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Research Article

Isolation, Application of Polyethene Degrading Bacteria Isolated from Garbage Dumping Sites of Daman & Vapi Area

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Abstract

Plastics have revolutionized industries such as electronics, packaging, healthcare, and transportation due to their affordability, adaptability, and durability. However, their environmental persistence has led to severe pollution, posing significant threats to ecosystems and human health. Conventional disposal methods, including recycling, incineration, and landfilling, are inadequate in addressing plastic waste accumulation. Microbial biodegradation uses bacteria to break down plastic with microplastic acting as surface for microbial growth, forming the "plastisphere". In this study, Bacillus spp. Were used to examine the Polythene degradation. The degradation assessed by viable cell count, cell density, weight loss and microscopy. Over 4 isolate the C4 show highest degradation of 0.55% and microscopy show the crack, pits and roughness on the Polythene that exposed to the microorganisms. Further research needed to examine which enzyme can be involved in the degradation

Keywords: Polythene, Biodegradation, Bacillus spp, Pollution, Enzyme, Plastic.

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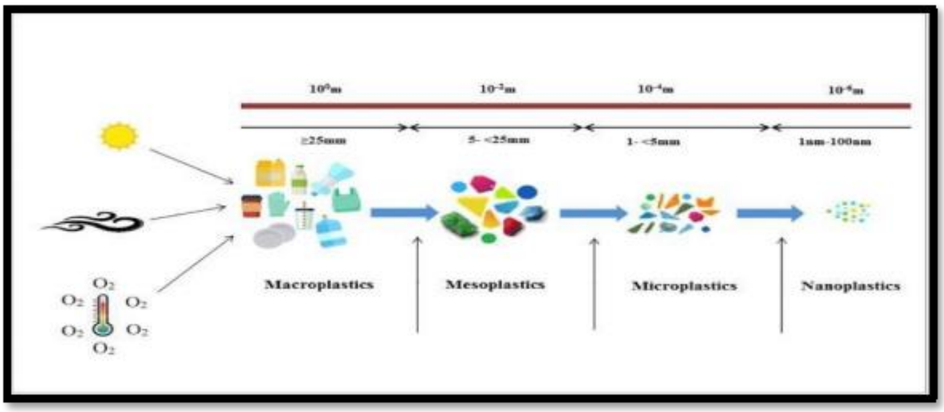
Introduction:

Brief introduction to plastics

Plastic is one of the most versatile and widely used materials in modern society. It is a synthetic polymer derived primarily from petrochemicals, although biodegradable and bio-based alternatives are emerging. The unique properties of plastic, including its durability, flexibility, lightweight nature, and resistance to water and chemicals, have made it indispensable in industries such as packaging, construction, healthcare, electronics, and automotive manufacturing (Thakur *et al;*2023). The invention of plastic dates back to the early 20th century, with Bakelite being one of the first fully synthetic plastics developed in 1907. Since then, advancements in polymer science have led to the production of a wide variety of plastics, including polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS), each designed for specific applications. Despite its benefits, plastic has also become a significant environmental concern due to its persistence in nature. Most conventional plastics take hundreds of years to decompose, leading to pollution in landfills, oceans, and ecosystems. The accumulation of plastic waste has prompted research into sustainable alternatives, such as biodegradable plastics and recycling technologies, to mitigate its environmental impact (Thakur *et al;*2023). In recent years, scientific efforts have focused on microbial degradation of plastic as a potential solution to plastic waste. Certain bacteria, such as *Bacillus cereus* and *Bacillus subtilis*, have demonstrated the ability to break down plastic components under specific conditions. This emerging field of research holds promise for reducing plastic pollution and contributing to a more sustainable future. (Thakur *et al;*2023)

Classification of plastic based on size



Plastic debris can be categorized in various ways, including its origin, size, shape, colour, polymer type, or original use. One widely accepted classification is based on size. Plastic enters aquatic

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environments in diverse sizes, ranging from several thousand meters in length to microscopic scales. (Silva *et al*;2018)

Fig 1.1 Classification of plastics based on size

Three commonly used size classifications for plastic pollution are:

