post-bacterial photochemical and thermal degradation of polyethylene (LDPE) was examined by Abrusci *et al*. (2013). It was found that these stearates increased the carbonyl index and decreased the molecular weight of LDPE, indicating enhanced degradation. The study supports the oxo-biodegradation theory, suggesting increased abiotic oxidation levels and decreased molecular weight.

The role of biofilm formation and bacterial colonization in the biodegradation of low-density polyethylene films by *Bacillus amyloliquefaciens* strains BSM-1 and BSM-2 was examined by Das *et al*. (2013). It was found that BSM-2 had superior colonization and biofilm formation capabilities, leading to enhanced polymer degradation. The study calls for further molecular-level investigations to understand these microbial behaviors.

A thermophilic bacterium, *Chelatococcus* sp. E1, capable of degrading low-molecular-weight polyethylene (LMWPE) from commercial PE, was isolated by Jeon *et al*. (2013). The bacterium mineralized LMWPE into CO_2, increasing its molecular weight and causing microbial-induced oxidation and dehydrogenation, highlighting its potential for degrading various LMWPE types.

Pseudomonas sp. AKS2 rapidly degrades low-density polyethylene (LDPE) within 45 days, as observed by Tribedi *et al*. (2013). The degradation is influenced by agents that modify hydrophobic interactions. Mineral oil enhances bacterial attachment, promoting biofilm formation and polymer degradation, while Tween 80 reduces bacterial attachment and consequently decreases degradation. *Pseudomonas* sp. AKS2's unique enzymatic activities enhance LDPE degradation.

Pseudomonas putida, isolated from garden soil, was found by Saminathan *et al*. (2014) to degrade plastic materials by up to 75.3% within a month. The milk cover showed the highest degradation rate, suggesting potential for use in bioremediation technologies to mitigate plastic pollution.

Chryseobacterium gleum EY1 was found to degrade polyethylene (PE) films under UV exposure by Jeon *et al*. (2014). Fe-stearate was identified as the most effective catalyst for photo-degradation, reducing tensile properties and molecular weight. The rate of decay was influenced more by temperature than UV intensity, indicating its potential for bioremediation of agricultural polyethylene waste.

The biofilm-mediated degradation mechanism of *Pseudomonas* sp. AKS2 on low-density polyethylene (LDPE) was investigated by Tribedi *et al*. (2015). It was hypothesized that AKS2 adapts successfully, leading to higher degradation levels. The viability and fitness of AKS2 cells in biofilm were examined, revealing increased hydrolytic activity, metabolic potential, functional diversity, and hydrophobicity.

Two bacterial strains, *Bacillus amyloliquefaciens* (BSM-1) and *Bacillus amyloliquefaciens* (BSM-2), were found by Das *et al*. (2015) to degrade low-density polyethylene (LDPE), a major environmental pollutant. The strains adhered to and grew on LDPE, with BSM-2 showing superior degradation. This suggests the potential of these strains for faster biodegradation, offering a potential solution to plastic pollution.

Naturally occurring soil microbes, specifically *Streptomyces* species, were found by Deepika *et al*. (2015) to have the highest capacity to degrade synthetic polymers and LDPE in India. The microbes, isolated from garbage soil in Andhra Pradesh and Telangana, were assessed for their biodegradation efficiency over 2, 4, and 6 months. The results showed that *Streptomyces* species were highly effective in degrading these materials, indicating their potential for environmental remediation.

Desulfotomaculum nigrificans and *Pseudomonas alcaligenes* were identified by Begum *et al*. (2015) as soil bacteria from plastic-contaminated soil. They were found to be more effective in degrading polythene bags over a 30-day period, resulting in significant weight loss. This suggests that *Pseudomonas alcaligenes* could be a cost-effective and eco-friendly method for reducing plastic waste in the environment.

The optimal conditions for the biodegradation of low-density polyethylene (LDPE) by bacterial isolates *Pseudomonas fluorescens, Pseudomonas aeruginosa*, and *Acinetobacter ursingii* were determined by HUSSAIN *et al*. (2015). After 15 days, Pseudomonas fluorescens showed the highest growth and CO_2 production, followed by *Pseudomonas aeruginosa* and *Acinetobacter ursingii*. FT-IR spectrum analysis revealed significant changes in LDPE

transmission bands, with *Pseudomonas fluorescens* showing the highest percentage of C-H group biodegradation.

Plastic accumulation poses a significant environmental threat due to pollution and ecosystem disruption. Traditional degradation methods are insufficient, and natural degradation is slow. Microbial degradation is the most eco-friendly solution. Bacteria capable of degrading LDPE, a major pollutant, were isolated and screened by Botre *et al*. (2015). Out of 20 cultures, 7 showed high LDPE degradation potential. Two promising isolates, M1 and SWG, from sewage and marine sources, belonging to the genus *Staphylococcus*, demonstrated significant LDPE degradation capability in laboratory conditions.

The biodegradation of LDPE films modified with food-grade dye-sensitized titania nanoparticles and starch blend was explored (Mehmood *et al*., 2016). Bacterial strains identified as effective degraders were *Pseudomonas aeruginosa, Burkholderia seminalis,* and *Stenotrophomonas pavanii*. CC18 showed the highest biofilm formation and hydrophobicity, confirming biodegradation. The study suggests LDPE-titania-starch blends could be used for environmentally friendly shopping bags.

The degradation of polythene, a major environmental pollutant, using microbial methods was investigated by Singh *et al*. (2016). Fifteen bacteria were isolated from soil and tested for polythene degradation. *Bacillus* sp. showed the highest degradation, reducing 42.5% of the polythene's weight. The study highlights the potential of microbes to effectively degrade polythene, offering a promising approach for environmental remediation. *Bacillus* sp. could provide a viable solution to polythene pollution.

The use of *Bacillus subtilis* and biosurfactants to accelerate polyethylene (PE) biodegradation was explored by Vimala *et al*. (2016). Pre-treated PE with UV radiation, the bacteria produced surfactin, which facilitated microbial attachment to hydrophobic PE surfaces, leading to faster PE degradation. Inoculating PE films with these biosurfactants resulted in a 9.26% weight loss in 30 days. The study suggests these biosurfactants could be a promising approach to plastic pollution mitigation.

The biodegradation of LDPE, a synthetic polymer, was investigated by Gupta *et al*. (2016) by isolating and screening bacterial strains from waste disposal sites in the Haridwar region. The clear zone method was used to assess biodegradation activity, with seven isolates showing positive results. *Bacillus* sp., *Pseudomonas* sp., and *Micrococcus* sp. were tentatively

identified. The study highlights the potential of waste disposal sites as polyethylene-degrading microbes and emphasizes the simplicity of the clear zone method for initial screening.

The creation of new microbial consortia from plastic waste processing areas to improve the degradation of low-density polyethylene (LDPE) was aimed at by Skariyachan *et al*. (2016). The best LDPE-degrading bacteria were formulated into bacterial consortia, resulting in significant weight reductions for LDPE strips and pellets over 120 days. The consortia, identified as *Enterobacter* sp. bengaluru-btdsce01, *Enterobacter* sp. bengaluru-btdsce02, and *Pantoea* sp. bengaluru-btdsce03, showed structural changes and bacterial film formation on degraded LDPE strips.

The screening of bacteria capable of degrading synthetic polyethylene plastic from soil samples taken from The Final Waste Process Area in Padang City, West Sumatra, Indonesia was conducted by Agustien *et al*. (2016). Soil bacterial isolation was performed using serial dilution and pour plate techniques on Nutrient Agar medium, resulting in 24 different bacterial isolates. Among these, 11 isolates showed potential for degrading polyethylene plastic. The bacterial isolate coded as BTS-5 demonstrated the highest degradation potential at 11.7% w/w, while BTS-9 and BTS-12 showed the lowest potential at 0.9% w/w.

The use of surfactants to improve polyethylene biodegradation during thermal oxidation was investigated by Mukherjee *et al*. (2017). Polyethylene treated with SDS showed a higher carbonyl index, indicating greater oxidation. Surfactants like SDS, SDBS, and sodium stearate increased oxidation levels. Bacterial treatment with *Lysinibacillus fusiformis* and peptone resulted in maximum weight losses and conversion of carbonyls into unsaturated hydrocarbons. This suggests that anionic surfactants can significantly enhance polyethylene biodegradation.

The degradation potential of bacterial strains (*Serratia* sp. KC1-MRL, *Bacillus licheniformis* KC2-MRL, *Bacillus* sp. KC3-MRL, and *Stenotrophomonas* sp. KC4-MRL) from a limestone cave in Pakistan was evaluated by Jada *et al*. (2017). The results showed that the highest bacterial growth and chemical changes in LDPE were observed in the presence of calcium and glucose, indicating that antibiotic-producing cave bacteria can degrade LDPE, especially in calcium-rich media.

The development of eco-friendly, safe, and cost-effective strategies for the degradation of low- and high-density polyethylene using thermophilic microbial consortia from cow dung was pursued by Skariyachan *et al*. (2017). Plastic-degrading bacteria were enriched from cow

dung samples and assessed for degradation ability using the zone of clearance method and weight reduction. The best isolates were characterized and used to form various microbial consortia. The study identified significant degradation rates of 75 ± 2% for LDPE strips, 55 ± 2% for LDPE pellets, 60 ± 3% for HDPE strips, and 43 ± 3% for HDPE pellets over 120 days at 55°C.

The use of vermicomposting to manage disposable paper cups, which are made of 90% high-strength paper and a 5% polyethylene coating, was explored by Arumugam *et al*. (2017). Two methods were investigated: Vermicompost (VC) and Vermicompost with bacterial consortium (VCB). Both methods reduced total organic carbon, total organic matter, and carbon-to-nitrogen ratios, with VCB demonstrating greater reductions. The VCB setup accelerated the degradation process, reducing the composting period from 19 to 12 weeks. The study also confirmed the degradation of carboxylic and aliphatic groups, disaggregation of cellulose and lignin, and cellulose degradation. Vermicomposting with microbial consortia significantly enhances the degradation rate of paper cup waste, producing nutrient-rich manure in a shorter time.

An eco-friendly method for degrading Low Density Polyethylene (LDPE) using bacterial isolates was investigated by Gupta *et al*. (2017). Three isolates, identified as ISJ51, ISJ55, and ISJ57, showed positive results for polyethylene degradation, indicating they can use LDPE powder as a carbon source. Future research will explore their hydrophobicity and biofilm formation abilities.

The biodegradation of polyethylene by soil microorganisms, *Staphylococcus aureus* and *Bacillus cereus*, was explored by Archana *et al*. (2017). *Staphylococcus aureus* showed higher degradation efficiency, causing a 32.2% weight loss in nutrient medium and 57.3% in minimal medium. The microbes used polyethylene as a carbon source and produced extracellular enzymes, aiding in the degradation process. This suggests an eco-friendly method for managing plastic waste.

India generates 1.5 million tonnes of plastic waste annually, with 1-4% being LDPE. Bioremediation as a cost-effective, eco-friendly alternative was explored by Niloufer *et al*. (2017). Eighteen bacterial isolates, including *Pseudomonas stutzeri* strain AT11, showed the highest LDPE degradation, highlighting its potential for effective biodegradation methods.

The biodegradation of low-density polyethylene (LDPE) using fungi and *actinobacteria* from a waste dumping site was explored by Abraham *et al*. (2017). Two potential strains, *Aspergillus* nomius and *Streptomyces* sp., were identified as capable of degrading LDPE, with *Aspergillus* nomius showing a weight loss of 4.9% and *Streptomyces* sp. showing 5.2%. Significant carbon utilization was observed in the films, with *Aspergillus nomius* producing 4.27 g/L and *Streptomyces* sp. producing 2.85 g/L. This suggests these microbes could be a promising avenue for plastic waste management.

The effectiveness of *Actinomycetes* strains from waste dump yards and virgin soil in biodegrading low-density polyethylene (LDPE) was investigated by Mohan *et al*. (2018). Strains from waste dump yards exhibited higher degradation rates than those from virgin soil. This indicates that *Actinomycetes* associated with plastic waste are more efficient at biodegradation than those from uncontaminated soil. Enhancing soil with these degraders could accelerate the degradation process, highlighting the potential of *Actinomycetes* for plastic bioremediation.

The presence of eight halophilic bacterial isolates in saltpans was investigated by Rafiq *et al*. (2018) in Kovalam, Chennai. Two isolates exhibited hydrophobicity and potential for low-density polyethylene (LDPE) biodegradation. After 60 days, LDPE films treated with these bacteria developed cracks and pits, unlike untreated control films. This study marks the first report of *Nesiotobacter exalbescens* and *Bacillus vietnamensis* in Chennai's hypersaline lakes, highlighting their role in LDPE biodegradation under hypersaline conditions.

The degradation of low-density polyethylene (LDPE) by bacterial strains *Bacillus krulwichiae, Bacillus pseudofirmus, Prolinoborus fasciculus,* and *Bacillus* sp. was revealed by Torre *et al*. (2018) without the need for thermal or oxidative pretreatments. Over 90 days, the weight reductions achieved were 9.9%, 8.3%, 5.1%, and 6.3%, respectively. This depolymerization process is correlated with microbial viability, indicating active metabolism. Further research on bacterial protein concentration and cell surface hydrophobicity could enhance biodegradation efficiency and reduce solid waste accumulation.

The efficient degradation of low-density polyethylene (LDPE) sheets by the dominant *cyanobacterial* species *Phormidium lucidum* and *Oscillatoria subbrevis* was demonstrated by Sarmah *et al*. (2018). Various analyses, including FT-IR, SEM, NMR, CHN content, and thermal and tensile strength tests, were used to monitor structural, morphological, and chemical changes in the polymer. The *cyanobacteria* utilized approximately 4% of the carbon from the

polyethylene, and their rapid growth on the PE surface suggested continuous energy gain. This indicates that these cyanobacterial species could provide a practical and efficient alternative for polyethylene waste management, potentially offering a viable solution to polyethylene pollution under natural conditions.

The isolation of eleven bacterial strains from landfill soil capable of degrading low-density polyethylene (LDPE) using UV radiation was reported by Montazer *et al*. (2018). The strains, including *Pseudomonas* and *Sphingobacterium*, were found effective in the abiotic degradation of LDPE films. FT-IR spectroscopy was used to analyze the abiotic degradation of LDPE films inoculated with these isolates. The most active bacterial isolate, IRN19, exhibited significant LDPE degradation, while IRN11 demonstrated the highest cell mass production. This indicates that combining UV radiation photo-oxidation with biodegradation using new bacteria can enhance polyethylene degradation in a natural and ecologically feasible manner.

The investigation of bacteria from plastic waste dumpsites in Ekiti State University and Ado-Ekiti metropolis for the biodegradation of low-density polyethylene (LDPE) was conducted by Oluwole *et al*. (2018). The bacteria, including *Lysinibacillus xylanilyticus* BN-13, *Pseudomonas aeruginosa* RI-1, *Pseudomonas aeruginosa* JAY2N, *Stenotrophomonas maltophilia* T7D7, *Pseudomonas aeruginosa* SMVIT-1, and *Achromobacter xylosoxidans* YEB RH5, demonstrated potential for LDPE degradation. This suggests their use for on-site plastic pollution remediation. Further research is recommended to understand the mechanisms of degradation, enzyme roles, plasmid involvement, and strain improvement methods.

The ability of *Bacillus* sp. BCBT21, a thermophilic bacterial strain from Vietnam, to degrade three types of plastic bags at 55°C over 30 days was demonstrated by Dang *et al*. (2018). The strain produced various extracellular hydrolase enzymes, resulting in significant changes in the weight, structure, surface morphology, and average molecular weight of the treated bags. This suggests its potential for developing an integrated method for plastic degradation using bacterial and fungal enzymes. The study also highlighted the varying degradability of biodegradable plastic bags in Vietnam, the Netherlands, and Vietnam Academy of Science and Technology (VAST).

Plastic, the second largest solid waste, is being researched for its environmental persistence. Bacteria from landfill soil, specifically SP2 and SP4, were found by Munir *et al*. (2018a) to significantly reduce the weight of low-density polyethylene (LDPE) film after four weeks of incubation. The treated LDPE surface became rough and cracked, with bacteria cells attached.

The findings suggest that these bacteria play a crucial role in degrading plastic materials in landfills.

Plastic-degrading microbes were screened using opaque methods, revealing four bacterial species and two fungal species. *Bacillus amylolyticus*, isolated from garbage soil, was identified by Patil *et al*. (2018) as having the highest degradation potential, reducing plastic weight by up to 32%. Further characterization and optimization could enhance plastic biodegradation levels.

Three *Bacillus amyloliquefaciens* isolates (HK1, GSDM02, and GSDM15) were tested for their effectiveness in biodegrading plastic films, with larger clear-zone strains exhibiting better biodegradation effects, as reported by Zhang *et al*. (2018). Polyvinyl alcohol (PVA) was identified as an effective substitute for polyethylene. Among the isolates, HK1 demonstrated superior biodegradation effects, though further research is needed to optimize the process and evaluate its impact on soil.

The need for environmentally friendly disposal policies for synthetic plastics, particularly Low-Density Polyethylene (LDPE), is highlighted by Devi *et al*. (2019). *Bacillus* sp. strains ISJ51, ISJ55, and ISJ57 were investigated for LDPE degradation, with ISJ55 showing the highest biodegradation rate and protein content, indicating its potential for effective LDPE degradation. Further molecular studies are recommended to enhance biodegradation processes.

The ability of 31 bacterial isolates from a dump yard in Bangladesh to degrade Low-Density Polyethylene (LDPE) was screened by Hossain *et al*. (2019). Ten isolates, identified as PDB, were recognized as effective polythene degraders. While all isolates initially increased biomass, a decrease was observed by the 15th day, with weight loss ranging from 0.42% to 3.17%.

The biodegradation potential of *Bacillus* spp. for LDPE was studied by Shrestha *et al*. (2019), who revealed that these bacteria significantly reduced the weight of LDPE and its pH, demonstrating their potential for environmental pollution-free waste management. The study suggests promoting these bacteria for effective LDPE waste management, highlighting their potential under optimal conditions.

Bacterial isolates capable of degrading plastic, specifically HDPE and LDPE, were identified from soil samples at the Tamangapa landfill in Makassar City by Haedar *et al*. (2019). Using Nutrient Agar supplemented with 2% Polyethylene Glycol and assessing

degradation in liquid Mineral Salt media, six isolates were identified, with isolate T7 demonstrating the highest degradation ability of 6% for HDPE and 8% for LDPE.

