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Actinomycetes as a Promising Tool for Plastic and Hydrocarbon Biodegradation

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Abstract

Plastics and hydrocarbons are among the most prominent contaminants causing severe harm to the environment. Low-density polyethylene is one of the most commonly used plastics in day-to-day life. The high surface area and hydrophobicity of this plastic material serve as a vector for the transfer of other organic pollutants, like hydrocarbons. Eliminating these pollutants helps combat climate change and provide a safer and more sustainable future for all. Addressing both plastic and hydrocarbon pollution together requires a combination of techniques that target both the contaminants to move toward more sustainable practices. Biodegradation is the most cost-effective and long-term way to deal with this pollution. The key to solving this problem may lie with microorganisms that have diverse metabolism with the ability to utilize complex polymers, and one such potent microbe is the actinomycetes. In this study, we isolated 50 actinomycetes from various plastic dumping sites of Rajkot, Gujarat, and tested them for low-density polyethylene (LDPE) degradation by growing them in to medium having LDPE as a sole carbon source. Furthermore, the clear zone assay was performed to confirm the LDPE degradation. Alkane degradation was confirmed by observing the growth of isolates using hexadecane as a sole carbon source. This LDPE degrading actinomycetes were used for LDPE sheet, and reportedly found 16.2 %, 15.5 %, 14.6 %, and 14 % of weight loss of LDPE sheets. In one month by isolates PUA 20, PUA 35, PUA 11, and PUA 6 were isolated, respectively. Subsequently, plastic deterioration was verified through FTIR analysis, which revealed chemical alterations in the structure of the LDPE sheets. Notably, new absorbance peaks were observed in the spectra of isolates PUA 20, PUA 35, and PUA 11 at 953.54 cm^{-1}, 1073.79 cm^{-1}, 1149.35 cm^{-1}, and 1251.59 cm^{-1}, indicating the formation of new functional groups associated with polymer degradation.

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Introduction

In the fields of chemistry and material sciences, the synthesis of polymers like plastic from crude oil was revolutionary. This discovery opened a new doorway for the production of the most resistant and durable material, such as plastic.[1] A popular plastic polymer, low-density polyethylene (LDPE) is renowned for its adaptability, low density, and flexibility. It is produced through the polymerization of ethylene monomers under high pressure (1000 to 2000 atmospheres), which results in a branched structure that contributes to its unique properties. Plastics are derived from petrochemicals, linking their usage to hydrocarbon extraction and processing, which are primary contributors to pollution.[2] As versatile materials, plastics find applications in various sectors, including packaging, construction, and consumer goods. However, their durability, while beneficial in many contexts, poses a substantial threat to ecosystems and human health due to their resistance to degradation.[3] Furthermore, the primary issue that is currently emerging is the conversion of this plastic trash into microplastics which has detrimental effects on the environment, society, and economy.[4-5] As per the Plastics Industry Association of India the annual LDPE production capacity was estimated to be around 1.5 million metric tons per year in 2023 and the production capacity could reach approximately 1.8 million metric tons by 2024.[6] The increasing production and consumption of plastics, particularly low-density polyethylene (LDPE), have raised significant environmental concerns globally.[7]

Hydrocarbons are organic compounds made up solely of hydrogen and carbon atoms. In many industrial operations, they are vital raw materials and the main components of fossil fuels, such as coal, oil, and natural gas.[8] Hydrocarbon pollution occurs due to the leakages and accidental spills that happen during the exploration, extraction, refinement, transportation, and storage of petroleum hydrocarbons.[9] Also, another source of hydrocarbon pollution is plastic, which can leach harmful hydrocarbons into the environment, especially when exposed to sunlight and heat.[2] These chemicals can contaminate soil and waterways, affecting both ecological and human health. Biodegradation is the most economical and environmentally friendly process that converts complex polymers into simpler products primarily by microbial activity.[10] Bacteria belonging to the actinomycetes class have a range of metabolic processes and the capacity to make secondary metabolites. They are known to produce a range of enzymes that can be utilised to break down hydrocarbons and low-density polyethylene.[11]