- Macro plastics: Particles larger than 20 mm in diameter
- Mesoplastics: Particles between 5 mm and 20 mm in diameter
- Microplastics: Particles smaller than 5 mm in diameter

However, no universal standard for these classifications exists, making it difficult to compare data across studies. Macro plastics have been documented in various environments. Since they are highly visible, Macro plastic pollution is often considered one of the most concerning forms of plastic contamination. Clean-up initiatives primarily target these larger items, but their distribution varies significantly across regions, complicating trend analysis. Additionally, Microplastics are often identifiable by their original use, such as packaging, fishing gear, or sewage-related waste (Silva *et al*;2018). Attributing the source of microplastics is more complex. While microplastics are generally defined as particles smaller than 5 mm, their lower size limit is often determined by the equipment used for sampling. In field studies, this limit is typically set by the mesh size of nets for collecting surface water samples or the sieves used for filtering beach sand. Some studies have identified plastic particles just a few microns in size using spectroscopy. Even smaller nano plastics are likely present in the environment, but current technology does not allow for their effective separation and identification from complex environmental mixtures (Silva *et al*;2018).

Source of plastics and plastic degrading bacteria

Plastics are primarily derived from fossil fuels such as crude oil and natural gas. These raw materials undergo chemical processes to produce monomers like ethylene, propylene, and styrene, which are then polymerized to create different types of plastics. Common fossil fuel-based plastics include polyethylene (PE), polypropylene (PP), and polystyrene (PS), which are widely used in packaging, construction, and consumer goods. In addition to fossil fuels, renewable resources like corn, sugarcane, and vegetable oils are used to produce bio-based plastics such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA), which are biodegradable and environmentally friendly alternatives. (Silva *et al*;2018)

Another significant source of plastics is recycled materials, which help reduce plastic waste and reliance on virgin raw materials. Recycled plastics come from post-consumer waste, such as used

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plastic bottles and containers, and post-industrial waste from manufacturing processes. These recycled materials are processed and transformed into new plastic products, contributing to a more sustainable approach to plastic production. By utilizing both fossil fuel-based and alternative sources, the plastic industry continues to meet global demand while exploring more eco-friendly solutions. The sources of plastic degrading bacteria include various natural environments where microorganisms have adapted to break down plastics. (Saritha, Sindgi, &Remade; 2021)

These bacteria are commonly found in:

1. **Soil and landfills** – Especially in areas with high plastic waste accumulation, bacteria such as *Bacillus cereus, Bacillus subtilis, Pseudomonas, and Rhod coccus* have been identified for their plastic-degrading abilities.
2. **Marine and freshwater ecosystems** – Certain bacteria like *Carnivora, Donella akinesis*, and *Pseudomonas aeruginosa* have been discovered in oceans, rivers, and lakes, where they degrade plastic pollutants.
3. **Compost and waste treatment facilities** – Microorganisms in compost heaps and sewage treatment plants, such as *Streptomyces* and *Actinobacteria*, contribute to the breakdown of biodegradable plastics.
4. **Extreme environments** – Bacteria found in hot springs, deep-sea hydrothermal vents, and other harsh conditions, such as *Thermomonospora* and *Thermobifida*, have shown potential in plastic degradation.

Role of various plastic-degrading microbes

Microbial degradation of plastics involves various microorganisms, including bacteria, fungi, and algae, that can break down synthetic polymers. (Lv, Li, Zhao, & Shao, 2024)

Bacteria

Bacteria are abundant in nature and play a significant role in plastic degradation

- *Pseudomonas* Species: Comprising approximately 21% of plastic-degrading bacteria, *Pseudomonas* spp. has been extensively studied. Notably, *Pseudomonas aeruginosa* has demonstrated the ability to degrade various plastics. For instance, strains isolated from the intestines of super worms exhibited daily weight loss rates of 0.64% for polyethylene (PE), 0.098% for polystyrene (PS), and 0.025% for polypropylene (PP). Additionally, *P. aeruginosa* strains from waste dumps showed PE degradation rates of 6.5% and 8.7% after 60 days in

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different media. Other species like *Pseudomonas putida* and *Pseudomonas fluorescens* have also been identified for their plastic-degrading capabilities.