A bacterial isolate, identified as *PaeniBacillus* sp., sourced from a Brazilian landfill and solid-waste incinerator, was examined by Bardají *et al*. (2019). The isolate demonstrated the ability to degrade LDPE, modifying and colonizing it as a carbon source after three months of incubation. Additionally, the isolate exhibited a low resistance profile against antimicrobials, suggesting its suitability for bioremediation efforts aimed at polythene degradation.

The effectiveness of two bacterial strains, *Pseudomonas aeruginosa* SKN1 and SKN2, in degrading LDPE from Yazd landfill waste samples was identified by Nourollahi *et al*. (2019). They showed a weight loss of 10.32% in LDPE strips, demonstrating their specialized ability to biodegrade LDPE waste. This suggests their potential for environmental and economic benefits, particularly in environments with rapidly accumulating plastic waste.

Bacillus tropicus, a bacterium found in landfill sites, was identified by Samanta *et al*. (2020) as effective in biodegrading low-density polyethylene (LDPE) films, resulting in a 10.15% weight reduction. The bacterium's properties were tested, showing reduced mechanical strength, altered transparency, and increased haze. This suggests *Bacillus tropicus* as a promising candidate for biodegrading thin LDPE films, offering a potential solution to reduce plastic pollution by utilizing bacteria commonly found in landfill sites.

Natural bacteria for plastic biodegradation were explored by Dey *et al*. (2020), identifying aerobic bacteria from municipal waste dumpsites and bentonite-based drilling fluids as effective in degrading low-density polyethylene (LDPE). *Stenotrophomonas* sp. and *Achromobacter* sp. were found to be capable of degrading LDPE, with the study revealing surface damage and increased nano-roughness on the LDPE films. This suggests further investigation into metabolic pathways and enzymatic reactions to optimize the biodegradation process, highlighting the potential of these bacteria for large-scale plastic bioremediation.

The biodegradation of low-density polyethylene (LDPE) by three *Bacillus* species, ISJ36, ISJ38, and ISJ40, was explored by Gupta *et al*. (2020). ISJ40 showed the highest affinity for PE degradation, forming a robust biofilm and exhibiting significant microbial biomass and metabolic activity. This suggests that ISJ40 can effectively use LDPE as a carbon and energy source, making it a promising eco-friendly solution for PE waste treatment.

The degradation capability of low-density polyethylene (LDPE) by *Pseudomonas aeruginosa* ISJ14, isolated from waste dump sites, was investigated by Gupta *et al*. (2020a). Results showed that *P. aeruginosa* ISJ14 is effective in degrading LDPE, forming biofilms, and utilizing LDPE as a carbon source. This suggests that biodegradation is a promising solution for eliminating plastic from the environment.

The potential of *Bacillus wudalianchiensis* UMT and *Pseudomonas aeruginosa* UMT for accelerating polyethylene degradation was identified by Bakht *et al*. (2020). These bacteria can significantly reduce plastic pollution faster than natural processes, making them suitable for large-scale plastic degradation at landfills and industrial settings. Further research could focus on cloning and expressing plastic-degrading genes.

The biodegradation of LDPE by *Enterobacter cloacae* AKS7, a non-biodegradable and harmful plastic, was explored by Sarker *et al*. (2020). AKS7 efficiently degrades LDPE, forming a biofilm and increasing microbial colonization. Mutants with reduced hydrophobicity compromised LDPE degradation, suggesting AKS7 as a potential sustainable solution for plastic waste management.

Plastic-degrading microorganisms were isolated from soil samples at dumping sites in Himachal Pradesh by Rana *et al*. (2020). Twenty-three isolates were assessed for their plastic-degrading activity, with PDBH1 and PDBM2 identified as elite degraders for further investigation. Environmental factors were found to significantly influence the activity of plastic-degrading bacteria. The selected isolates demonstrated efficient LDPE degradation over 45 days, suggesting their potential for consortium formation.

The biodegradation of Low-Density Polyethylene (LDPE) by bacteria from Nigeria was investigated by Chigor *et al*. (2020). *Pseudomonas aeruginosa* and *Micrococcus* sp. exhibited steady growth on LDPE at 37°C and 50°C, with *Micrococcus* sp. showing the highest growth rates. The study highlights the significant potential of these bacteria for environmental remediation of plastic pollution, although further research is recommended to enhance their degradation capabilities and optimize waste management practices.

In Iran, Soleimani *et al*. (2021) found that *Actinobacteria* from various plastic landfills effectively degrade low-density polyethylene (LDPE). The bacteria, primarily *Streptomyces*, *Nocardia*, and *Rhodococcus*, significantly reduced the weight and tensile strength of LDPE

films. This research highlights the importance of environmental conditions in influencing microbial diversity and biodegradation efficiency.

Jayashree *et al*. (2021) identified *Pseudomonas putida* and *Pseudomonas fluorescens* as effective LDPE degraders from plastic dump sites. These bacteria possess the alkB gene, which is crucial for transforming xenobiotic compounds, including LDPE. The study demonstrates that soil-native organisms can degrade plastic with appropriate methods and time, offering promise for environmental remediation. *Pseudomonas putida* exhibited a high LDPE degradation rate of 40% at 37°C.

Kavitha *et al*. (2021) identified ligninolytic bacteria capable of degrading polyethylene from plastic waste-polluted soil. Two isolates, PE2 and PE3, achieved the highest polyethylene weight reduction of 6.68% after 30 days of incubation, with *Bacillus* sp. strain PE3 being the most effective. This strain formed a biofilm on the polyethylene surface and produced a lipopeptide biosurfactant in both mineral salt and synthetic media. The study suggests that exploring microorganisms from natural resources can lead to effective polyethylene waste degradation and the production of valuable by-products like biosurfactants.

Fibriarti *et al*. (2021) tested local *Bacillus* sp. strains BP4 and BP6, isolated from waste soil, for their ability to degrade Low-Density Polyethylene (LDPE). The results showed that BP4 and BP6 degraded LDPE by 7.23 ± 0.64% and 8.19 ± 0.12%, respectively. The study demonstrated that these bacteria could significantly reduce plastic waste accumulation in soil, with BP6 showing particular promise as a plastic decomposition agent.

Nanthini *et al*. (2021) evaluated the microplastic degradation efficiency of bacterial isolates from the Vaigai River in Madurai, India. The study tested *Bacillus* sp., *Bacillus cereus* (BC), *Bacillus* sp., and *Bacillus paramycoides* (BP) for their ability to degrade UV-treated polyethylene and polypropylene over 21 days. *Bacillus paramycoides* and BC demonstrated the highest degradation rates, suggesting that these bacteria offer a cost-effective and environmentally friendly solution to microplastic pollution.

Zahari *et al*. (2021) investigated the biodegradation of low-density polyethylene (LDPE) and starch-based plastics (SBP) using thermophilic microorganisms, specifically *Bacillus subtilis* and *Candida tropicalis*. The study found that *C. tropicalis* was more effective in degrading SBP compared to LDPE, achieving a significant weight loss of 22.9% and showing notable biofilm development on SBP surfaces. The study also observed surface bubbling on

LDPE and the formation of cracks and holes on SBP, indicating differential degradation patterns between the two types of plastics.

Jeon *et al*. (2021) discovered that *Lysinibacillus* sp. JJY0216 effectively decomposes petroleum-based plastics such as polyethylene and polypropylene without the need for pretreatment. Over a period of 26 days, the strain achieved weight reductions of 4% for polyethylene and 9% for polypropylene. These findings suggest that *Lysinibacillus* sp. JJY0216 holds the potential for developing soil remediation techniques to address plastic contamination. However, further research is required to optimize and fully understand its biodegradation capabilities.

Radinski *et al*. (2021) investigated bacterial plastic biodegradation in Alberta, Canada, emphasizing the role of carbon source restriction in enhancing plastic degradation. The study found that *Pseudomonas* sp. exhibited a significant increase in low-density polyethylene (LDPE) biodegradation when carbon sources were limited to plastic. This suggests that controlling carbon availability in specialized composting environments could effectively stimulate microbial lytic activity and improve plastic waste management. The research highlights the potential for optimizing composting conditions to reduce plastic pollution through targeted microbial action.

Sarker *et al*. (2021) demonstrated that *Enterobacter cloacae* AKS7 has significant potential for enhancing the bioremediation of low-density polyethylene (LDPE). The study found that the hydrophobic properties of AKS7, especially under specific concentrations of glucose and ammonium sulfate, notably improved microbial colonization and LDPE degradation. This suggests that *Enterobacter cloacae* AKS7 could be effectively utilized in bioremediation efforts to manage plastic waste.

Maroof *et al*. (2021) explored an environmentally friendly method for degrading low-density polyethylene (LDPE) using bacteria isolated from waste disposal sites. Among the six strains tested, including *Bacillus siamensis* and *Bacillus wiedmannii*, significant biodegradation was observed over 90 days. The study employed various techniques, such as FE-SEM, FT-IR, and XRD, to analyze the degradation process. Future research should aim to optimize enzyme expression to enhance LDPE biodegradation further.

Nadeem *et al*. (2021) identified four bacterial strains from waste dumpsites in Faisalabad, Pakistan, as potential candidates for low-density polyethylene (LDPE) biodegradation.

Serratia sp. demonstrated the highest biodegradation capability, achieving a 40% weight loss of LDPE plastic pieces after 150 days. *Stenotrophomonas* and *Pseudomonas* species achieved 32% and 21% weight loss, respectively. The study highlights the need for further research to enhance plastic waste management and environmental protection.

Khandare *et al*. (2021) identified four marine bacterial strains, H-237, H-255, H-256, and H-265, for their ability to biodegrade low-density polyethylene (LDPE) films over 90 days without chemical treatment. These bacteria formed biofilms and achieved a maximum weight loss of 1.72%. Their biodegradation capabilities could aid in mitigating plastic pollution in marine environments.

Skariyachan *et al*. (2021) analyzed the biodegradation potential of bacterial consortia from cow dung samples, finding that Consortium CB3 significantly reduced the weight of low-density polyethylene (LDPE) and polypropylene (PP) while forming biofilms. The consortium was identified through microbiological methods and 16S rRNA gene sequencing. Its potential for use in cost-effective industrial biodigesters suggests an innovative approach to managing plastic waste.

Hamzah *et al*. (2022) identified six microorganisms from garden soil capable of degrading low-density polyethylene (LDPE), a significant environmental pollutant. Among them, strain A3, identified as *Riemerella anatipestifer*, demonstrated the highest growth rate and optimal conditions for LDPE degradation. This suggests that *Riemerella* sp. holds significant potential for effective LDPE biodegradation.

Zadjelovic *et al*. (2022) investigated the biodegradation of polyethylene (PE) by the marine bacterium *Alcanivorax* sp. 24 using high-throughput proteomics. The study found that *Alcanivorax* sp. 24 efficiently utilizes leachate from both weathered and pristine PE, leading to a reduction in the molecular weight distribution and mass of LDPE films. This suggests that hydrocarbon-degrading bacteria, such as *Alcanivorax* sp. 24, could play a significant role in the biodegradation of polyethylene.

Saeed *et al*. (2022) explored the degradation of PVC and polyethylene-derived synthetic polymers using fungi and bacteria isolated from plastic waste. The study found that bacterial strains *Bacillus licheniformis* and *Achromobacter xylosoxidans* demonstrated significant degradation capabilities, with polyethylene samples showing 32.2% and 40% degradation in

just 4 weeks. This research highlights the potential of these bacteria and fungi as effective bioremediation candidates for managing plastic waste.

Hou *et al*. (2022) explored the use of polyethylene (PE)-degrading bacteria to address pollution from discarded PE products. The study focused on two *Pseudomonas* strains, *P. knackmussii* N1-2 and *P. aeruginosa* RD1-3, which were found to effectively degrade PE mulching film. This degradation helps reduce "white" pollution in agriculture. The research provides valuable insights into the mechanisms of PE degradation and highlights the potential of these bacteria for environmental management.

Sharma *et al*. (2022) identified *Microbacterium barkeri* SH20 (TN2) as a novel strain with significant polyethylene waste degradation capabilities. Over 30 days, TN2 effectively degraded both low-density polyethylene (LDPE) and high-density polyethylene (HDPE) films, with the highest degradation observed in HDPE. This research suggests promising applications for TN2 in solid plastic waste management, addressing a critical global environmental challenge.

Oluwole *et al*. (2022) investigated six bacterial strains capable of degrading low-density polyethylene (LDPE) water sachet bags over 56 days. The study observed significant changes in the functional groups and elemental composition of the treated films, indicating that these bacteria have the potential for plastic waste bioremediation. This approach offers a promising method for environmental cleanup and plastic waste management.

Mohammadi *et al*. (2022) explored a novel biotreatment process for degrading low-density polyethylene (LDPE) films using a bacterial consortium that produces peroxidase. Over 12 months, the process significantly reduced the molecular weight of LDPE, with hydrogen peroxide (H_2O_2) biostimulating the degradation. This research highlights the potential of this biotechnological approach for sustainable plastic pollution reduction.

Kalia *et al*. (2022) investigated the ability of *Lysinibacillus fusiformis* to degrade low-density polyethylene (LDPE) waste. The bacterium demonstrated promising degradation, achieving 9.51% degradation of 30 µm LDPE films and 1.45% of 50 µm films over 30 days. Xylene pretreatment significantly enhanced degradation efficiency, resulting in 12.09% degradation of 30 µm films and 1.97% of 50 µm films. The study highlights *Lysinibacillus fusiformis* potential for LDPE waste degradation, with xylene pretreatment notably improving its efficiency, particularly for thicker LDPE films.

Emmanuel-Akerele *et al*. (2022) conducted a study in Lagos State, Nigeria, and found that microorganisms such as *Staphylococcus* aureus, *Streptococcus* sp., *Bacillus* sp., and *Micrococcus* sp. are capable of degrading polyethylene and polystyrene, leading to a reduction in their weight. The study recommends strategies for enhancing bioremediation efforts, including minimizing plastic production, promoting the use of degradable plastics, raising public awareness, improving waste disposal systems, and extracting enzymes on a large scale.

Maroof *et al*. (2022) investigated the biodegradation potential of *Exiguobacterium* sp. strain LM-IK2, isolated from plastic waste, for Low-Density Polyethylene (LDPE). The study demonstrated that over a 90-day period, the strain achieved a weight loss of 5.70±0.7%, indicating significant chemical changes and increased crystallinity in the LDPE. Genetic analysis identified the presence of Laccase and Alkane Hydroxylase genes, which are crucial for the degradation process.

Naaz *et al*. (2022) analyzed the biodegradation of polythene bags using microorganisms isolated from waste soil. The study identified *Bacillus* sp., *Arthrobacter* sp., and *Pseudomonas* sp. as efficient degraders. Among these, *Bacillus* species demonstrated superior effectiveness in degrading plastic bags. The natural soil at Jamia Millia Islamia University was found to support this degradation process.

Postolachi *et al*. (2022) optimized a mineral salt medium (MSM) for the biodegradation of low-density polyethylene (LDPE) by *Pseudomonas fluorescens* CNM-PFB-01. Four media with varying salt content and N:P and C: N ratios were evaluated. The strain showed weak positive reactions in MSM 4 (with N:P ratio of 4.30:1 and a C: N ratio of 0.29:1) supplemented with LDPE, increasing media pH and stimulating biomass accumulation. MSM 4 was identified as the optimal medium for enhancing LDPE biodegradation.

Chofifawati *et al*. (2023) explored the potential of *Pseudomonas* sp. for plastic degradation using its inducible operon system. The study demonstrated that *Pseudomonas* sp. could degrade plastics by 2-19% and convert them into simpler molecules within 1-3 months. This suggests that *Pseudomonas* sp. has the potential to provide a sustainable solution for plastic waste through scientific innovation.

Ghosh *et al*. (2023) investigated the potential of halophilic bacteria from Digha, West Bengal, to degrade low-density polyethylene (LDPE). The study identified two isolates with significant potential, demonstrating that 66% of them could depolymerize LDPE. These

bacteria can utilize LDPE as a carbon source, breaking down the polymer into oligomers and monomers. This indicates their potential contribution to plastic waste biodegradation.

Devi *et al*. (2023) found that *Alcaligenes faecalis* strain ISJ128, isolated from partially degraded polyethylene (PE) film, demonstrated significant growth and effectively degraded 10.40% of the PE film within 60 days. The strain's stability and effectiveness were confirmed through scanning electron microscope and FT-IR spectroscopy studies. This highlights its potential as a promising candidate for eco-friendly PE waste disposal and underscores the effectiveness of indigenous microbes in plastic degradation.

Shilpa *et al*. (2023) demonstrated that *Pseudomonas aeruginosa* effectively degraded low-density polyethylene (LDPE), achieving a 9.2% weight reduction in LDPE films after 100 days at 37°C. The study identified the biochemical pathway involved in LDPE degradation and suggested further exploration of microbial consortia to enhance degradation efficiency.

Al-Imara *et al*. (2023) examined the biodegradation capabilities of five bacterial species, including *Bacillus subtilis*, on oil-based plastics such as LDPE, PS, and PVC. The study found that *Bacillus subtilis* was the most effective in degrading LDPE and PVC, with UV irradiation enhancing microbial adhesion. The findings suggest that *Bacillus subtilis* and similar bacteria could be eco-friendly tools for breaking down plastic waste.

Makut *et al*. (2023) investigated the biodegradation of Low-Density Polyethylene (LDPE) by bacteria isolated from dump sites in North Central Nigeria. Bacteria such as *Pseudomonas aeruginosa* and *Providencia stuartii* demonstrated significant weight loss of LDPE over an 8-week period at 30°C. The study highlights the potential of these bacteria for polyethylene waste degradation and recommends developing commercial bioreactors to enhance the biodegradation process.

Shilpa *et al*. (2023) identified a novel bacterial strain, *Pseudomonas aeruginosa* WD4, that effectively degrades LDPE films by using LDPE as a nutritional source. Analytical tests confirmed its biodegradation potential, suggesting it as a cost-effective and eco-friendly solution to global LDPE waste and plastic pollution. The study recommends further research to enhance LDPE biodegradation efficiency.

Mallisetty *et al*. (2023) analyzed the biodegradation of LDPE by aerobic bacteria from offshore RK Beach, Visakhapatnam. Six strains were identified as effective in degrading LDPE, with *PaeniBacillus* sp. and *Serratia* sp. demonstrating the highest efficiency. These

bacteria utilized LDPE as their sole carbon and energy source, indicating their potential for environmental applications in plastic waste management.