There have been several reports that confirm the microbial degradation of LDPE and hydrocarbons by several microbial species. The bacterial species reported for LDPE degradation include: Acinetobacter *Brevibacillus*, *Rhodococcus*, *Micrococcus*, and *Pseudomonas*.[12-14] Fungus genera such as *Aspergillus*, *Mucor*, *Cunninghamella*, and *Penicillium* were also reported as potent polyethylene degraders.[15] Hydrocarbon-degrading bacterial species include *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Azotobacter chroococum*, *Pseudomonas putida*, and *Streptococcus faecium*.[16,17] Recent studies related to the biodegradation of hydrocarbons revealed that the use of actinomycetes for the biodegradation of hydrocarbons is relatively scarce. Some species of actinomycetes reported for hydrocarbon biodegradation were Streptomyces sp. MOE6, *Micromonospora* sp., *Nocardia* sp., *Gordonia* sp., and *Rhodococcus* sp.[18] In recent studies, it has been reported that hydrocarbon-degrading bacteria from various oil-contaminated sites can degrade plastic such as polyethylene (PE), polystyrene (PS), but very few studies have reported the potential of plastic-dumping sites harbouring plastic-degrading bacteria that can target both plastic and hydrocarbon simultaneously.[19,20] The main aim of this study is to explore the LDPE and hydrocarbon-degrading abilities of actinomycetes isolated from the plastic dumping sites of Rajkot, India. Actinomycetes, that able to degrade natural and synthetic plastics, include Streptomyces sp., *Rhodococcus* sp..[11] The potential of each isolates were assed for LDPE and hydrocarbon degradation. The confirmation of degradation was confirmed by the clear zone assay for both LDPE and hydrocarbon. Furthermore, the biodegradation of commercially available LDPE sheets was performed to determine the real-time application of isolates, and the confirmation for the degradation was obtained by observing the chemical changes of LDPE sheets by FTIR spectroscopy.

Materials and Methods

Review literature

To conduct research and review literature, a comprehensive search was conducted across major

scientific databases such as PubMed, ScienceDirect, SpringerLink, Google Scholar, and ResearchGate to find relevant literature. Several keywords were used: "LDPE biodegradation," "plastic degrading bacteria," "actinomycetes and polyethylene," "bioremediation of plastics," "plastic dumping site microbiota," "hydrocarbon degrading microorganisms," as well as "FTIR in polymer degradation." Following an initial screening of titles and abstracts, about 50 papers were chosen for full-text review. The most significant 36 studies were rigorously analysed and cited in the current study.

Isolation of Actinomycetes

To isolate actinomycetes, soil samples were collected from numerous garbage disposal sites in Rajkot, India. Four distinct locations inside Rajkot City were selected for sample collection, which were Aji Dam, Vagodad, Navagam, and Sadhuvasvani Road. The sample collection was done by collecting soil from the three to five centimetres, and the samples were collected in a sterile zip bag. The samples of soil were dried at 70 °C for two hours for the isolation of the actinomycetes from the soil. In addition, 1 g of the heated samples was serially diluted and applied to sterile starch casein agar plates (10 g of soluble starch, 2 g K_2HPO_4, 2 g KNO_3, 0.3 g casein, and 0.05 g $MgSO_4.7H_2O$, 0.01 g $FeSo_4.7H_2O$, 0.02 $CaCO_3$ in 1 L of distilled water). The actinomycetes colonies were recognized by physical characteristics and Gram staining following seven days of incubation at 30 °C. They were then streaked further to preserve them on starch casein agar plates.[11,21]