- *Bacillus* species: Species such as *Bacillus cereus*, *Bacillus safensis*, and *Bacillus subtilis* have shown effectiveness in degrading various plastics. For example, *B. cereus* isolated from mangroves resulted in weight losses of 1.6% for PE, 6.6% for PET, and 7.4% for PS over 40 days. *B. subtilis* strains have demonstrated degradation of low-density polyethylene (LDPE) with weight losses ranging from 1.54% to 1.75% over periods of 30 to 90 days.
- **Other bacterial species:** Various other bacteria, including *Lysinibacillus spp*., *Achromobactin dentifrices*, and *Alcaligenes faecalis*, have demonstrated the capacity to degrade plastics like PE, PP, and PVC, with degradation rates varying based on species and conditions.

Fungi

Fungi contribute significantly to plastic degradation due to their filamentous structures, allowing them to infiltrate substrates effectively. Marine fungi such as *Rhodiola mucilaginous*, *Penicillium* spp., and *Alternaria alternate* have been identified for their ability to degrade plastics like PE by forming biofilms and secreting degrading enzymes. Terrestrial fungi, including *Aspergillus spp*. and *Trichoderma spp*., have also shown notable plastic-degrading capabilities.

Algae

Although less studied, certain algae have demonstrated potential in plastic degradation. For example, the microalga *Urinoma africanus* was found to degrade LDPE sheets within 30 days, exhibiting surface corrosion and abrasion. Cyanobacteria like *Phormium lucidum* and *Oscillatoria sub brevis* have been observed colonizing and degrading LDPE. Additionally, freshwater *Spirulina* isolates have shown the ability to degrade PET and PP after 112 days of interaction.

Application of plastic degradation

Plastics are indispensable materials in modern society, valued for their durability, lightweight nature, flexibility, and resistance to chemicals and moisture. These properties make plastics a preferred choice across numerous industries, where they contribute to efficiency, cost reduction, and enhanced product performance.

Packaging Industry

Plastics play a fundamental role in the packaging sector, where they are used to manufacture plastic bags, bottles, food containers, and wrapping films. Their lightweight nature reduces transportation costs, while their ability to create airtight and moisture-resistant barriers helps preserve food and other

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perishable goods, extending their shelf life and minimizing waste. Plastic packaging is also commonly used for pharmaceuticals, cosmetics, and household products, ensuring safety, hygiene, and convenience. (Silva & Pålsson;2022)

Construction and building materials

Plastics are widely used in the construction sector due to their strength, durability, and resistance to environmental factors such as moisture, corrosion, and chemical exposure. Materials like PVC and polyethylene are essential for manufacturing pipes, insulation panels, roofing sheets, flooring, window frames, and wall coverings. These plastic-based materials offer cost-effective and long-lasting solutions for infrastructure projects, improving energy efficiency and sustainability. Additionally, plastic composites and reinforced polymer materials are increasingly being used in bridges, buildings, and roadways for their structural resilience and reduced maintenance needs. (Haigh;2023)

Healthcare and medical applications:

The medical industry relies heavily on plastics for the production of a wide range of healthcare products and devices. Plastics such as polyethylene, polypropylene, and polycarbonate are used to manufacture disposable syringes, IV bags, blood storage containers, catheters, surgical gloves, and prosthetic limbs. Their sterility, flexibility, and affordability make them ideal for ensuring patient safety, reducing infection risks, and enhancing medical advancements. Additionally, 3D-printed medical implants and biodegradable plastic-based sutures are revolutionizing surgical procedures and patient care. (Ong, Thiel, & Singh;2024)

Textile and consumer goods

Synthetic fibres derived from plastics, such as polyester, nylon, and acrylic, are widely used in the textile industry to produce clothing, upholstery, carpets, and industrial fabrics. These materials offer durability, water resistance, and wrinkle resistance, making them popular in fashion, sportswear, and outdoor gear. Plastics also play a crucial role in the manufacturing of household items, furniture, kitchenware, toys, and sports equipment, providing consumers with lightweight, affordable, and aesthetically appealing products. (Løvbak Berg *et al*;2023)