Ali *et al*. (2023) isolated bacteria from sewage wastewater capable of degrading plastic, which poses environmental and health risks. Strains *Pseudomonas* sp. SH5B and *Pseudomonas aeruginosa* SH6B demonstrated 25% plastic degradation after 120 days. FT-IR analysis revealed bond stretching, bending, and new bond formation, suggesting potential for green chemistry solutions in plastic waste management.

Vinuthna *et al*. (2023) found that *Bacillus vallismortis* can effectively biodegrade polyethylene, a prevalent environmental pollutant. Under optimal conditions, the strain achieved significant LDPE degradation, with 33.79% and 29.42% reductions in 50 µm and 75 µm films, respectively, and produced substantial CO_2. This suggests *B. vallismortis* is a promising solution for sustainable plastic waste management.

Ji *et al*. (2023) explored the use of low-temperature plasma treatment to enhance the biodegradation of low-density polyethylene (LDPE), a significant environmental pollutant. The treatment induced oxidation, creating new chemical bonds and altering the surface properties of LDPE. When combined with LDPE-degrading bacteria, plasma-treated films showed improved adhesion and colonization efficiency. This suggests that plasma pretreatment can facilitate bacterial adhesion to plastic surfaces, offering a potential strategy to mitigate plastic pollution, especially in agricultural environments.

Alamer *et al*. (2023) identified two bacterial strains, APCK5 and APCZ14, as effective in biodegrading low-density polyethylene (LDPE) in plastic-contaminated water samples from Al-Ahsa, Saudi Arabia. These strains demonstrated significant LDPE degradation capabilities, highlighting their potential for environmentally friendly plastic removal.

Nademo *et al*. (2023) investigated bacterial strains from Addis Ababa's Koshe municipal solid waste dumping site and found that KS35, KS14, and KS119—identified as *Methylobacterium radiotolerans*, *Methylobacterium fujisawaense*, and *Lysinibacillus fusiformis*, respectively—demonstrated significant LDPE weight loss. These strains show potential for degrading LDPE bags, suggesting they could be used for effective plastic waste management.

Szczyrba *et al*. (2023) identified twelve bacterial strains with the ability to biodegrade low-density polyethylene (LDPE), a significant environmental concern. While these strains did not

exhibit an increase in biomass, they caused chemical changes in the LDPE and formed biofilms. Pretreatment with nitric acid notably improved the biodegradability of LDPE, highlighting the potential of these bacteria for managing polymer waste through biofilm formation and the production of extracellular enzymes and metabolites.

Tao *et al*. (2023) studied the microbial degradation of polyethylene (PE) and identified *Rhodococcus* strain A34 as a capable degrader, achieving a 1% weight loss of PE after 30 days. The study pinpointed key enzymes involved in the oxidation and depolymerization of PE. This information can aid in optimizing conditions for plastic degradation and in designing synthetic microbial communities to enhance biodegradation efficiency. The research suggests the potential for refining degradation conditions and improving enzyme efficiency through protein engineering, advancing the understanding and application of bacterial PE degradation.

Hossain *et al*. (2024) discovered that *Bacillus cereus* SHBF2, a floc-forming bacterium from an aquaculture farm, effectively degraded microplastics such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) over a 60-day period. The bacterium achieved a 6.87% weight loss for PE and demonstrated the highest growth efficiency with PP at 6.77%. These findings highlight *B. cereus* SHBF2's potential for sustainable green aquaculture applications, although achieving complete removal of microplastics remains a challenge.

Febria *et al*. (2024) investigated the biodegradation potential of marine bacterial isolates for low-density polyethylene (LDPE), a significant oceanic plastic pollutant. Four strains—*Lysinibacillus* sp. IBP-1, *Bacillus* sp. IBP-2, *Bacillus paramycoides* IBP-3, and *Bacillus cereus* IBP-4—were isolated from plastic samples and tested with LDPE powder over 35 days. The study reported a 10-15% weight loss and a reduction in LDPE mass, indicating effective bacterial degradation. This research underscores the potential of these bacterial isolates for LDPE biodegradation and suggests further exploration to optimize the process and elucidate the underlying mechanisms.

Banerjee *et al*. (2024) investigated the degradation of low-density polyethylene (LDPE) plastic beads using selected bacterial isolates. Over 60 days, a significant weight reduction in LDPE beads was observed, with degradation rates increasing when 10% starch was present. The degradation was confirmed through scanning electron microscopy and FT-IR analyses, highlighting the effectiveness of the bacterial isolates in breaking down LDPE and suggesting that starch can enhance the degradation process.

Ji *et al*. (2024) identified *Bacillus safensis* BS-10L as an efficient bacterium for degrading low-density polyethylene (LDPE) from plastic waste. Plasma treatment was found to significantly increase the bacterial population and viability, resulting in notable surface damage to the LDPE. This study demonstrates that plasma technology can effectively enhance the plastic-degrading capabilities of microorganisms, suggesting it as a promising approach for managing plastic waste and mitigating environmental pollution.

Seong *et al*. (2024) introduced a novel bioprocess utilizing pseudo-resting cells of *Acinetobacter nosocomialis*, isolated from landfills containing polyethylene (PE), to effectively degrade low-density polyethylene (LDPE). The bioreactor operation resulted in noticeable holes on the LDPE film, indicating degradation. Analytical techniques such as ATR-FT-IR and XPS revealed an increase in oxygen-containing functional groups and enhanced hydrophilicity on the LDPE surface, suggesting that this biocatalytic approach holds promise for addressing global plastic waste issues.

Nehal *et al*. (2024) identified *Bacillus* sp. MZ540316, *Bacillus* sp. MZ540327, and *BreviBacillus borstelensis* MZ562352 as effective in degrading plastics and synthetic dyes. While *Bacillus* sp. MZ540316 and *Bacillus* sp. MZ540327 exhibited moderate decolorization effects against synthetic dyes and effectively degraded LDPE and HDPE pellets, *BreviBacillus borstelensis* MZ562352 showed lower degradation rates for plastics but achieved high decolorization rates against synthetic dyes.

Mazaheri *et al*. (2024) explored the biodegradation of low-density polyethylene (LDPE) by two strains from landfill soil in Hamadan province, Iran. *Stenotrophomonas* sp. and *Alcaligenaceae* bacterium showed significant weight loss and detoxification after three months, with LDPE degradation end-products detected. This suggests the bacteria's potential for environmentally friendly plastic bioremediation.

Fulke *et al*. (2024) studied the biodegradation potential of microorganisms associated with plastic surfaces in marine environments. Four *Pseudomonas* strains (ABFPD01, ABFPD02, ABFPD03, ABFPD05) were identified to significantly degrade polyethylene strips. This highlights marine environments as a valuable source for plastic-degrading microorganisms, offering a potential solution to plastic pollution.

ii. Fungal Biodegradation

Raghavan *et al*. (1992) utilized *Aspergillus niger* to degrade thermoplastic polyethylene films, observing a decrease in sample amorphocity. The presence of sucrose in the growth medium significantly influenced the process. FT-IR analysis revealed that biotic-treated polyethylene followed a distinct degradation pathway, characterized by unique double bond peaks. The study found that adapted *A. niger* was crucial for effective biodegradation, with sucrose supporting fungal growth and enhancing the bio-oxidation of amorphous polyethylene units.

Otake *et al*. (1995) studied the biodegradation of polymers under soil conditions and revealed significant degradation in low-density polyethylene (LDPE) thin films, characterized by hyphae growth and FT-IR spectra. This suggests that LDPE can undergo significant biodegradation under bioactive conditions, particularly in shallow soil with high aerobic activity, where hyphae and filamentous fungi contribute to the process.

Zahra *et al*. (2010) evaluated the biodegradation of low-density polyethylene (LDPE) by fungi from a landfill in Tehran. The fungi, including *Aspergillus fumigatus*, formed a biofilm and degraded LDPE. The process was monitored for optimal moisture and pH levels. The study is the first to report LDPE degradation by *A. fumigatus* under solid waste conditions, highlighting the potential for LDPE biodegradation and composting.

The biodegradation of low-density polyethylene (LDPE) by two fungal strains, *Mucor circinilloides* and *Aspergillus flavus*, was investigated by Pramila *et al*. (2011). Colonization studies, SEM analysis, and the Sturm test were used to assess the fungi's ability to degrade LDPE, revealing their potential to grow on LDPE as the sole carbon source.

The diversity and load of heterotrophic fungi involved in polyethylene degradation in polluted sites in Chennai, Tamil Nadu, were examined by Raaman *et al*. (2012). *Aspergillus japonicus* demonstrated a higher degradation potential of 12% compared to 8% for *A. niger* within one month, highlighting the significant role of these fungi in LDPE degradation.

The biodegradation of low-density polyethylene (LDPE) using fungal species was investigated by Jyoti *et al*. (2014). Six species, including *Aspergillus, Fusarium, Mucor,* and *Penicillium*, were cultured with LDPE as a carbon source. *Fusarium* and *Penicillium* exhibited the most effective degradation, resulting in a weight loss of 16% to 36% over four weeks, suggesting a promising microbial approach to tackle plastic pollution.

The biodegradation of plastics was compared between fungal species *Aspergillus niger* and bacterial species *Bacillus weihenstephanensis, Burkholderia cepacia*, and *Escherichia coli* by Mukherjee *et al*. (2014). *Bacillus weihenstephanensis* demonstrated strong degradation capabilities against thick plastic bags, while *Burkholderia cepacia* was particularly effective against thin plastics. These findings suggest the potential for developing commercial biodegradable and eco-friendly plastic products.

The isolation of microorganisms capable of degrading low-density polyethylene (LDPE) from soil samples of an aged landfill in Tehran, Iran, was carried out by Esmaeili *et al*. (2014). The study identified two super strains, *Lysinibacillus xylanilyticus* XDB9 and *Aspergillus niger,* following enrichment culture procedures. The results indicated increased fungal biomass and bacterial growth, suggesting the potential of preoxidized PE as a carbon source for these isolates. This highlights their potential for bioremediation applications.

The biodegradation of polyethylene films by *Aspergillus* species from Niger Delta mangrove soil was investigated by Immanuel *et al*. (2014). The *Aspergillus* species, namely *Aspergillus japonicus* and *Aspergillus terreus*, utilized the films as their carbon source, resulting in a reduction of dry weight by 10.70%-22.54% after 45 and 60 days of incubation. The study suggests that environmental microbes have potential in addressing plastic accumulation, although further research is needed to identify the specific enzymes and genes involved in the degradation process.

The study by Sheik *et al*. (2015) explores the biodegradation of plastic waste using endophytic fungi from *Psychotria flavida* and *Humboldtia brunonis*, which produce enzymes capable of degrading plastics. The fungi were inoculated on polyethylene and polypropylene films, which were subjected to varying doses of radiation and incubated for 90 days. The results demonstrated that *Aspergillus* sp., *Paecilomyces lilacinus* from *H. brunonis*, and *Lasiodiplodia theobromae* from *P. flavida* effectively degraded gamma-irradiated LDPE. Additionally, only *L. theobromae* from *P. flavida* was able to degrade irradiated polypropylene, resulting in a 0.3 mg weight loss.

The study by Devi *et al*. (2015) investigated the biodegradation of high-density polyethylene (HDPE) from marine coastal waste using two fungal strains, *Aspergillus tubingensis* VRKPT1 and *Aspergillus flavus* VRKPT2. Identified through ITS region sequence analysis, both strains demonstrated effective biofilm formation, viability, and enzyme activity. Among them, A. flavus VRKPT2 showed superior performance in colonization, biofilm

formation, and HDPE degradation compared to *A. tubingensis* VRKPT1. The study suggests that future research should focus on genomics and proteomics to further enhance the degradation rates.

The study by Vignesh *et al*. (2016) addressed the environmental threat posed by plastic and polythene waste by examining the biodegradability of Low-Density Polyethylene (LDPE). Microorganisms, including both bacteria and fungi, were isolated from soil samples collected from Pallikaranai and the harbor in Chennai and screened for their ability to degrade plastics using the opaque method. The study identified three bacterial species (*Bacillus* sp., *Pseudomonas* sp., and *Streptococcus* sp.) and three fungal species (*Aspergillus* sp. and two *Fusarium* sp.) as the most effective degraders. Notably, *Bacillus* sp. from petroleum soil degraded LDPE plastic by 23%, while *Fusarium* sp. II achieved a 44% degradation over 30 days in laboratory shaker culture. Fungal species exhibited a faster degradation rate compared to bacterial species, suggesting potential complete degradation in approximately 75 days for fungi and 120 days for bacteria. The study recommends further biochemical tests and characterization of these microbes to enhance plastic biodegradation efficiency.

The study by Awasthi *et al*. (2017) explores the biodegradation of low-density polyethylene (LDPE) using the fungal isolate *Rhizopus oryzae* NS5. This strain adhered to and grew on LDPE surfaces, leading to an 8.4 ± 3% weight reduction and a 60% decrease in tensile strength. The findings highlight the potential of *Rhizopus oryzae* NS5 for eco-friendly and sustainable LDPE degradation, underscoring the value of discovering microorganisms capable of breaking down artificial plastics.

Hikmah *et al*. (2018) explored the biodegradation potential of *Trichoderma* spp. isolates for low-density polyethylene (LDPE) plastic. Five isolates were screened for their ability to degrade LDPE, with TL1, TL4, and TL5 demonstrating the most potential. The degradation percentages achieved were 4.87%, 7.12%, and 7.51%, respectively. Scanning electron microscopy (SEM) revealed visible surface damage and unevenness after the process. The findings indicate that three out of five lignolytic *Trichoderma* spp. isolates from Zalacca plantation show promise for LDPE plastic biodegradation.

Muhonja *et al*. (2018) identified bacteria and fungi capable of degrading low-density polyethylene (LDPE) from the Dandora dumpsite. Degradation was evaluated using weight loss analysis, FT-IR , and GC-MS. Fungi, particularly *Aspergillus oryzae*, demonstrated higher degradation compared to bacteria. The presence of aldehyde, ether, and carboxyl groups

confirmed biodegradation. Enhancing microbial activity and enzyme production, along with environmentally friendly pre-treatments, is suggested to improve the commercial application of polyethylene biodegradation.

Molecular and biochemical characteristics of LDPE-degrading fungi and bacteria from a Nairobi dumpsite were analyzed by Muhonja *et al*. (2018a). *Aspergillus oryzae* strain A5,1 demonstrated the highest fungal degradation capacity, while *Bacillus cereus* strain A5,a and *BreviBacillus borstelensis* strain B2,2 exhibited the highest bacterial degradation. The identification of the alkane hydroxylase-encoding gene AlkB in four bacterial samples suggests its role in LDPE degradation. These findings indicate the potential use of these fungi and bacteria for LDPE bioremediation.

The potential of fungi to degrade low-density polyethylene (LDPE) was investigated by Munir *et al*. (2018). Nine fungal isolates from landfill soil were cultured in LDPE powder broth. *Trichoderma viride* (RH03) and *Aspergillus nomius* (RH06) showed the best growth response, significantly reducing the weight and tensile strength of LDPE films after 45 days. These findings highlight the potential of RH03 and RH06 in mitigating LDPE pollution.

Kunlere *et al*. (2019) revealed that low-density polyethylene (LDPE) can be biodegraded by two fungal species and eight bacterial species, using LDPE as both nitrogen and carbon sources. The research involved heat-sterilized ground LDPE in media with varying carbon and nitrogen sources. The findings challenge the idea that LDPE is entirely resistant to biodegradation, emphasizing the need for further research to understand the mechanisms, optimal conditions, and microbial genes involved. The study monitored biodegradation using gravimetric methods and Fourier transform infrared spectroscopy, demonstrating potential for LDPE biodegradation.

Sáenz *et al*. (2019) found that *Aspergillus niger* and *Aspergillus terreus* from an Ecuadorian mangrove can degrade low-density polyethylene (LDPE) without requiring additional nutrients or photothermal treatment. They achieved up to 30% weight reduction of LDPE, with observable cracks and biomass growth on the polymer's surface. This suggests that fungal degradation initiates at the surface of the polymer.

Ogunbayo *et al*. (2019) investigated the biodegradation of plastic waste using *Aspergillus niger* (fungi), *Pseudomonas* sp. (bacteria), and a combination of both microorganisms from a dumpsite at the University of Lagos. Over 60 days, *Aspergillus niger* demonstrated superior

degradation efficiency, and the combination of both microorganisms exhibited the highest degrading efficiency. This suggests a synergistic effect between the fungi and bacteria in plastic degradation.

Hyder *et al*. (2021) identified four fungal isolates capable of degrading low-density polyethylene (LDPE) plastics in Peshawar, Pakistan. The weight loss percentages observed were 22.9% for *Aspergillus niger*, 16.1% for *Aspergillus flavus*, 18.4% for brown rot fungi, and 22.7% for white rot fungi. The study recommends conducting large-scale research to improve the degradation of substantial polyethylene concentrations.

El-Sayed *et al*. (2021) investigated the biodegradation of low-density polyethylene (LDPE) sheets using fungi from landfill sites in Sharqiyah Governorate, Egypt. *Aspergillus carbonarius* and *A. fumigatu*s MF 276893 were identified as effective for LDPE biodegradation. The mixed culture of these fungi resulted in higher weight loss percentages of LDPE sheets. Physical and chemical treatments improved biodegradation, with thermal treatment enhancing it by 39.1%, HNO_3 treatment by 17.76%, and gamma irradiation by 5.79%. The study emphasizes the growing issue of plastic overconsumption and presents biodegradation as an eco-friendly solution.

Abbasi *et al*. (2021) reviewed microbial species capable of effective plastic biodegradation and compared pretreatment strategies. The review highlights that *Pseudomonas* and *Arthrobacter* species degrade high-density polyethylene (HDPE) by 15% and 12%, respectively. *Aspergillus glaucus* and *Aspergillus niger* are noted for their efficiency in degrading low-density polyethylene (LDPE) by 28.80% and 12%, respectively, while *Bacillus subtilis* and *Rhizopus oryzae* degrade LDPE by 9.26% and 8.4%, respectively.

Fachrul *et al*. (2021) tested an indigenous microbial consortium for its ability to degrade low-density polyethylene (LDPE) plastics. The consortium and fungi showed resistance to LDPE under controlled conditions, with the highest degradation occurring at 30°C. These findings underscore the consortium's potential environmental impact and its significance in plastic waste management.