Screening for LDPE Degradation

Screening of the isolated actinomycetes was carried out by spreading them on sterile minimal salt medium (MSM) containing $NaNo_3$ (2 g), $MgSo_4$ (0.5 g), Kcl (0.5 g), $FeSo_4$ (0.01g), KH_2Po_4 (0.14 g), K2HPo4 (1.2 g), and Yeast extract (0.02 g) adding 1% LDPE powder as a carbon source. Followed by observing the growth of isolates in MSM broth with 1 % LDPE powered at 600nm till 7 days at 30 °C in shaking conditions at 120 rpm.[11,22] Further to confirm the LDPE degradation, the clear zone assay was performed using polyethylene glycol as a sole carbon source in minimal salt agar. Coomassie Brilliant Blue (w/v), made with 40% methanol and 20% acetic acid, was used to dye the plates after 7 days for 20 minutes. The stain was eliminated, and 20% acetic acid and 40% methanol were used to remove the stain. The plates were checked for the zone of clearance in a blue background.[21,23]

Screening For Alkane Degradation

Screening of isolated actinomycetes for alkane degradation was carried out by observing the growth of isolates in minimal salt broth, adding 1% n-hexadecane as a model alkane at 600nm for 7 days at 30 °C in shaking conditions at 120 rpm.[19,24] Alkane degradation was further verified by inoculating a loop full of culture of actinomycetes on a minimal salt agar plate along with 1% n-hexadecane, and the zone of clearance was observed after 7 days of incubation.[25]

Lab Scale Degradation LDPE Sheets

The lab-scale degradation of LDPE Sheets regularly used for packaging purposes was carried out by cutting the LDPE sheets into 2 by 2 cm, followed by exposing the LDPE sheets to the potent isolates for 30 days in sterile MSM medium at 30 °C in shaking conditions at 120 rpm. Followed by the incubation period, LDPE sheets were washed with 2% SDS for 3-4 Hours, and afterwards it was cleaned up with deionized water and dried at 60 °C overnight. After the incubation, the dry weight of the sheets was measured by the following formula.[25]

Weight loss in %= (Initial Weight-Final weight)/(Initial weight)×100

FTIR analysis of LDPE Sheets

The changes in the chemical structure of the LDPE sheets were observed by comparing the control and LDPE sheets inoculated with the potent isolates by FTIR spectroscopy at 500-4000 cm^{-1}.[26]

Results

Isolation of Actinomycetes

Researchers have identified plastic dumping sites as important repositories of diverse microbiota, many of which can break down a variety of environmental contaminants, including plastics and hydrocarbons. In this work, 50 actinomycete strains were identified from soils at four separate dumping sites in Rajkot, India. Soil samples were collected and put on sterile Starch Casein Agar (SCA) plates. After a 7 to 14 day incubation period, separate colonies were discovered and isolated: 14 from Aji Dam, 15 from Vagodad, 11 from Navagam, and 10 from Sadhu Vasvani

Road. The isolates were identified by microscopic examination as non-motile, filamentous, Gram-positive bacteria that belonged to the actinomycetes group. The colouration of the colonies ranged from grey (e.g., PUA 11, PUA 35), light greyish (e.g., PUA 9, PUA 15), to white (e.g., PUA 6, PUA 20). This variance in colony morphology and pigmentation gives preliminary information about the taxonomic richness of the isolated strains, which may correlate with differences in biodegradation capacity.