Toxicity of plastics

Plastics are widely used in everyday life due to their convenience and versatility, but they pose significant risks to both human health and the environment because of their toxic properties and persistence. Toxicity arises from chemicals used during plastic production—such as bisphenol A (BPA), phthalates, and heavy metals—as well as from the harmful byproducts released when plastics degrade. These substances can leach into the environment and accumulate in ecosystems. Humans are exposed to these toxins through ingestion, inhalation, or skin contact, which has been linked to

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endocrine disruption, developmental and reproductive issues, cancer, and immune system problems. Wildlife is similarly affected, as animals can ingest or become entangled in plastic waste, leading to injury, death, or long-term health consequences.

Microplastics have become a major environmental concern, having been detected in water, soil, and even air, with the potential to enter the food chain. To address these challenges, strategies such as banning toxic additives, improving plastic waste management, promoting biodegradable alternatives, and increasing public awareness have been recommended. Overall, the toxicity of plastics represents a serious global issue that demands collaborative efforts in science, policy, and public behaviour to mitigate its widespread effects. (Maddela, *et al*;2023)

Review of Literature:

Liu *et al;*2022 focused on the effects of microplastic particle size and concentration, using polyethylene microplastics (PE-MPs) on maize (*Zea mays*) and cucumber (*Cucumis sativus*). Their experimental results demonstrated that larger particle sizes and higher concentrations of PE-MPs significantly reduced seed germination rates and inhibited seedling growth. Importantly, they also highlighted that chemical leachate from MPs, such as heavy metals and organic additives, further exacerbated phytotoxic effects. This dual threat—physical and chemical—provides a more complex picture of how MPs impair seed and seedling performance. Both studies complement each other by revealing that microplastic impacts on seed germination and seedling development are highly dependent on the physical attributes and environmental concentrations of MPs. Lozano *et al;*2022 provide valuable insights into shape-related stress mechanisms, while Liu *et al*;2022 demonstrate that both the size and abundance of MPs, along with their chemical toxicity, play vital roles in determining plant health outcomes. Together, these findings strengthen the evidence that microplastic pollution poses multifaceted risks to agricultural productivity and soil ecosystems. He focused on the effects of PE-MP particle size and concentration on the seed germination and early growth of maize (*Zea mays*) and cucumber (*Cucumis sativus*). Their results showed that larger microplastic particles and higher concentrations significantly suppressed germination potential and reduced shoot length in both crops. Notably, Liu *et al;*2022 highlighted not only the physical obstruction caused by PE-MPs but also the role of chemical leachates, such as heavy metals and organic additives, which further compromised seedling development.

Sahasa *et al*;2022 examined the effect of PE-MPs on the germination and seedling development of black gram (*Vigna mungo*) across different soil types, specifically loamy and clay loam soils. Their findings revealed that increasing concentrations of PE-MPs consistently reduced germination rates, regardless of soil type. However, the extent of the impact varied, with loamy soils showing relatively

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better tolerance compared to clay loam soils. Furthermore, both root and shoot growth deteriorated progressively as microplastic concentration increased, indicating that soil texture modulates the phytotoxicity of PE-MPs. This study underscores the importance of considering soil properties when assessing microplastic risks in agricultural systems.

Sahasa *et al;*2022 and Liu *et al*;2022 collectively highlights the complex interactions between microplastics, soil environments, and plant biology. By integrating insights from both soil variability and microplastic characteristics, future research can better predict and mitigate the impacts of plastic pollution on crop production and soil health.

Research Methodology:

Materials: [Yao *et al*. (2022)]

The polythene was purchased from a nearby store Vapi [U.T]. In Luria Bertani (LB) broth [sodium chloride (10 g/I), tryptone (10 g/l), and yeast extract (5 g/l), pH-7.4] bacterial cells were cultivated. Polythene was subjected to a microbial degradation assay using Bushnell-Haas (BH) broth [Cal (0.02 g/I), MgSO4 (0.20 g/l), NHNO3 (1 g/l), K2HPO4 (1 g/l), KH2PO4 (1 g/I), and FeCl3 (0.05 g/l), pH 7]. Acetone, chloroform, and ethanol were among the organic solvents utilized for sample processing and analysis.