Ayeni *et al*. (2022) investigated the microbial degradation of polyethylene from a Nigerian dumpsite and found that fungal isolates such as *Aspergillus nidulans*, *Eurotium repens*, and *Penicillium chrysogenum* effectively biodegrade the material. These results suggest potential

for developing eco-friendly waste management strategies to address environmental pollution from improper polyethylene disposal.

Ali *et al*. (2022) analyzed microorganisms from plastic-polluted sites to determine their ability to biodegrade low-density polyethylene (LDPE) films. The findings revealed that *Fusarium equiseti* and *BreviBacillus parabrevis* showed the most colonization on LDPE films, achieving up to 60% and 65% weight loss, respectively, confirming their effectiveness in degrading LDPE films.

Obaid *et al*. (2023) examined *Aspergillus niger* biodegradation on low-density polyethylene (LDPE) in Iraq. The fungus showed adaptability and growth in LDPE media at various concentrations, temperatures, and pH levels. Environmental factors significantly influenced *A. niger* ability to degrade LDPE, emphasizing the importance of time and adaptation in initiating the process.

Gong *et al*. (2023) identified *Cladosporium* sp. CPEF-6 as a potent fungal strain capable of degrading low-density polyethylene (LDPE), a non-degradable plastic causing environmental issues. The strain-induced weight loss in LDPE, which increased after heat treatment (T-LDPE), and produced laccase during T-LDPE degradation, suggesting its potential for large-scale LDPE waste management and environmental remediation efforts.

Chaudhary *et al*. (2023) investigated the biodegradation potential of a *Cephalosporium* strain NCIM 1251 on pre-treated LDPE and HDPE films. After 56 days, significant weight reductions were observed, indicating effective biodegradation. Physicochemical changes, FT-IR, TGA, and SEM images confirmed the biodegradation, highlighting the potential of the *Cephalosporium* strain in plastic waste management. The synergistic effect of UV and acid treatment was also emphasized.

Kučić Grgić *et al*. (2023) assessed a microbial consortium from activated sludge, river sediment, and compost for degrading LDPE/TPS. Eight bacteria (e.g., *Bacillus* species) and five molds (e.g., *Aspergillus* species) were identified. Two experiments (E1 and E2) demonstrated effective degradation of polymeric materials, with complete degradation of TPS observed in E1-2 and E2-2. Weight loss percentages for LDPE and composite films were higher in E2 than in E1. SEM showed moderate surface degradation in LDPE, while FT-IR analysis indicated more intense degradation in E2. The study highlights the consortium's potential but

suggests further research on pure culture efficiency and new biodegradable materials is needed to address plastic pollution effectively.

Wróbel *et al*. (2023) isolated microorganisms capable of degrading polyethylene and polypropylene from landfill environments. Seven bacterial and seven fungal strains showed potential for PE degradation, while six bacterial and six fungal strains demonstrated PP degradation ability. Scanning electron microscopy revealed biofilm formation and surface changes, highlighting the effectiveness of isolating plastic-degrading microorganisms.

Mechanism of biodegradation

i. Oxidative Degradation

The LDPE biodegradation process initiates with oxidative processes, in which microbial enzymes or reactive oxygen species (ROS) produced by bacteria oxidize the polymer surface (Albertsson et al., 1987; Tokiwa & Calabia, 2004).

Microbial attachment refers to the process by which microorganisms, specifically bacteria, and fungi, establish colonies on the surface of a polymer. The attachment is made possible by synthesizing extracellular polymeric substances (EPS), which aid in adhesion and enzymatic activity (Lear et al., 2021).

ii. Enzymatic Hydrolysis

Microorganisms secrete extracellular enzymes (Table 1), such as lipases, esterases, and peroxidases, which break down the polymer chains of LDPE into smaller fragments (Pathak and Navneet, 2018; Hadad et al., 2005). Hydrolysis of esters in LDPE is catalyzed by esterases, producing hydroxyl and carboxyl groups (Hadad et al., 2005; Bano et al., 2017).

iii. Penetration and Internal Deterioration

Enzymes and microbial activity infiltrate the polymer matrix, causing internal degradation (Tokiwa and Calabia, 2004).

Random Chain Scission: Microbial enzymes randomly cleave the polymer chains, leading to the production of oligomers and polymers with low molecular weight (Albertsson et al., 1987; Hadad et al., 2005).

Table 1: Enzymes involved in LDPE degradation

Enzyme	**Microorganism**	**References**
Lipase	*Penicillium citrinum*	Khan et al., 2023
Esterase	*Penicillium citrinum*	Khan et al., 2023
Laccase	*Penicillium citrinum, Aspergillus terreus, Penicillium sp., Bacillus sp., Neopestalotiopsis phangngaensis, Oscillatoria subbrevis, Phormidium lucidum*	Sarmah et al., 2018; Khruengsai et al., 2022; Eldin et al., 2022; Khan et al., 2023
Manganese peroxidase	*Penicillium citrinum, Neopestalotiopsis phangngaensis, Oscillatoria subbrevis, Phormidium lucidum*	Sarmah et al., 2018; Khruengsai et al., 2022; Khan et al., 2023
Peroxidase	*Aspergillus terreus, Penicillium sp., Bacillus sp.*	Eldin et al., 2022
Lignin peroxidase	*Neopestalotiopsis phangngaensis*	Khruengsai et al., 2022
Alkane monooxygenase	*Paenibacillus sp.*	Bardají et al., 2019

iv. Metabolism

Metabolism of Degradation Products refers to the process by which the breakdown products of a substance are transformed and processed within an organism. Microorganisms utilize the breakdown products, including oligomers and monomers, as a source of carbon and energy for their growth (Pathak and Navneet, 2010).

Metabolite Generation: Microorganisms produce metabolic intermediates and by-products, such as organic acids and gases, during the absorption process (Tokiwa and Calabia, 2004; Hadad et al., 2005).

v. Complete mineralization

Microorganisms can fully mineralize breakdown products into carbon dioxide, water, and biomass under favourable conditions (Tokiwa and Calabia, 2004).

Factors Affecting Biodegradation

i. Temperature

The temperature has a crucial impact on the process of LDPE biodegradation by affecting the activity of microorganisms and the efficiency of enzymes. Elevated temperatures typically expedite the deterioration process by augmenting the metabolic rates of bacteria. The most

suitable temperatures for the biodegradation of LDPE generally fall within the range of 25°C to 45°C, which can vary based on the specific microbial species and ambient factors. Temperatures outside this range can hinder microbial activity and decomposition. Studies have shown that thermophilic microorganisms, which flourish in high temperatures, can break down LDPE more because their enzymatic activity is heightened in these settings (Tokiwa and Calabia, 2004; Albertsson et al., 1987).

ii. UV Radiation

UV exposure causes the oxidative breakdown of LDPE by breaking down polymer chains and producing reactive oxygen species (ROS). Photodegradation enhances the polymer's vulnerability to microbial attack by inducing surface oxidation and rendering the polymer more susceptible to enzymatic degradation. Exposure to ultraviolet (UV) radiation can significantly accelerate the process of degradation in low-density polyethylene (LDPE), particularly in areas with intense UV levels. UV exposure causes the degradation of LDPE, resulting in the formation of lower molecular weight fragments and microplastics. These particles can remain in the environment for a long time and contribute to the problem of plastic pollution (Andrady, 2011).

iii. Oxygen Availability

The breakdown of LDPE is significantly affected by oxygen, with aerobic circumstances generally promoting faster and more effective biodegradation compared to anaerobic settings. Aerobic degradation is a process where aerobic bacteria utilize oxygen to enzymatically oxidize LDPE polymer strands. These bacteria secrete enzymes such as peroxidases and oxygenases that degrade LDPE into smaller, more biodegradable bits. In environments lacking oxygen, such as anaerobic conditions, the degradation rates are slower because the metabolic activity of anaerobic microbes is lowered (Tokiwa and Calabia, 2004; Pathak and Navneet, 2018).

iv. Moisture Content

Microbial growth and enzymatic activities involved in the breakdown of LDPE require moisture. Sufficient moisture levels encourage the colonization of LDPE surfaces by microorganisms and enhance the enzymatic hydrolysis and oxidation of polymer chains. Inadequate moisture levels can impede microbial activity and enzymatic degradation, decelerating the biodegradation process. Moisture has a crucial role in preserving the structural integrity of microbial biofilms on LDPE surfaces. These biofilms shield microorganisms from

environmental pressures and improve their ability to break down substances (Shyam et al., 2023).

v. Microbial Diversity

A wide range of microorganisms capable of breaking down LDPE is essential for biodegradation. Various microbial species synthesize a diverse range of enzymes, including lipases, esterases, and peroxidases, which facilitate the degradation of LDPE polymer chains. The presence of a wide range of microorganisms guarantees that different stages of LDPE degradation, including initial attachment, polymer hydrolysis, and mineralization of degradation products, are effectively performed. Research has demonstrated that microbial communities with a wide range of metabolic abilities are more efficient at breaking down low-density polyethylene (LDPE) than single-species cultures (Hadad et al., 2005).

vi. Enzymatic Activity

Enzymatic activity refers to the ability of enzymes to catalyze chemical reactions. Microorganisms produce extracellular enzymes that are crucial for the enzymatic breakdown of LDPE. These enzymes are accountable for decomposing the polymer into tiny fragments that can be easily used by microorganisms as supplies of carbon. Lipases and esterases are enzymes that break down ester bonds in LDPE, forming hydroxyl and carboxyl groups. These groups make it easier for microorganisms to break down the LDPE further. Comprehending the enzymatic pathways associated with LDPE degradation is crucial for enhancing biodegradation procedures and advancing biotechnological solutions for managing plastic waste (Pathak and Navneet, 2018; Albertsson et al., 1987).

vii. Biofilm Formation

Microorganisms frequently develop biofilms on the LDPE surface, which increases microbial adhesion and enzymatic destruction. Biofilms shield microorganisms from various environmental pressures, such as UV radiation and desiccation, while also enabling the gradual breakdown of LDPE. Biofilm development on LDPE surfaces enhances microbial colonization and enzymatic activity, facilitating the effective degradation of polymer chains. The characteristics and makeup of biofilms can differ based on environmental factors and types of microorganisms, which can impact the speed and extent of LDPE biodegradation (Hadad et al., 2005).

viii. Nutrient Availability

Sufficient availability of nutrients, such as carbon and nitrogen sources, is crucial for facilitating the growth of microorganisms and the generation of enzymes during the degradation of LDPE. Microorganisms rely on nutrition to produce the enzymes necessary to break down polymer chains and sustain their metabolic processes. Insufficient nutrient availability might impede the growth of microorganisms and the breakdown of enzymes, therefore decelerating the biodegradation process. The presence of abundant nutrients stimulates the growth of microbes that can break down LDPE plastic, leading to more effective ways of cleaning up plastic trash (Tokiwa and Calabia, 2004).

Materials and methodology

Sample collection

Six partially degraded plastic samples were collected from a waste dumpsite adjacent to M hostel, Thapar Institute of Engineering and Technology, Patiala. (Location: 30.352963 °N, 76.360151°E) (Devi et al., 2023).

Isolation

Partially degraded plastic samples were taken, aseptically cut into small pieces, and suspended in 10mL Bushnell Haas Broth medium (HiMedia) (Appendix I) in a test tube, and incubated for 7 days at 37ºC. After seven days, 100µL of the media was added in a 900µL sterile 0.9% saline solution to make 1:10 dilution, and it was further serially diluted until 1:100000 dilution and spread on Nutrient Agar (Appendix I) enrichment medium and incubated at 37ºC overnight. Each colony was picked from a 1:100000 dilution spread plate and streaked on Nutrient Agar enrichment solid agar media plates to obtain the pure culture of the bacteria (Hadar et al., 2004).

Colony morphology and Gram staining

Isolates bacteria were classified based on their colony morphology by following Microbiology a laboratory manual. Pearson (Cappuccino, J. 2018).

Gram characterization: A bacterial smear was created from actively developing cells and spread on a glass slide, followed by heat-fixing. After that, it was flooded with crystal violet for 10 seconds followed by washing gently with water to remove any remaining crystal violet stain. It was then flooded with gram iodine for 10 seconds and rinsed with water. Decolorization was done with ethyl alcohol until the moving dye front reached the lower edge, which was washed immediately with tap water. It was counterstained with safranin for 15 seconds and then washed with water to eliminate any leftover stains. Finally, the slide was air-dried and examined under a microscope at various magnification levels.

Screening of plastic-degrading bacteria

Zone of clearance-based screening

Polyethylene Glycol (PEG) plate preparation: 5% of PEG-4000 was prepared in the Bushnell Haas Agar media (HiMedia) (Appendix I). The agar concentration was 2% in Bushnell Haas Agar media, which did not interfere with the solidification of 5% of PEG-4000 and autoclaved the media at 121ºC for 15 minutes at 15 psi (Nademo et al., 2023).

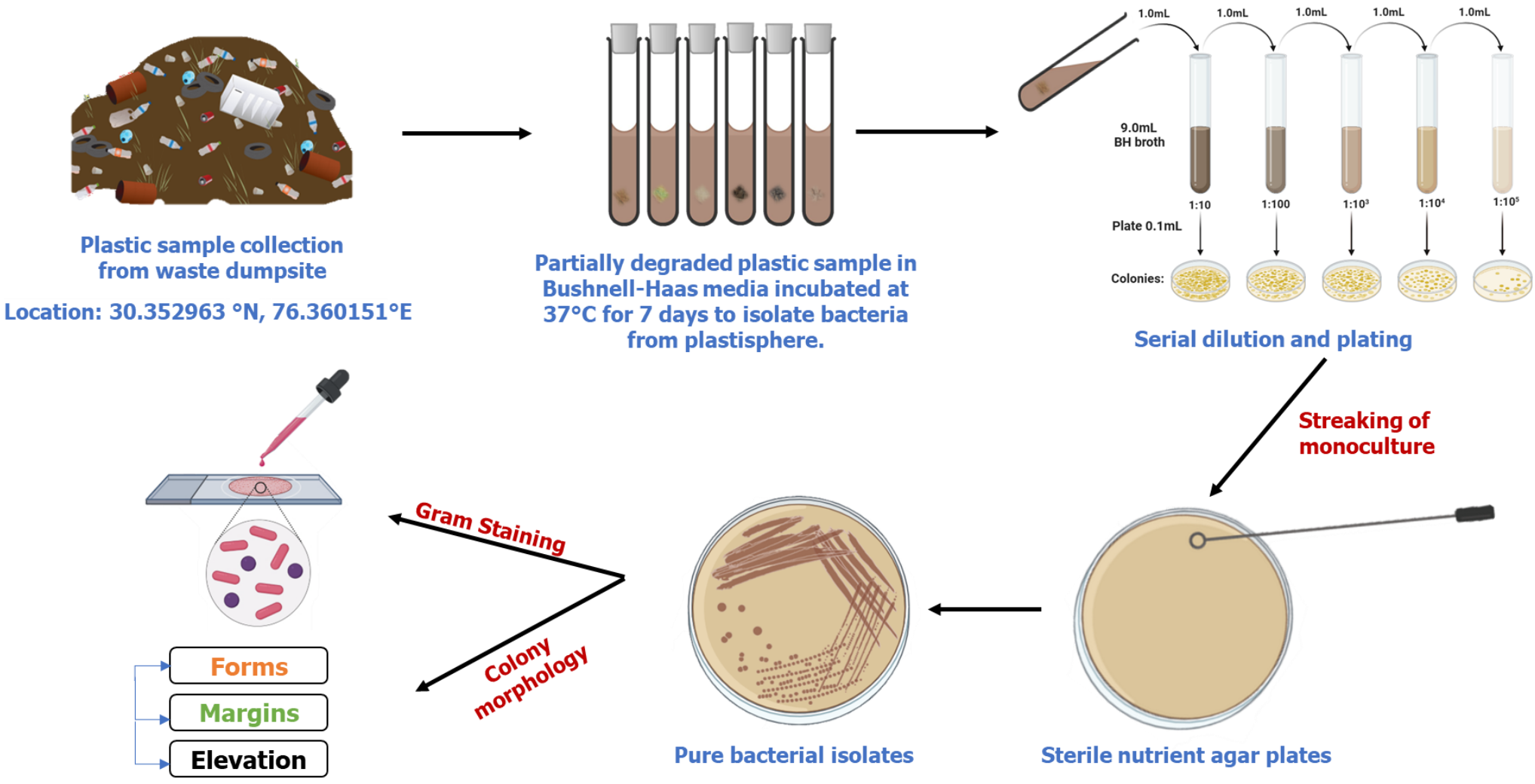

Fig 1: Isolation of LDPE degrading bacteria from plastisphere

Inoculation and screening: All isolates were inoculated on 5% PEG-4000 plates using an inoculation loop and incubated at 37ºC for 21 days (Devi et al., 2023). After 21 days, the PEG-4000 plates were stained with Coomassie Brilliant Blue R-250 (0.1% Coomassie R-250, 40% methanol, 10% Glacial Acetic acid, 50% distilled water) for 20 minutes. Stained plates were destained using the destaining solution (40% methanol, 10% Glacial Acetic acid, 50% distilled water) for 20 minutes. The zone of clearance was checked to identify the isolate with the potential for biodegradation activity (Mahalakshmi et al., 2014; Devi et al., 2023).

Polyethylene glycol (PEG) utilization-based primary screening

PEG standard curve preparation: To prepare the standard curve of PEG-4000, a series of solutions were made with concentrations of 0%, 0.2%, 0.4%, 0.6%, 0.8%, 1%, 2%, 4%, and 6% by dissolving the required amounts of PEG-4000 in Bushnell Haas broth. For each solution, 1mL of the PEG-4000 solution were taken and mixed with 50 µL of Coomassie Brilliant Blue R-250 stain. The mixtures were then incubated for 30 minutes at room temperature to allow for adequate binding between the dye and PEG-4000 molecules. After incubation, 1 mL of each stained solution was transferred to clean cuvettes, and the optical density (OD) was measured at 592 nm using a spectrophotometer. The OD readings corresponding to each PEG-4000 concentration were recorded and used to plot a standard curve, establishing a reference for quantifying PEG-4000 in subsequent assays. All measurements were performed in triplicate to ensure accuracy and reproducibility.

Polyethylene Glycol (PEG) containing media preparation: 0% (control), 1% and 5% of PEG-4000 was added in 15mL Bushnell Haas broth and autoclaved the media at 121ºC for 15 minutes at 15 psi.