Isolation and Screening of Bacterial Isolates [Yao *et al.* (2022)]

From plastic-contaminated soil, bacterial strains capable of degrading polyethylene were isolated; these isolates were previously identified as Bacillus spp. The bacterial cultures were kept on nutrient agar and nutrient broth for the biodegradation studies that followed.

Preparation and sterilization of Polyethylene Films [Yao *et al*. (2022)]

The plastic sheets used in this experiment were cut into uniform 3 × 3 cm squares and then carefully washed with distilled water to get rid of all remaining contaminants after 30 minutes in a washing solution containing 70% ethanol. After a one-hour drying process at 60°C, treated polyethylene pieces were carefully stored for later use. [10]

Studies on Biodegradation at the laboratory Scale [Vimala & Mathew (2016)]

Soaked plastic films in 100 ml of BHM medium, the degradation potential of the bacterial isolates was examined simultaneously. The inoculum was made by centrifuging 10 ml of an overnight Bacillus culture at 12,000 rpm for two minutes at 4°C. Distilled water was then used to resuspend the cells. There were two stages of this washing procedure to get rid of any leftover LB media. Finally, the cleaned cells were inoculated. Flasks with polyethylene that had not been inoculated with bacteria

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were used as the control. For 30 days, the flasks were shaken and incubated at 37°C. At regular intervals, polyethylene film weight loss analysis and surface characterization were carried out.

Study of Bacterial Viability on Polyethylene Surface [Lev *et al.*2024)]

The colony-forming units (CFU/cm2) were used to measure the viability of bacterial isolates attached to polyethylene films. To track the growth of the cells, the optical density of the culture broth was measured at 600 nm (OD600) every two days. The colony counting approach was also used to determine the number of viable cells. This was accomplished by spreading 100 µl of the culture broth on LB agar plates and incubating them for 24 hours at 37°C. The colony-forming units per millilitre (CFU/mL) was used to represent the viable cell count.

Determination of Polyethylene Weight Loss [Nadeem *et al.* (2023)]

The level of polyethylene degradation was measured by measuring the weight loss of plastic films. After being carefully taken out of the culture media, the films were rinsed with 2% sodium dodecyl sulphate (SDS) to get rid of any remaining microorganisms and then cleaned with distilled water. The films were dried at 60°C for 1 h and weighed. The percentage of degradation was calculated using the following equation:

Weight loss (%) = initial weight − final weight × 100%.

____________________________ Initial weight

Surface Characterization of Polyethylene Degradation [Nadeem *et al.* (2023)]

The surfaces of Polythene film samples were examined using an optical microscope at 1000× magnification. The analysis included three sample types: the untreated Polythene film (blank), the negative control (Polythene film cultured without microbes), and the experimental samples (Polythene film cultured with bacterial cells).

Result

In this study, we aimed to degrade plastic using polyethene degrading bacteria obtained from soil samples collected from two different dumping areas of Daman (U.T) and Utada (Gujarat). From the above samples, four different isolates C1, C4, C5, C7, C10 and C12were selected from nutrient agar medium and selective medium. All four isolates were further screened by primary screening with morphological and biochemical characteristics and identified by standard protocol. (Holt *et al.*, 1994). The obtained isolates were identified as *Bacillus spp*. by comparing the above results with Bergey's

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Manual. The results of screening, plastic degradation and gram staining are given below for isolates C4 and C10 respectively.

Table 4.1 Screening of bacterial isolate for plastic degradation

Sr. No	Region	Sample	Bacterial isolates	Screening on mineral salt medium seeded with plastic
1	Daman	Soil sample – 1	C1	Positive
2		Soil sample – 1	C2	Negative
3		Soil sample – 1	C3	Negative
4		Soil sample – 1	C4	Positive
5		Soil sample – 1	C5	Positive
6		Soil sample – 1	C6	Negative
7		Soil sample – 1	C7	Positive
8		Soil sample – 1	C8	Negative
9	Adovada	Soil sample – 2	C9	Negative
10		Soil sample – 2	C10	Positive
11		Soil sample – 2	C11	Negative
12		Soil sample – 2	C12	Positive
13		Soil sample – 2	C13	Negative
14		Soil sample – 2	C14	Negative

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Fig 4.1: C4

Fig 4.2: C10

Fig 4.1 and 4.2: Bacterial isolates C4 and C10 showing the zone of clearance on the mineral salt medium seeded with plastic. Transparent zone shows that bacterial isolate has capability of plast degradation.

Fig 4.3: C4   Fig 4.4: C10

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Fig 5.3 and 5.4: Gram staining of bacterial isolates C4 and C10

Hydrophobicity of bacterial isolates

Table 4.2 Hydrophobicity of bacterial isolates

Isolates	O. D	% of Hydrophobicity
C1	0.15	21.05%
C4	0.12	36.84%
C5	0.17	10.26%
C7	0.16	15.78%
C10	0.13	31.57%
C12	0.15	21.05%

In the present study 6 bacterial isolates which have given positive results on MSM (Mineral Salt Medium) were selected and subjected for evaluating their % hydrophobicity by BATH method to determine the interaction between bacterial cell surfaces and the surface of plastics [Devi *et al*;2019]. The results showed variable levels of hydrophobicity among the bacterial isolates at the BATH test. The isolates C1, C4, C5 and C10, has shown 21.05%, 36.84%,10.26%, and 31.57% hydrophobicity percentage respectively. The isolate C4 has given highest percentage of hydrophobicity i.e. 36.84% suggesting greater potential for surface interaction with a hydrophobic plastic whereas isolate C12 has given comparative low hydrophobicity level of 5.26%, suggesting even less affinity compared to other isolates.

Study of bacterial viability on plastic surfaces

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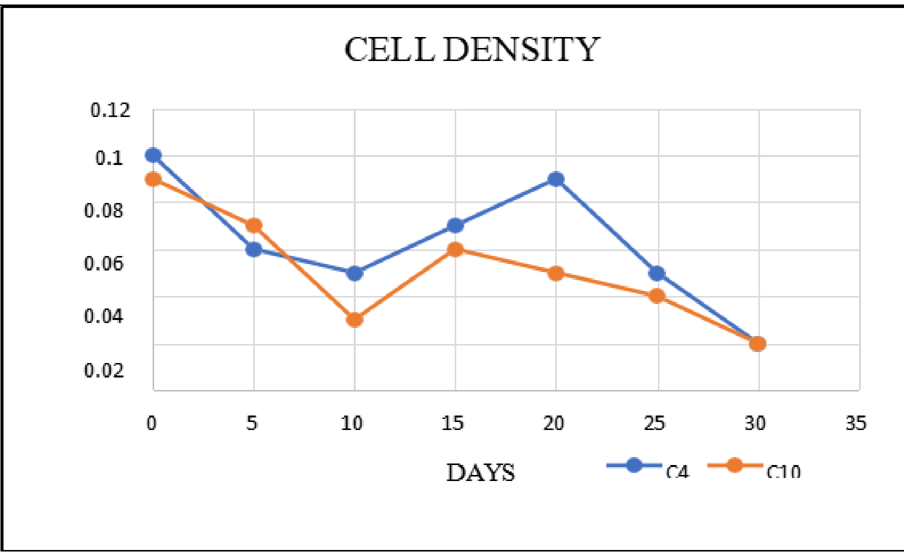
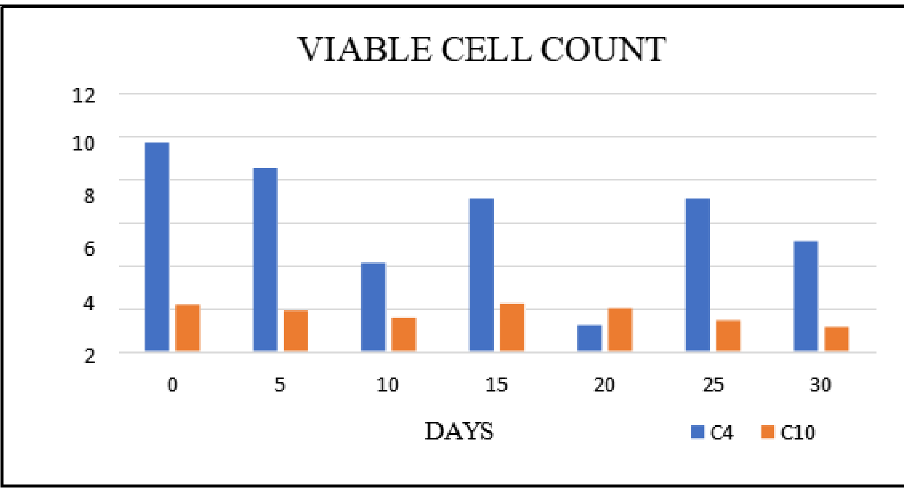