Inoculation: All the isolates were inoculated in 25 mL Nutrient Broth Medium (HiMedia) (Appendix I) and grown overnight at 37ºC. After incubation, the cultures were centrifuged at 6000 rpm for 10 minutes to obtain bacterial cell pellets and washed with sterile 0.9% saline solution. OD was adjusted at 0.8 McFarland unit at 600 nm. 10% inoculum was added to the 10mL PEG-containing broth and incubated at 37ºC for 20 days.

Screening: Bacterial biomass and remaining PEG concentration in broth were determined using the spectrophotometric method after 20 days. 1.2 mL of the sample was drawn from the test tube and centrifuged at 8000 rpm for 7 minutes. 1mL supernatant was transferred to fresh Eppendorf, and 50 µL Coomassie Brilliant Blue R-250 staining solution was added and

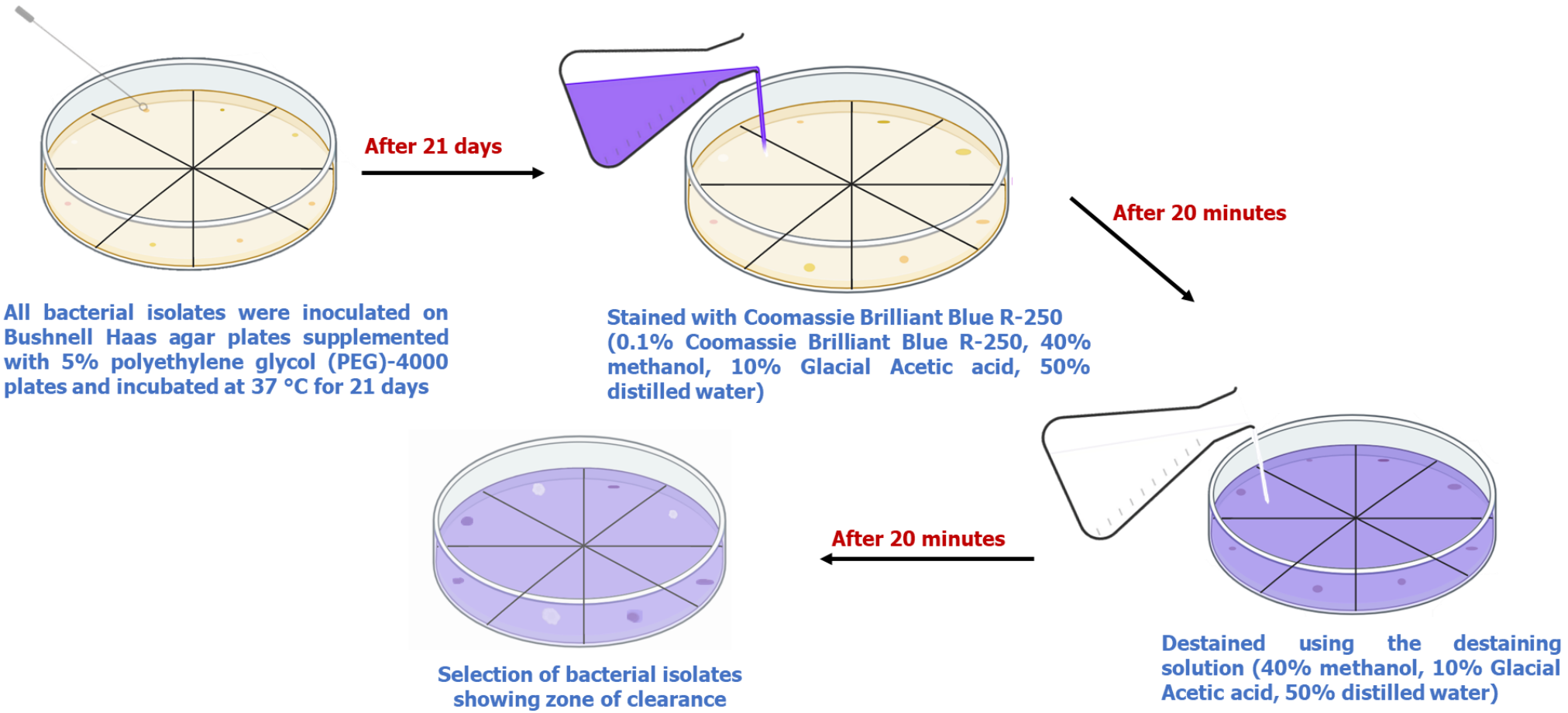

Fig 2: Zone of clearance based qualitative assay

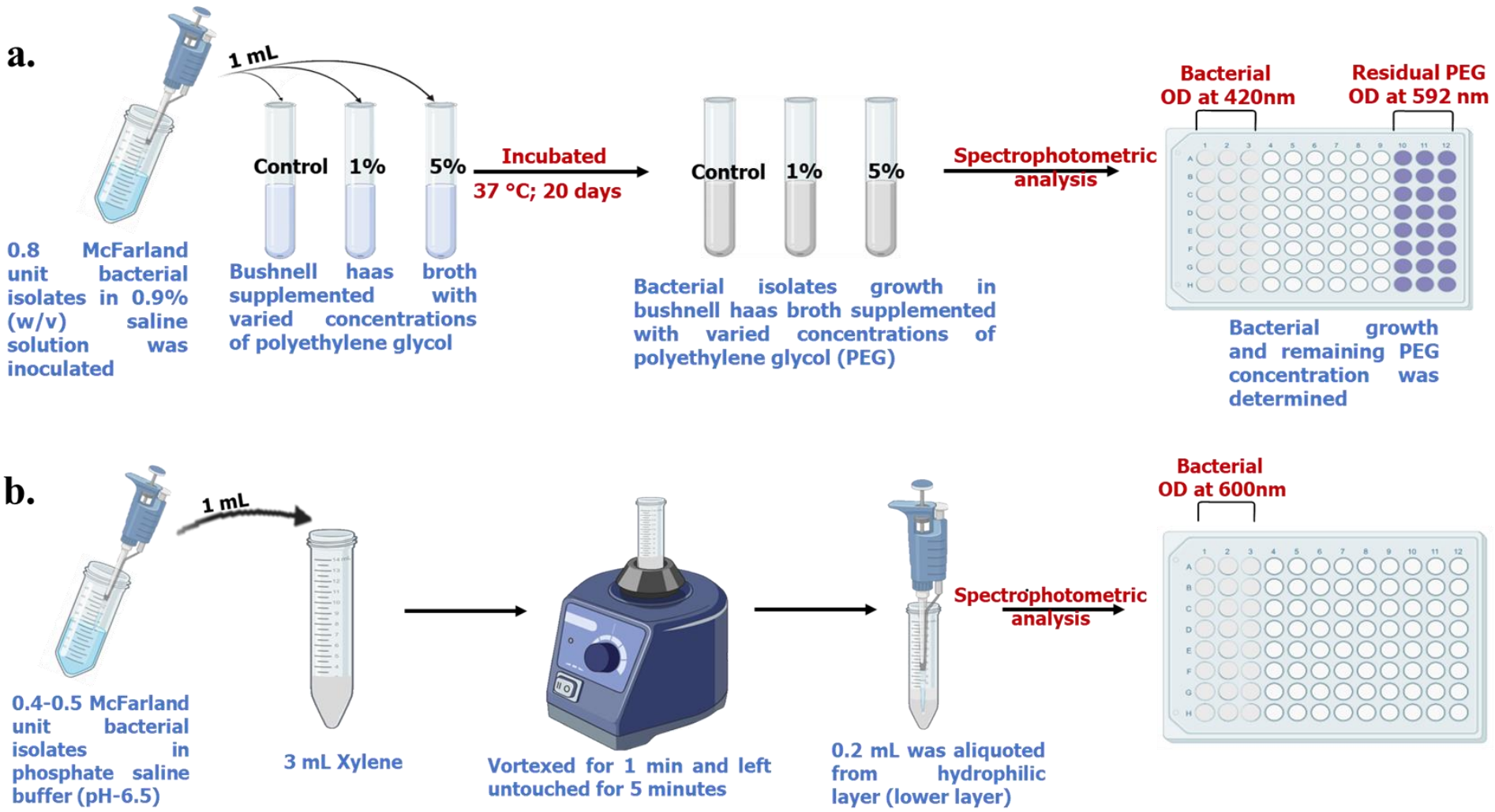

Figure 3: Screening based on polyethylene glycol in liquid medium **(a.)** and microbial adhesion to hydrocarbon (MATH) assay **(b.)**

incubated at room temperature for 30 minutes, and OD was measured at 592 nm. The pellet was resuspended in 200 µL supernatant, and OD was measured at 420 nm using a 96 microtiter well plate reader, and the remaining concentration of the sample was determined from the standard curve (Appendix II)

Microbial adhesion to hydrocarbon (MATH) assay

Bacterial suspension in Potassium phosphate buffer saline (pH-6.5) (Appendix II) was adjusted with an initial OD of 0.4-0.5 at 600 nm. Then, 1 mL bacterial suspension was taken, and 3 mL xylene was added to it and vortexed for 1 minute for uniform mixing. After vortexing, it was left untouched for 5 minutes, which led to the formation of two layers: hydrophobic (upper layer) and hydrophilic (lower layer). Then, the OD of the hydrophilic layer was noted down. The % hydrophobicity can be measured by the following equation (Salas-Tovar et al.,2021):

$$\% \text{ hydrophobicity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where, A_0: Initial OD of bacteria suspension

A_1: Final OD of bacterial suspension in the hydrophilic layer

Biodegradation Assay

Preparation of Plastic Film: Two types of Plastic film (5cm*4cm) of thickness 44mm (P1) and 22mm (P2) were cut and washed using 2% SDS solution for 4 hours at 120 rpm followed by repeated aseptic washing with sterile distilled water and finally subjected to ethanol washing for 2 hours at 120 rpm to ensure the sterilization of plastic film. Plastic film was then air dried and gravimetric weight was taken (Shilpa et al., 2023).

UV Treatment: After preparing the plastic film, one set of each plastic type was subjected to 24 hours of UV treatment. After treatment, the plastic film was washed with ethanol for 30 minutes at 120 rpm followed by sterile distilled water washing (Ghatge et al., 2020).

Inoculation: Selected isolates were inoculated in 25 mL Nutrient Broth Medium (HiMedia) and grown overnight at 37ºC. After incubation, the cultures were centrifuged at 6000 rpm for 10 minutes to obtain bacterial cell pellets and washed with sterile 0.9% saline solution. OD was adjusted at 0.8 McFarland unit at 600 nm. According to the experimental design (Table 2), 10% inoculum and the plastic film were added to the 250mL Bushnell Haas broth and incubated at room temperature for 30 days with intermittent shaking (Shilpa et al., 2023).

Table 2: Experimental design

Control	Inoculant + Media
	Only Media
Treatment of Plastic Film 1 (P1)	UV treated Plastic film + Inoculant +Media
	Untreated Plastic film + Inoculant +Media
	UV treated Plastic film +Media
	Untreated Plastic film +Media
Treatment of Plastic Film 2 (P2)	UV treated Plastic film + Inoculant +Media
	Untreated Plastic film + Inoculant + Media
	UV treated Plastic film +Media
	Untreated Plastic film +Media

Analytical method

Gravimetric weight loss determination: After the 30-day incubation period, plastic film was taken out from the culture aseptically, and biomass on the surface of the plastic film was scraped off which was attached to the plastic film. Plastic film was washed with distilled water, followed by 2% SDS, 70% ethanol, and distilled water washing. The plastic film was air-dried then to take the gravimetric weight. Gravimetric weight loss was determined by the following formula (Devi et al., 2023):

$$\%\ \text{weight loss:}\ \left(\frac{A_i - A_f}{A_i}\right) \times 100$$

Where, A_f: Gravimetric weight loss after biodegradation assay

A_i: Gravimetric weight loss before biodegradation assay

SEM analysis: The plastic film was cut into 1*1 cm, the gold coating was done to make it conductive, and the cracks and biofilm formation on the surface of the plastic film were analyzed (Mahalakshmi et al., 2014)

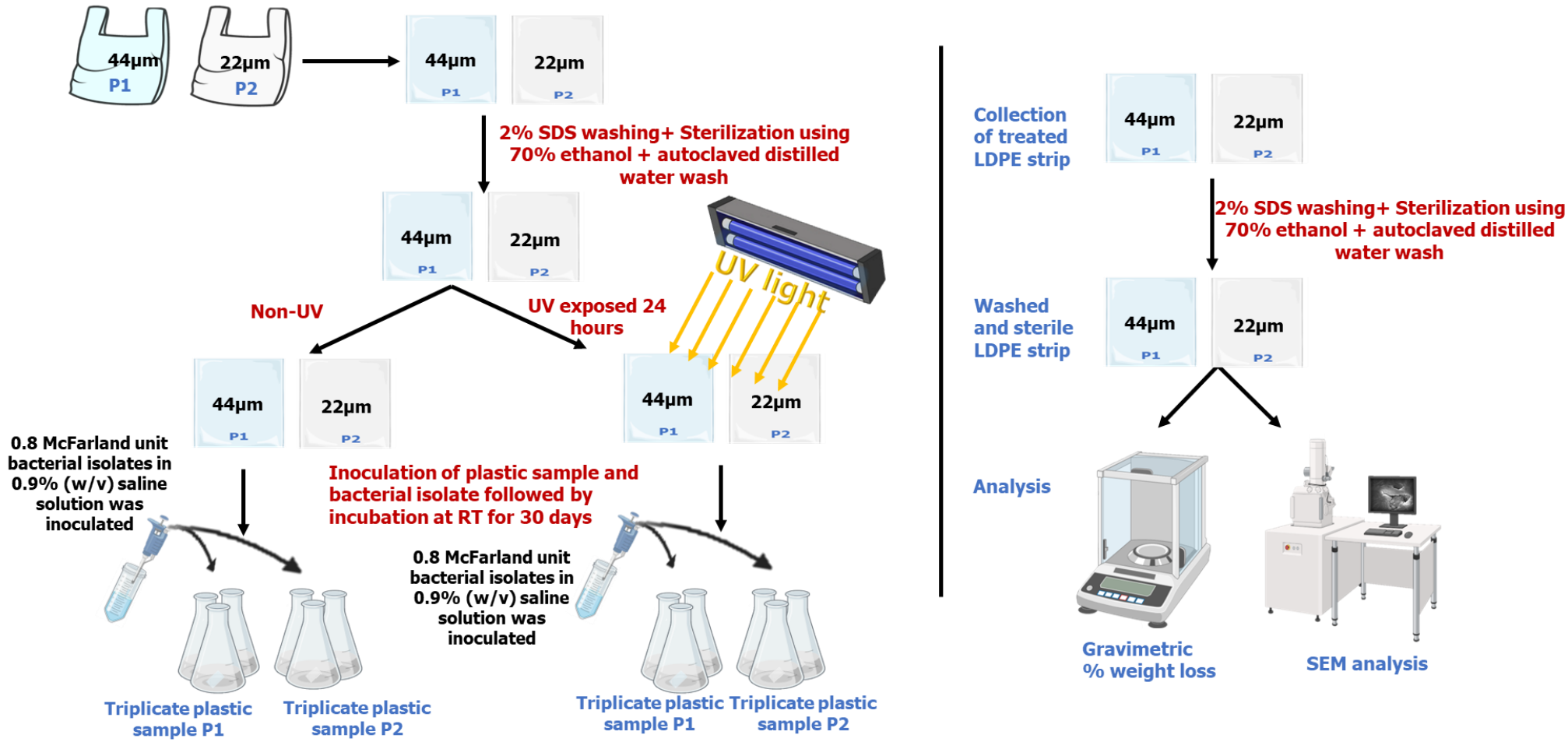

Figure 4 : Biodegradation assay of LDPE by DGK4 and DGK7

Results and Discussion

Sample collection and isolation of plastic-degrading bacteria

Six partially degraded plastic samples (Fig 5) were collected from plastic dump sites near M Hostel at Thapar Institute of Engineering and Technology. From these samples, 24 bacterial isolates with potential plastic degradation capabilities were successfully isolated (Fig 6-Fig *7*). Each isolate underwent colony morphological analysis and Gram characterization, the results of which are presented in Table 3.

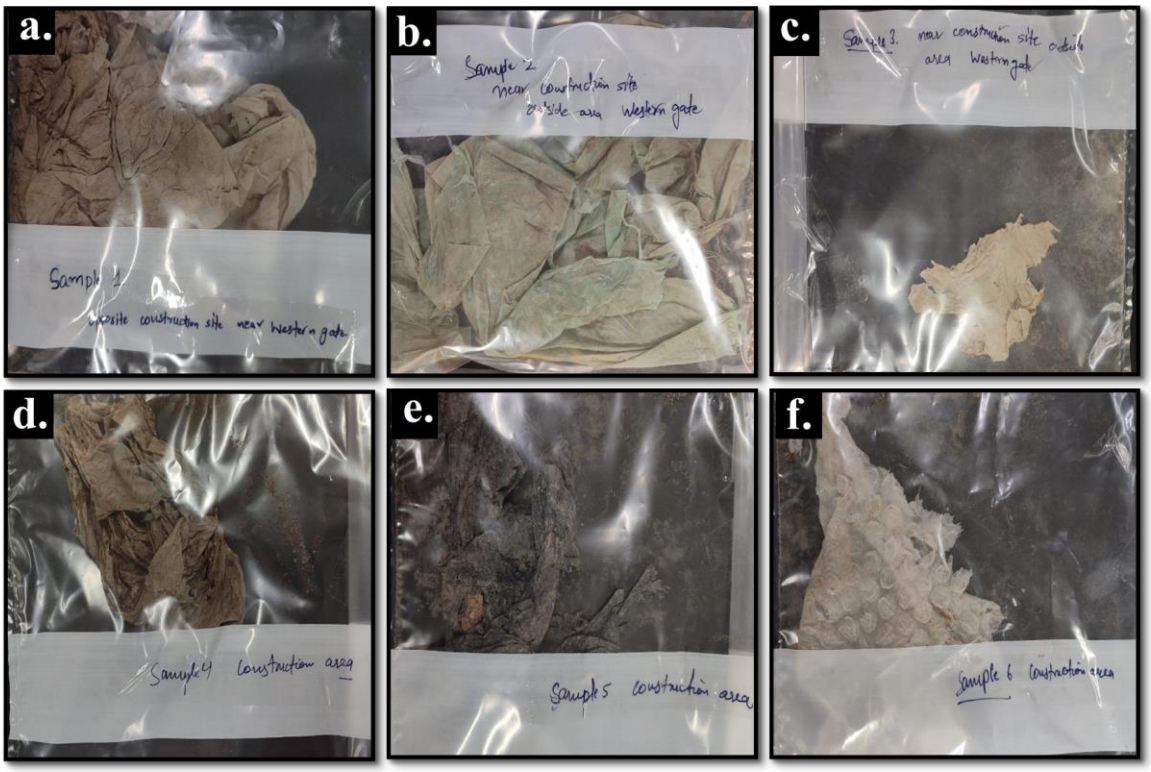

Fig 5: Partially degraded samples collected from plastic waste dump sites (Sample a-f)

Screening of plastic-degrading bacteria

Zone of clearance-based assay

All 24 bacterial isolates isolated from six plastic samples were screened for the potential of plastic biodegradation using a zone of clearance-based assay in which polyethylene glycol (PEG-4000) was used as a carbon source at 5% concentration in Bushnell Haas agar. The zone of clearance assay was performed using Bushnell Haas agar plates supplemented with 5% polyethylene glycol (PEG) as the carbon source. The zone of clearance assay revealed that 9 out of 24 isolates produced significant clear zones, indicating their ability to degrade PEG (Fig 8) (Table 4).