Graph 4.1: Cell density of bacterial isolate C4 and C10



Graph 4.2: Viable cell counts of bacterial isolate C4 and C10

To study the viability of bacterial isolate on plastic surface the cell density and viable cell count were examined. The isolates C1, C4, C5, C7, C10 and C12 shows the absorbance at 600 nm which are recorded as 0.09, 0.09, 0.09, 0.12, 0.11 and 0.12 respectively and viable count were 2.5×10^4, 9.7×10^6, 2.17×10^6, 1.01×10^6, 2.17×10^4, and 2.67×10^6 respectively after inoculation. The absorbance and viable cell count were decreased over next 10 days after bacterial inoculation. As the incubation continued the absorbance and viable cell count started to increase between 10th to 25th day indicating bacterial adaptation and utilisation of plastic as sole carbon source. The maximum growth was seen between the 15th to 25th day. The growth of bacterial isolates was increased with the absorbance and viable cell count.

At the beginning and during the lag phase, the bacteria were unable to utilize plastic as a carbon source, leading to a decrease in absorbance values and viable cell counts. However, once the bacterial log

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phase began, the cells adapted to using plastic as a carbon source. This adaptation resulted in an increase in bacterial cell numbers as they were able to degrade the plastic and utilize it for growth. Among all the isolates, C4 showed the most significant increase in both optical density and viable cell count, indicating the strongest potential for plastic degradation under the given conditions, [Lev *et al;*2024].

A comparative result of cell density and viable cell count for isolate C4 and C10 is represented in graph 4.1 and 4.2 respectively.

Determination of plastic weight loss

Table: 4.3 Plastic degradation with weight loss over the time in percentage

DAYS	% Weight loss given by different bacterial isolates					
	C1	C4	C5	C7	C10	C12
0	0%	0	0%	0%	0%	0%
10	0.25%	0.22%	0%	0%	0.33%	0%
20	0.25%	0.55%	0.11%	0.37%	0.44%	0%
30	0.37%	0.55%	0.22%	0.22%	0.44%	0.12%

To check the degradation of plastics the weight loss was measured at regular time interval. The above Table no: 5.4 shows the % weight loss of bacterial isolates. The weight loss after 30 days was 0.37%, 0.55%, 0.22%, 0.22%, 0.44%, and 0.12% for C1, C4, C5, C7, C10, and C12 respectively. The results, along with an increase in viable cell counts, suggest that the bacteria were able to use the plastic as a carbon source for growth. Among all the isolates C4 and C10 showed the highest degradation potential which was 0.55% and 0.44% respectively.