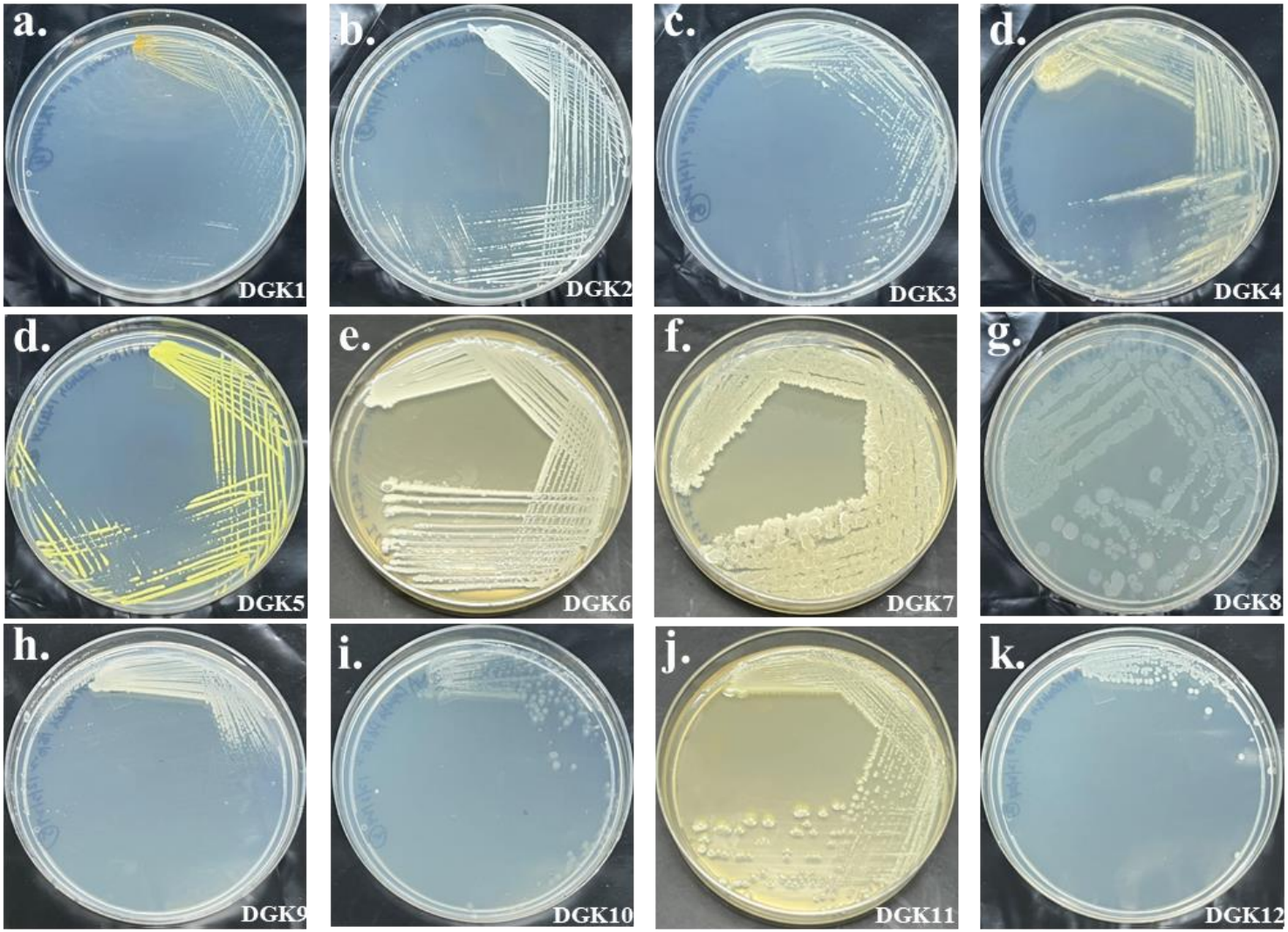

Fig 6: Bacterial isolates from plastic samples on Nutrient Agar at 37 ºC (DGK1-12)

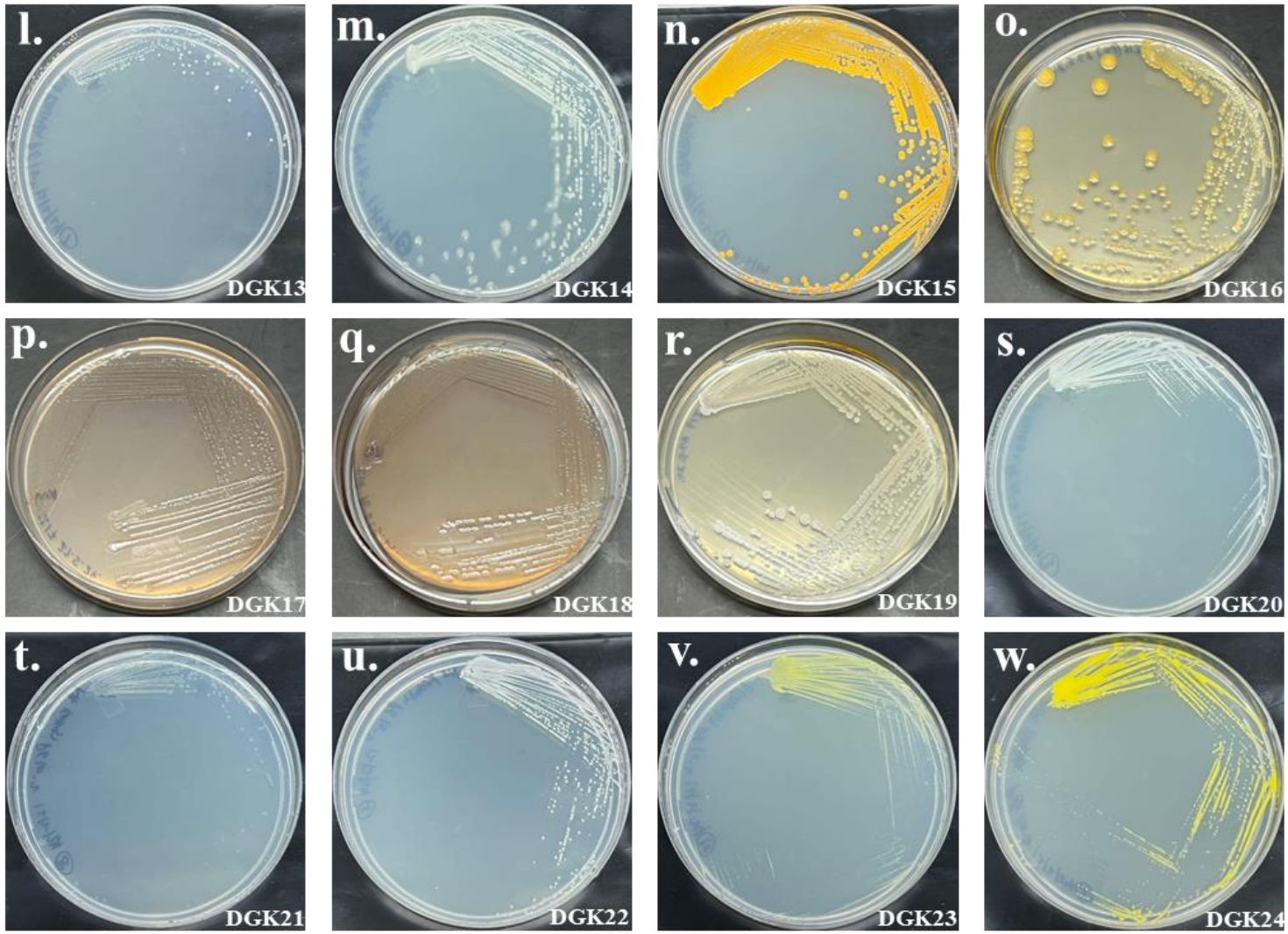

Fig 7: Bacterial isolates from plastic samples on Nutrient Agar at 37 ºC (DGK13-24)

Table 3: Colony morphology and Gram character of bacterial isolates

Isolate	Forms	Elevation	Margins	Gram character/ Shape
DGK 1	Circular	Raised	Entire	Positive; cocci shaped
DGK 2	Circular	Convex	Entire	Positive; cocci shaped
DGK 3	Irregular	Umbonate	Undulate	Negative; rod shaped
DGK 4	Irregular	Umbonate	Undulate	Negative; rod shaped
DGK 5	Circular	Raised	Entire	Negative; rod shaped
DGK 6	Irregular	Raised	Undulate	Positive; rod shaped
DGK 7	Irregular	Flat	Lobate	Positive; rod shaped
DGK 8	Circular	Flat	Entire	Negative; rod shaped
DGK 9	Circular	Raised	Entire	Negative; rod shaped
DGK 10	Circular	Convex	Entire	Positive; cocci shaped
DGK 11	Circular	Convex	Entire	Negative; cocci shaped
DGK 12	Circular	Flat	Entire	Positive; rod shaped
DGK 13	Circular	Flat	Entire	Negative; cocci shaped
DGK 14	Circular	Convex	Entire	Negative; cocci shaped
DGK 15	Circular	Convex	Entire	Negative; cocci shaped
DGK 16	Circular	Flat	Entire	Negative; cocci shaped
DGK 17	Circular	Raised	Entire	Positive; rod shaped
DGK 18	Circular	Raised	Entire	Positive; rod shaped
DGK 19	Circular	Flat	Entire	Negative; rod shaped
DGK 20	Circular	Flat	Entire	Negative; rod shaped
DGK 21	Circular	Raised	Entire	Positive; cocci shaped
DGK 22	Circular	Convex	Entire	Negative; rod shaped
DGK 23	Circular	Flat	Entire	Negative; cocci shaped
DGK 24	Circular	Raised	Entire	Positive; cocci shaped

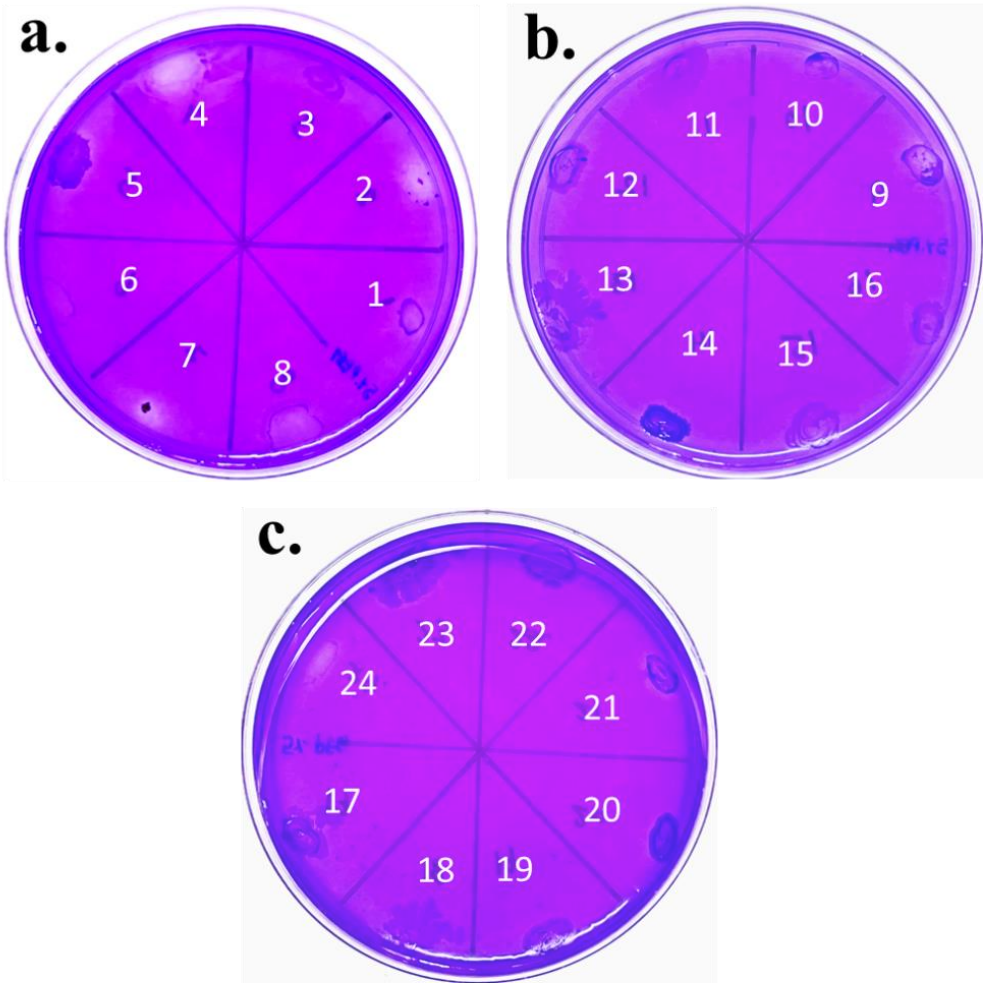

Fig 8: Zone of clearance by different bacterial isolates in Bushnell Haas medium supplemented with 5% polyethylene glycol (PEG)

Given the similarity in degradation pathways, these isolates likely possess the enzymes required for polyethylene degradation as well. The formation of clear zones in the PEG-based assay indicates the presence of enzymatic activity capable of degrading PEG. The degradation pathways of PEG and polyethylene share significant similarities, particularly the involvement of oxidative enzymes such as laccases and peroxidases. These enzymes catalyse the breakdown of both polymers, suggesting that the isolates capable of degrading PEG might also be effective in degrading polyethylene. The oxidative cleavage of PEG and polyethylene involves similar enzymatic mechanisms, which supports the use of PEG as a carbon source for screening potential polyethylene-degrading bacteria.

Table 4: Zone of clearance by different bacterial isolates in Bushnell Haas medium supplemented with 5% polyethylene glycol (PEG)

Isolate	Zone of clearance	Isolate	Zone of clearance	Isolate	Zone of clearance
DGK 1	+	**DGK 9**	+	**DGK 17**	-
DGK 2	+	**DGK 10**	-	**DGK 18**	-
DGK 3	-	**DGK 11**	-	**DGK 19**	-
DGK 4	+	**DGK 12**	-	**DGK 20**	+
DGK 5	+	**DGK 13**	-	**DGK 21**	+
DGK 6	-	**DGK 14**	-	**DGK 22**	-
DGK 7	+	**DGK 15**	-	**DGK 23**	-
DGK 8	+	**DGK 16**	-	**DGK 24**	+

+: zone of clearance was observed; -: Zone of clearance was not observed

Polyethylene glycol (PEG) utilization-based primary screening

Primary screening for LDPE degrading bacteria was performed using PEG-4000 as a carbon source in Bushnell Haas broth media at concentrations of 1% and 5%. Table 5 depicts the residual concentration of PEG (1% and 5%) after 20 days. At the 1% PEG-4000 concentration, DGK4 showed a significant reduction from 0.98±0.01 to 0.56±0.02, indicating its high biodegradation potential. DGK7 also demonstrated substantial PEG-4000 consumption, with a reduction from 0.97±0.01 to 0.58±0.01. Other notable performers include DGK2 and DGK24, which reduced PEG-4000 from 0.97±0.01 to 0.60±0.01 and from 0.97±0.02 to 0.59±0.01, respectively.

In the 5% PEG-4000 concentration, DGK4 again showed the highest reduction, from 4.90±0.02 to 2.75±0.02. Similarly, DGK7 exhibited a significant decrease from 4.94±0.06 to 2.94±0.02. DGK2 and DGK24 also displayed notable reductions, from 4.86±0.04 to 2.94±0.02 and from 4.92±0.02 to 2.94±0.02, respectively. Conversely, isolates DGK1 and DGK9 showed relatively low reductions in PEG-4000 concentrations at both initial concentrations, indicating lower biodegradation activity. Specifically, DGK1 reduced PEG-4000 from 0.98±0.01 to 0.84±0.01 in the 1% concentration and from 4.84±0.10 to 4.16±0.05 in the 5% concentration, while DGK9 reduced PEG-4000 from 0.96±0.01 to 0.85±0.01 and from 4.85±0.03 to 4.30±0.02, respectively.

Overall, isolates DGK4 and DGK7 exhibit the highest significant potential for biodegradation of PEG-4000, showing substantial reductions at both 1% and 5% concentrations (Fig 9), and should be prioritized for further research and application in biodegradation processes.

Table 5: Residual concentration of Polyethylene glycol (PEG) (1% and 5%) after 20 Days

Bacterial Isolate	**PEG (1%)**		**PEG (5%)**	
	0 day	**20th day**	**0 day**	**20th day**
DGK1	0.98±0.01	0.84±0.01	4.84±0.10	4.16±0.05
DGK2	0.97±0.01	0.60±0.01	4.86±0.04	2.94±0.02
DGK4	0.98±0.01	0.56±0.02	4.90±0.02	2.75±0.02
DGK 5	0.96±0.01	0.70±0.01	4.84±0.01	4.05±0.05
DGK7	0.97±0.01	0.58±0.01	4.94±0.06	2.94±0.02
DGK9	0.96±0.01	0.85±0.01	4.85±0.03	4.30±0.02
DGK20	0.99±0.03	0.73±0.02	4.89±0.02	4.10±0.01
DGK21	0.96±0.01	0.62±0.01	4.87±0.08	3.97±0.03
DGK24	0.97±0.02	0.59±0.01	4.92±0.02	2.94±0.02

Bacterial growth, measured by optical density (OD) at 420 nm, and utilization of 1% and 5% polyethylene glycol (PEG) supplemented in Bushnell Haas medium revealed significant variability among the different isolates over a 20-day period (Table 6). Isolate DGK1 demonstrated moderate growth in both 1% (1.530 ± 0.01) and 5% PEG (1.83 ± 0.01) with relatively low utilization rates of 14.60 ± 0.97% and 14.07 ± 1.08%, respectively. Conversely, isolate DGK2 exhibited substantial growth in 1% (3.74 ± 0.05) and even higher growth in 5% PEG (5.10 ± 0.06), coupled with high utilization rates of 37.64 ± 0.48% in 1% PEG and 39.58 ± 0.15% in 5% PEG, indicating its robust metabolic capabilities despite the increased substrate concentration.

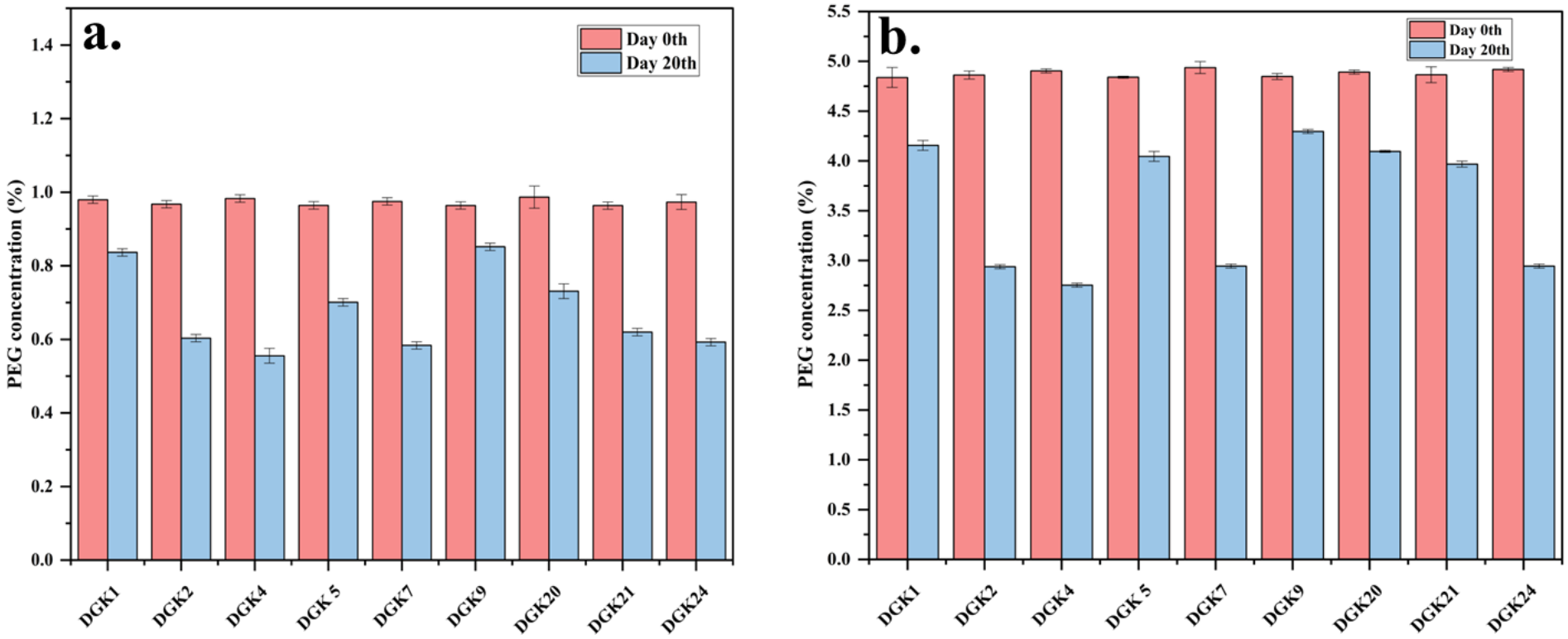

Fig 9: Residual Concentration of polyethylene glycol (PEG) (1% and 5%) after 20 Days

Bacterial isolates in Bushnell Haas medium supplemented with 1% PEG **(a.)** and 5% PEG **(b.)**

Table 6: Bacterial growth and % utilization of polyethylene glycol (PEG) in Bushnell Haas medium after 20 Days

Isolate	Bacterial growth (OD_{420}) in 1% PEG	Bacterial growth (OD_{420}) in 5% PEG	Utilization of 1% PEG (%)	Utilization of 5% PEG (%)
DGK1	1.530±0.01	1.83±0.01	14.60±0.97	14.07±1.08
DGK2	3.74±0.05	5.10±0.06	37.64±0.48	39.58±0.15
DGK4	5.48±0.03	6.27±0.03	43.47±0.96	43.87±0.39
DGK 5	3.23±0.03	2.39±0.02	27.31±1.53	16.41±1.00
DGK7	5.66±0.11	6.04±0.12	40.13±0.32	40.36±0.79
DGK9	1.48±0.01	1.62±0.02	11.63±1.02	11.38±1.06
DGK20	4.74±0.07	2.37±0.03	25.91±1.26	16.27±0.45
DGK21	6.17±0.07	2.30±0.03	35.68±1.12	18.44±0.81
DGK24	4.93±0.07	6.06±0.09	39.11±1.20	40.13±0.61

Among all isolates, DGK4 recorded the highest bacterial growth in both 1% (5.48 ± 0.03) and 5% PEG (6.27 ± 0.03), along with the highest utilization rates of 43.47 ± 0.96% and 43.87 ± 0.39% in 1% and 5% PEG, respectively. This isolate's efficiency can be attributed to its ability to maintain balanced metabolic processes and effectively convert PEG into energy and biomass, even at higher concentrations. Isolate DGK5 showed lower growth in 5% PEG (2.39 ± 0.02) compared to 1% PEG (3.23 ± 0.03) and exhibited moderate utilization rates of 27.31 ± 1.53% in 1% PEG and 16.41 ± 1.00% in 5% PEG, likely due to osmotic stress and substrate inhibition at higher concentrations which hindered the bacterial metabolism. Notably, bacterial isolate DGK7 demonstrated high growth rates in both 1% (5.66 ± 0.11) and 5% PEG (6.04 ± 0.12) and utilization rates of 40.13 ± 0.32% in 1% PEG and 40.36 ± 0.79% in 5% PEG, indicating its metabolic pathways are well-suited to handle varying substrate levels. Isolate DGK9 showed the least growth and utilization among the isolates, with values of 1.48 ± 0.01 in 1% PEG and 1.62 ± 0.02 in 5% PEG, and utilization rates of 11.63 ± 1.02% and 11.38 ± 1.06%, respectively, likely due to limited metabolic efficiency and substrate inhibition at higher PEG concentrations. Isolate DGK20 exhibited higher growth in 1% PEG (4.74 ± 0.07) compared to 5% PEG (2.37 ± 0.03), with utilization rates of 25.91 ± 1.26% in 1% PEG and 16.27 ± 0.45% in 5% PEG, indicating that higher substrate levels may inhibit its metabolic

activity. Isolate DGK21 displayed the highest growth in 1% PEG (6.17 ± 0.07), but significantly lower growth in 5% PEG (2.30 ± 0.03), with utilization rates of 35.68 ± 1.12% and 18.44 ± 0.81% in 1% and 5% PEG, respectively, suggesting substrate inhibition and osmotic stress at elevated PEG levels. Lastly, isolate DGK24 exhibited high growth rates in both 1% (4.93 ± 0.07) and 5% PEG (6.06 ± 0.09) and utilization rates of 39.11 ± 1.20% in 1% PEG and 40.13 ± 0.61% in 5% PEG, indicating its metabolic pathways are optimized to handle varying substrate concentrations without significant inhibition.

The results indicate that isolates DGK4 and DGK7 are particularly effective at utilizing PEG as a carbon source, showing both high growth and high utilization rates (Fig 10) in both concentrations of PEG. These isolates demonstrate robust metabolic capabilities, efficient substrate conversion, and resilience to substrate inhibition and osmotic stress, making them promising candidates for further studies focused on the biodegradation of polyethylene materials.

Microbial adhesion to hydrocarbon (MATH) assay

The Microbial Adhesion to Hydrocarbons (MATH) test, was conducted to evaluate the potential of bacterial isolates to adhere to hydrophobic substrates, which is indicative of their ability to degrade polyethylene (PE). The results from this assay provide insight into the bacteria's affinity for hydrophobic surfaces, which is a crucial factor in polyethylene degradation.

The hydrophobicity assay results are summarized in (Table 7). The hydrophobicity assay was performed following the PEG degradation assay to further characterize the bacterial isolates potential for polyethylene degradation. The PEG degradation assay, which identified bacterial isolates capable of degrading polyethylene glycol (PEG), served as an initial screening step. The hydrophobicity assay provided additional evidence of the isolates' potential to degrade polyethylene, given that hydrophobic interactions play a significant role in the degradation process.

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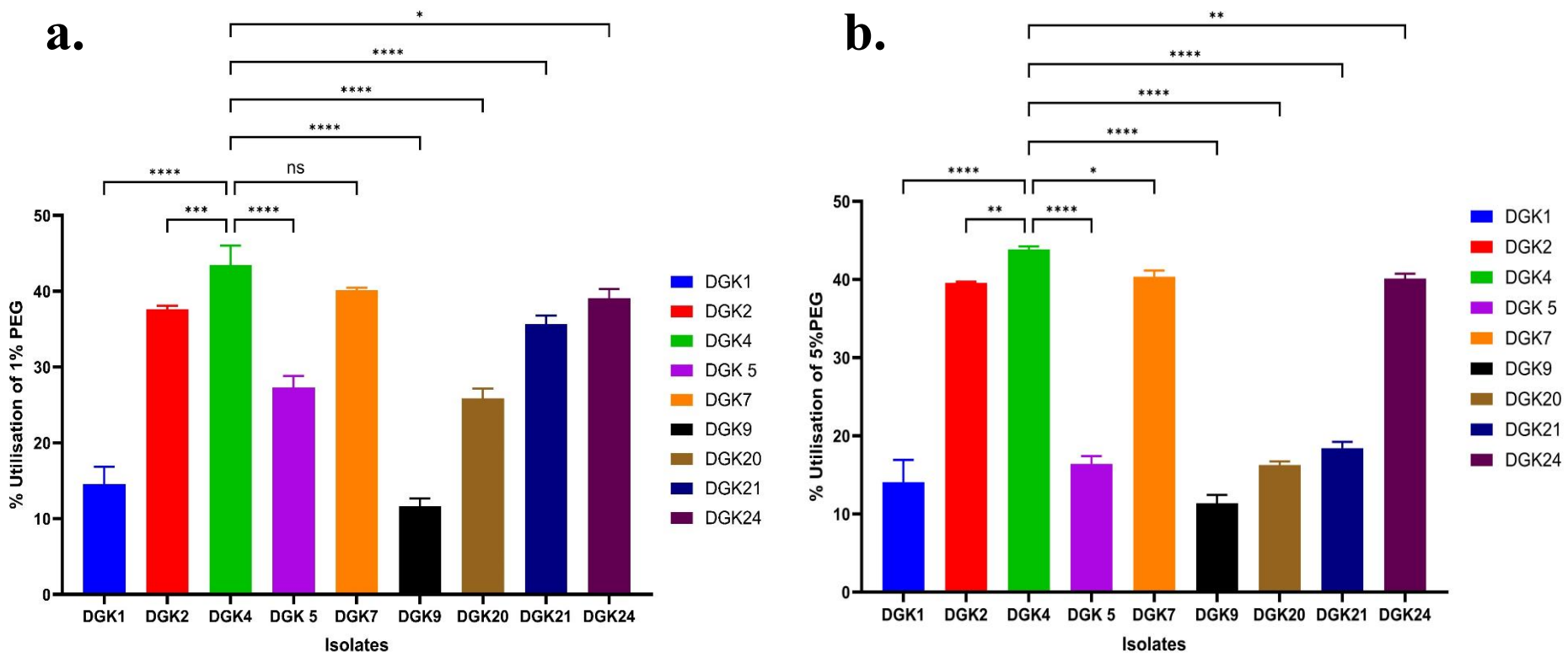

Fig 10: % utilization of 1% **(a.)** and 5% **(b.)** polyethylene glycol (PEG) in Bushnell Haas medium by different isolates after 20 Days

Data represent mean ± SD; Statistical significance was assessed using a one-way ANOVA followed by Tukey's HSD post-hoc test; Asterisks indicate significance levels: **** ($p \leq 0.0001$), *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), and ns (not significant).

Table 7: Microbial adhesion to hydrocarbon (MATH) assay

Isolate	Hydrophobicity (%)
DGK1	17.38 ± 0.65
DGK2	27.03 ± 0.63
DGK4	48.79 ± 0.51
DGK5	8.42 ± 0.50
DGK7	50.14 ± 0.28
DGK9	15.48 ± 0.78
DGK20	7.88 ± 0.85
DGK21	17.39 ± 0.67
DGK24	25.17 ± 0.61

Isolate DGK1, with a hydrophobicity of 17.38 ± 0.65%, exhibited low adherence properties. Isolate DGK2, showing a hydrophobicity of 27.03 ± 0.63%, demonstrates a relatively higher degree of hydrophobicity, potentially enhancing its interaction with hydrophobic substrates. Isolate DGK4, with a hydrophobicity of 48.79 ± 0.51%, exhibited high hydrophobicity, which suggests a strong ability to adhere to and interact with hydrophobic materials. Similarly, isolate DGK7, with a hydrophobicity of 50.14 ± 0.28%, has the highest significant hydrophobicity, indicating a strong adherence capability. High hydrophobicity suggests a strong affinity for hydrophobic substrates like polyethylene, implying that these strains possess surface properties for effective adhesion and subsequent degradation of polyethylene. The ability of bacteria to adhere to and form biofilms on polyethylene surfaces enhances their degradation efficiency. Isolate DGK5, with a hydrophobicity of 8.42 ± 0.50%, showed lower hydrophobicity, potentially limiting its effectiveness in interacting with hydrophobic substrates. Isolate DGK9, at 15.48 ± 0.78%, and isolate DGK20, at 7.88 ± 0.85%, exhibited lower hydrophobicity, which may reduce their adhesion efficiency. Isolate DGK21, with a hydrophobicity of 17.39 ± 0.67%, and isolate DGK24, at 25.17 ± 0.61%, also showed low levels of hydrophobicity, indicating moderate adherence capabilities.

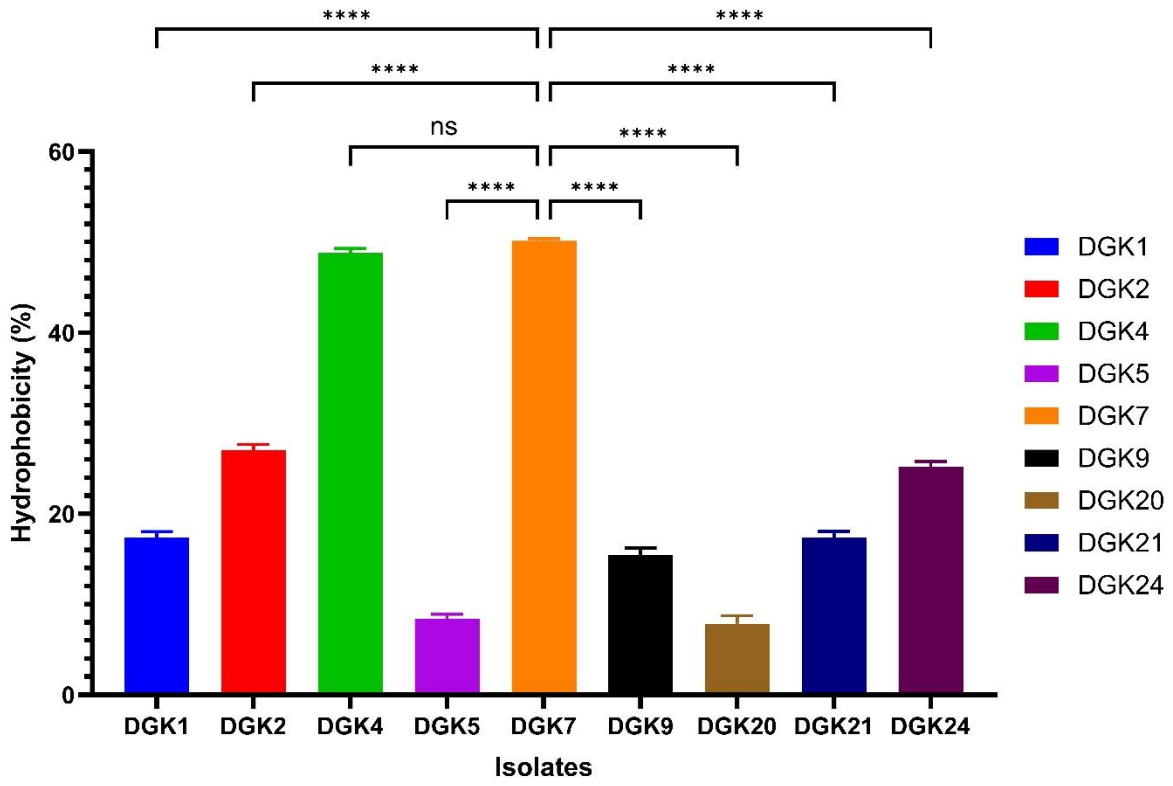

Fig 11: Microbial Adhesion to Hydrocarbon (MATH) Assay

Data represent mean ± SD; Statistical significance was assessed using a one-way ANOVA followed by Tukey's HSD post-hoc test; Asterisks indicate significance levels: **** ($p \leq 0.0001$), *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), and ns (not significant)

Considering both the hydrophobicity data (Fig **11**) and the performance in PEG degradation isolates DGK4 and DGK7 stand out as the most promising candidates. DGK4 and DGK7 not only exhibit the highest hydrophobicity but also show strong overall performance in PEG degradation. This combination of high hydrophobicity and effective substrate utilization suggests that these isolates have superior potential for applications involving the degradation of hydrophobic materials like polyethylene. Their enhanced adhesion properties are likely to contribute significantly to their efficiency in breaking down polyethylene waste

Analytical method

Gravimetric weight loss determination

The biodegradation results for LDPE samples treated under UV and non-UV conditions using the microbial cultures DGK4 and DGK7 are presented in Table 8. These results reveal a significant enhancement in the biodegradation of LDPE with UV treatment across both microbial cultures.