Surface characterization of plastic degradation

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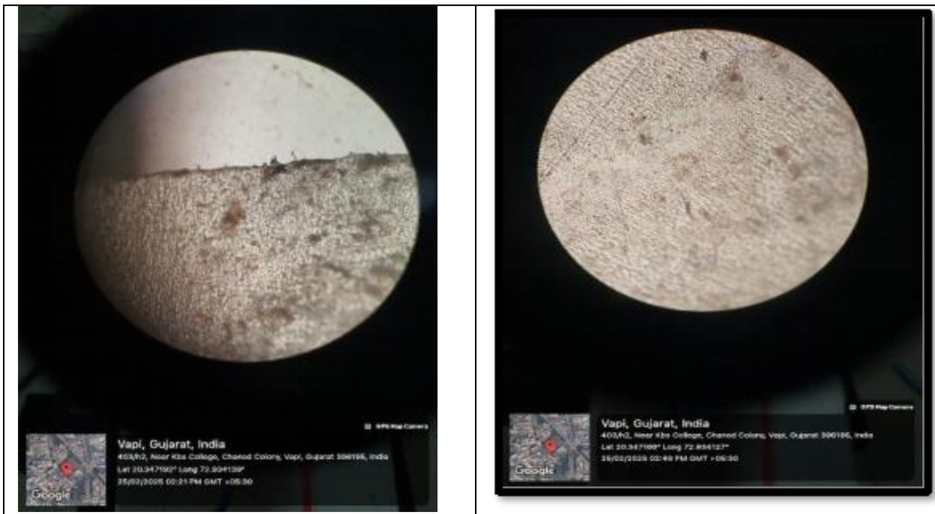
A	B

Fig 4.5: Morphology of plastic inoculated with bacterial isolate C4 under optical microscope: A, showing the edge of degraded plastic; B showing the surface of degraded plastic



A	B

Fig 4.6: Morphology of plastic inoculated with bacterial isolate(C10) under optical microscope: A, showing the edge of degraded plastic; B showing the surface of degraded plastic

After 30 days of incubation with the selected bacterial strain, noticeable changes were observed on the plastic film surface under microscopic examination. The edges of the plastic films appeared eroded, and the overall surface exhibited significant roughness compared to the untreated control samples. In some areas, visible degradation patterns such as cracks, pits, and irregular textures were evident. These morphological alterations indicate the biodegradative activity of the bacteria, suggesting that the strain was effective in initiating the breakdown of the plastic polymer structure over the incubation period. [Nadeem *et al*;2023]

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Discussion / Analysis

Bacterial isolate C4 showed superior hydrophobicity (36.84%) compared to % hydrophobicity demonstrated by Devi *et al.* in 2019 which was maximum 20.3%. This could be likely due to differences in bacterial strains, assay conditions, and environmental factors. These results shows that bacterial cell surface hydrophobicity is an important characteristic contributing to attachment and interactions with hydrophobic polymer surfaces, such as plastic.

Upon comparing the current data with Yao *et al.*2022 a similar result was given by *B. subtilis* ATCC6051 and *B. licheniformis* ATCC14580 after 30 days of incubation. *B. subtilis* ATCC6051 and *B. licheniformis* ATCC14580 exhibited measurable growth indicated by increases in OD600 and viable cell counts.

Upon comparing the present results with [Lev *et al;*2024] who used *Bacillus subtilis* and *Bacillus licheniformis* gave higher reduction of about 3.49% and 2.83% as compared to the reduction given by the isolates C4 and C10 which was 0.55% and 0.44% respectively. Overall, *Bacillus* strains remain effective degraders, but bacterial isolate could offer superior performance in plastic degradation by further modification.

Upon comparing surface morphology with Yao *et al.*2022 a similar result was given by *Subtilise* ATCC6051 and *B. licheniformis* ATCC14580 after 30 days of incubation. Treated films displayed surface roughness, edge erosion, and visible degradation features such as cracks, pits, and peeling, indicating active microbial degradation. [Yao *et* al;2022]

Conclusion:

The results demonstrate that *Bacillus* spp. can colonize and degrade polythene under controlled laboratory conditions. The increase in bacterial biomass, viability, and polythene weight loss over time confirms microbial degradation activity. These findings highlight the potential of Bacillus species in bioremediation applications for reducing plastic pollution. Further studies utilizing advanced spectroscopic and chromatographic techniques may provide deeper insights into the biochemical mechanisms of polythene degradation by these bacteria.

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