Table 8: Gravimetric weight loss of LDPE samples (P1, P2) after 30 days of treatment with DGK4 and DGK7

Isolate	P1 UV	P1 Non-UV	P2 UV	P2 Non-UV
DGK4	0.46±0.09	0.18±0.023	5.19±0.17	2.89±0.52
DGK7	4.95±0.21	2.58±0.28	12.13±0.56	11.44±0.47

For LDPE films with a thickness of 44 µm (P1), DGK4 demonstrated a weight loss of 0.46 ± 0.09% under UV treatment, which is significantly higher than the 0.18 ± 0.023% weight loss observed in non-UV-treated samples. This difference is statistically significant, as indicated by the **** marker ($p < 0.0001$), suggesting that UV treatment plays a crucial role in enhancing microbial degradation efficiency for thicker LDPE films. Similarly, DGK7 exhibited a weight loss of 4.95 ± 0.21% under UV treatment for P1, whereas the non-UV-treated samples showed a weight loss of 2.58 ± 0.28%. The statistical significance of this difference further confirms that UV treatment significantly improves the degradation process in DGK7 for thicker films. For thinner LDPE films with a thickness of 22 µm (P2), the results show a more pronounced effect of UV treatment. DGK4 exhibited a weight loss of 5.19 ± 0.17% under UV conditions, in contrast to 2.89 ± 0.52% without UV treatment, with the difference being statistically significant (****). DGK7, on the other hand, showed an even higher weight loss of 12.13 ± 0.56% for P2 under UV treatment, compared to 11.44 ± 0.47% for non-UV treatment. Although the difference between UV and non-UV treatments in DGK7 for P2 is not statistically significant (ns), the overall trend suggests that UV exposure generally enhances microbial degradation, particularly for thinner films. The higher weight loss observed for P2 samples under UV treatment, particularly with DGK7, suggests that UV exposure enhances the microbial degradation process more effectively for thinner films. The enhanced degradation with UV treatment can be attributed to the formation of free radicals and oxidative groups on the LDPE surface. UV irradiation induces the formation of reactive species, such as carbonyls and hydroxyls, which modify the polymer surface and make it more susceptible to microbial attack. These oxidative groups create additional sites for microbial colonization and enzymatic activity, leading to increased weight loss and more effective degradation of the

polymer. The free radicals formed during UV exposure create sites for microbial colonization and enzymatic action, facilitating further degradation.

Both DGK4 and DGK7 demonstrated increased biodegradation efficiency with UV-treated samples. DGK7, in particular, showed a higher capacity for degrading both P1 and P2 samples, with the most significant degradation observed in P2 UV samples (Fig 12). The results indicate that the thickness of LDPE films significantly influences the biodegradation process. Thinner films, like P2 (22 µm), exhibit higher weight loss rates compared to thicker films (P1, 44 µm) under both UV and non-UV conditions, with statistical significance supporting these observations. This is likely due to the larger surface area-to-volume ratio of thinner films, which provides a more extensive surface for microbial colonization and enzymatic action. UV treatment further amplifies this effect by increasing the availability of oxidative sites on the polymer surface, thus optimizing the biodegradation process. Consequently, the combination of UV treatment and thinner film thickness enhances the overall efficiency of LDPE degradation, as observed with both DGK4 and DGK7 isolates.

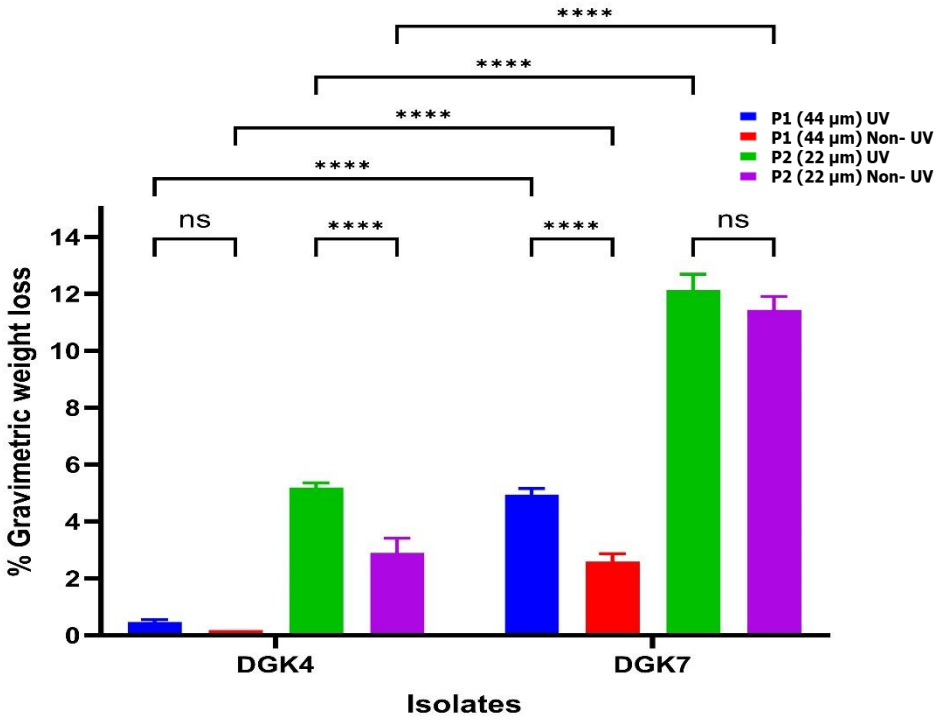

Fig 12: Gravimetric weight loss of LDPE samples (P1, P2) after 30 days of treatment with DGK4 and DGK7

Data represent mean ± SD; Statistical significance was assessed using a one-way ANOVA followed by Tukey's HSD post-hoc test; Asterisks indicate significance levels: **** ($p \leq 0.0001$), *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), and ns (not significant)

The thickness of the LDPE samples also significantly influences the biodegradation process. Thinner films, such as P2 (22 µm), exhibit higher weight loss rates compared to thicker films (P1, 44 µm) under both UV and non-UV conditions. This is likely due to the larger surface area-to-volume ratio of thinner films, which provides a more extensive surface for microbial colonization and enzymatic action. The UV treatment further amplifies this effect by increasing the availability of oxidative sites on the polymer surface, thus optimizing the biodegradation process. Consequently, the combination of UV treatment and thinner film thickness enhances the overall efficiency of LDPE degradation, as observed with both DGK4 and DGK7 isolates.

SEM analysis

The SEM images of the control plastic sample 1 (44 µm) exhibit a smooth and uniform surface with minimal signs of degradation. No significant morphological changes or surface irregularities are observed, indicating the absence of microbial activity or environmental degradation. In contrast, the UV-treated plastic sample 1 shows considerable surface modifications. The SEM images reveal the formation of cracks, pits, and increased surface roughness, which are indicative of photodegradation initiated by UV exposure. The generation of free radicals during UV treatment likely facilitated the breakdown of polymer chains, creating a more accessible surface for microbial attack. The non-UV-treated plastic sample 1, subjected to microbial degradation alone, displays moderate surface irregularities compared to the control. The SEM images show signs of microbial colonization, including small pits and grooves, suggesting enzymatic degradation of the polymer. However, the extent of degradation is less pronounced than in the UV-treated samples, underscoring the synergistic effect of UV treatment in enhancing biodegradation.

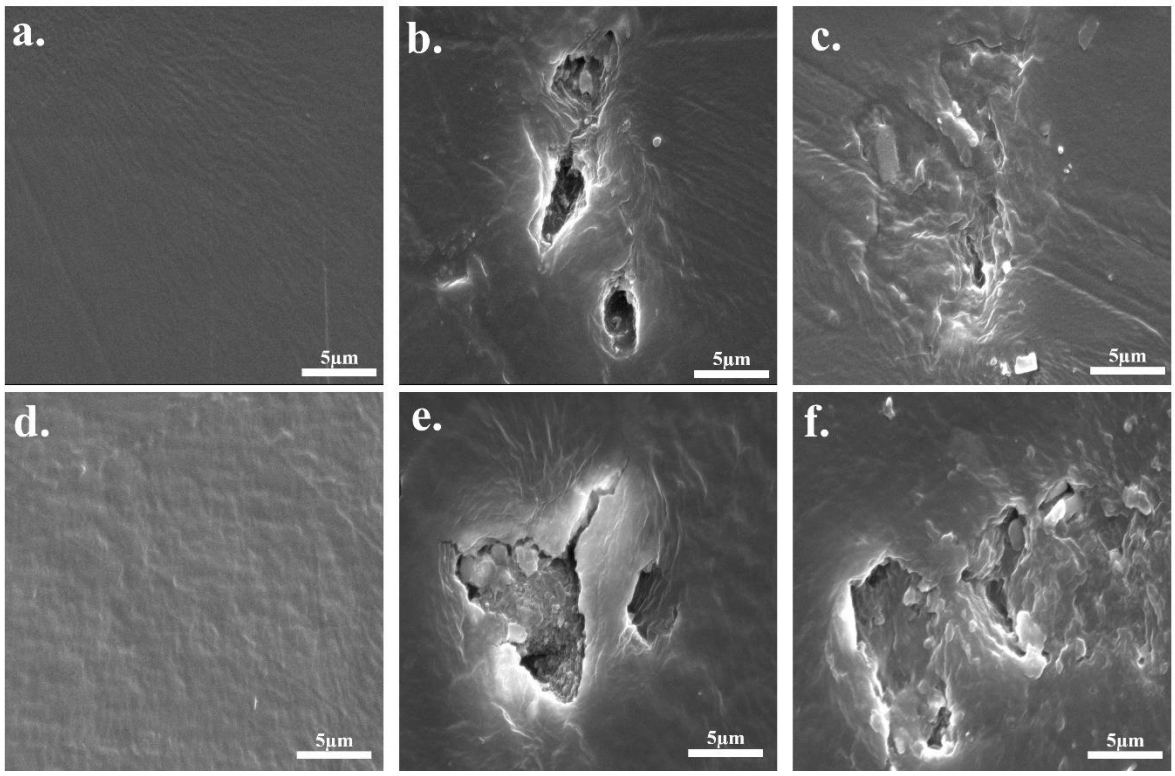

Fig 13: SEM analysis of LDPE treated by DGK7 (5000X)

a. P1-UV-exposed untreated; **b.** P1-UV-exposed treated; **c.** P1- non-UV treated; **d.** P2-UV-exposed untreated; **e.** P2-UV-exposed treated; **f.** P2- non-UV treated

Similar to the control of sample 1, the control plastic sample 2 (22 µm) maintains a smooth and intact surface in the SEM images. The thinner plastic sample does not exhibit any significant degradation features, confirming its stability in the absence of UV treatment and microbial activity. The UV-treated plastic sample 2 demonstrates substantial degradation, with SEM images showing extensive cracking, and increased surface roughness. These morphological changes are more pronounced compared to the UV-treated sample 1, possibly due to the thinner structure of sample 2, which is more susceptible to UV-induced photodegradation. For the non-UV-treated plastic sample 2, the SEM images reveal moderate signs of microbial degradation. Unlike non-UV-treated plastic sample 1, larger pits and surface irregularities are observed, indicating microbial activity. However, the degradation is less extensive compared to the UV-treated plastic sample 2, highlighting the importance of UV pretreatment in facilitating microbial degradation.

While comparing the UV-treated samples, the thinner plastic sample (22 µm) shows more extensive degradation features than the thicker sample (44 µm). The SEM images of the 22-micron sample reveal deeper cracks and more significant fragmentation, suggesting that

thinner plastics are more prone to UV-induced degradation. This enhanced degradation in thinner samples can be attributed to the higher surface area-to-volume ratio, which allows more UV penetration and interaction with the polymer chains. The non-UV-treated samples of both thicknesses exhibit similar degradation patterns, with the presence of microbial colonization and surface irregularities. However, the thinner sample (22 µm) shows slightly more pronounced degradation features than the thicker sample (44 µm). This difference suggests that the thinner plastic is more accessible to microbial enzymes, leading to more effective biodegradation.

Conclusion

This study provides significant insights into the potential of bacterial isolates for degrading low-density polyethylene (LDPE), a major environmental pollutant. Out of 24 bacterial isolates screened from partially degraded plastic samples, DGK4 and DGK7 emerged as the most promising candidates based on their performance in several assays. The zone of clearance assay, PEG utilization in Bushnell Haas medium, and hydrophobicity tests were used in selecting these isolates.

DGK7 demonstrated superior biodegradation capabilities compared to DGK4, as evidenced by its high PEG utilization rates of 43.87 ± 0.39% for 1% PEG and 40.13 ± 0.32% for 5% PEG, and its high hydrophobicity value of 50.14 ± 0.28%. This suggests that DGK7 has a strong affinity for hydrophobic materials such as LDPE, which likely enhances its ability to degrade plastic. Gravimetric weight loss measurements further confirmed DGK7's effectiveness, showing significant degradation with 4.95 ± 0.21% weight loss for Plastic Sample 1 (44 µm) UV and 12.13 ± 0.56% weight loss for Plastic Sample 2 (22 µm) UV. In contrast, DGK4, while effective, showed lower degradation rates with 0.46 ± 0.09% for Plastic Sample 1 (44 µm) UV and 5.19 ± 0.17% for Plastic Sample 2 (22 µm) UV. The SEM analysis of LDPE samples degraded by DGK7 revealed notable surface erosion and morphological changes, highlighting the strain's ability to break down plastic at a microscopic level. These findings underline DGK7's potential as a highly effective strain for bioremediation applications aimed at mitigating plastic pollution.

Overall, DGK7 stands out as a promising candidate for future research and application in environmental cleanup efforts. The study not only identifies effective microbial agents for plastic degradation but also underscores the importance of leveraging microbial solutions to tackle the growing issue of plastic waste. Further investigations into the specific enzymatic

processes employed by DGK7, as well as the optimization of bioremediation protocols, could enhance our ability to manage and reduce plastic pollution on a larger scale.

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Appendix I

A. Nutrient Broth

Ingredients	Quantity (g/L)
Peptone	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50

B. Nutrient Agar

Ingredients	Quantity (g/L)
Peptone	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	1.50

C. Bushnell Haas Broth

Ingredients	Quantity (g/L)
Magnesium sulphate	0.20
Calcium chloride anhydrous	0.02
Potassium dihydrogen phosphate	1.00
Dipotassium hydrogen phosphate	1.00
Ammonium nitrate	1.00
Ferric chloride	0.05

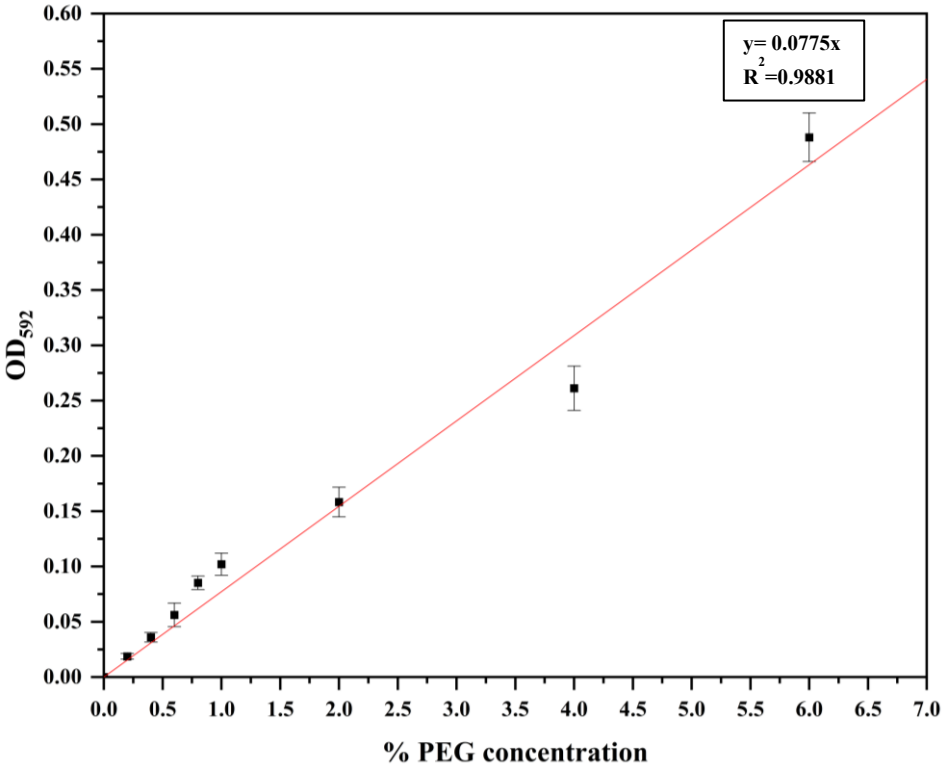
D. Bushnell Haas Agar

Ingredients	Quantity (g/L)
Magnesium sulphate	0.20
Calcium chloride anhydrous	0.02
Potassium dihydrogen phosphate	1.00
Dipotassium hydrogen phosphate	1.00
Ammonium nitrate	1.00
Ferric chloride	0.05
Agar	20.00

Appendix II

A. 1M Potassium phosphate buffer (pH: 6.5)

Ingredients	Quantity (g/L)
Potassium Phosphate Monobasic	95
Potassium Phosphate Dibasic	52.5



Standard curve of Polyethylene glycol-4000

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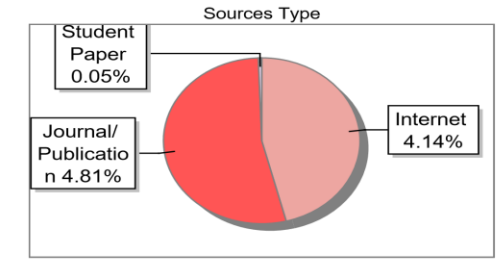

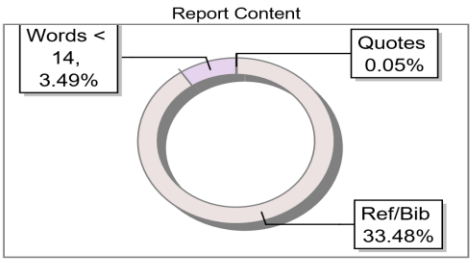

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