Screening of Isolates for LDPE Degradation

The primary screening for LDPE degradation was carried out by spreading the culture of isolates on MSM plates containing 1% LDPE as the only source of carbon. The findings of primary screening revealed that out of 50 different actinomycetes from the plastic dumping sites, only 29 isolates were able to grow well on MSM with LDPE powder. Whereas 12 isolates showed moderate to little growth in the presence of LDPE powder, 8 isolates were unable to use LDPE powder as a source of carbon; hence, in MSM plates, these isolates showed no signs of growth. Secondary screening for LDPE degradation was carried out by observing the growth of isolates in MSM broth with 1% LDPE powder at 600nm up to 7 days. Out of 29 isolates, only 10 actinomycetes showed a potential growth in the presence of LDPE, which is represented in Figure 1. The maximum growth O.D observed initially was by PUA 11 (0.071 + 0.004) and PUA 20 (0.071+ 0.01). At the end of the incubation the maximum growth was observed in PUA 35 (0.378 + 0.006) followed by PUA 20 (0.370 + 0.005), PUA 11 (0.352 + 0.002), PUA 1 (0.337 + 0.007), PUA 32 (0.328 + 0.02), PUA 6 (0.321 + 0.002) and PUA 16 (0.321 + 0.01) respectively. Further, the clear zone assay was performed using PEG as a carbon source, and the maximum zone of clearance was observed in isolates PUA 20, PUA 35, PUA 11, and PUA 6 after staining with Coomassie brilliant blue, which is shown in Figure 2.

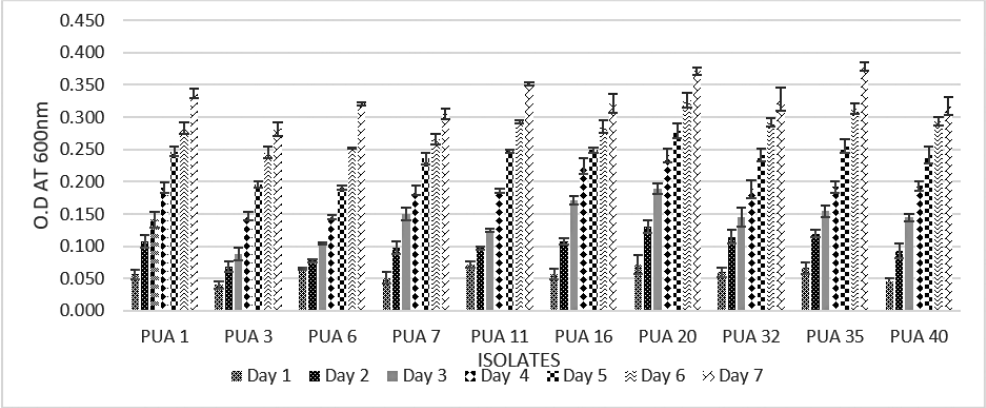


Fig. 1: Observation of Growth at 600 nm in 1% LDPE Containing Medium

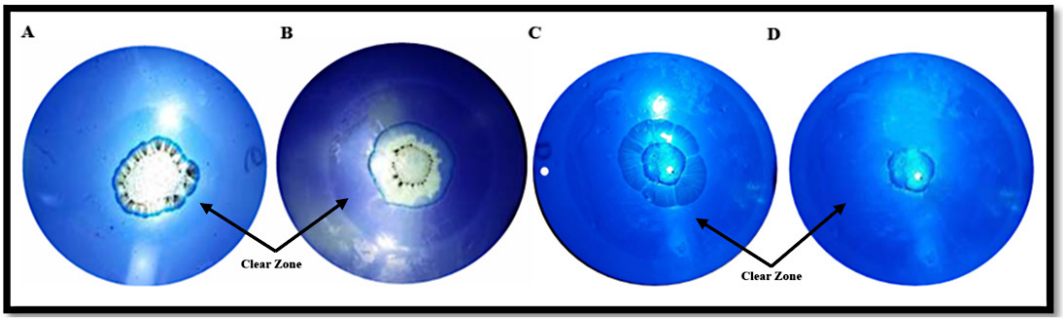


Fig. 2: Zone of clearance formed by isolates on MSM with 1% PEG, A. PUA 6, B. PUA 11, C. PUA 20, D. PUA 35

Screening of Isolates for Hydrocarbon Degradation

Hydrocarbon degradation is generally carried out by using a model alkane such as hexadecane, crude oil, or any other long-chain alkane. In this study n hexadecane was used as a model hydrocarbon. To screen out the hydrocarbon degrading potential of isolated actinomycetes (LDPE degrading) their growth was observed at 600nm in the presence of 1 % n hexadecane in MSM broth for 7 days at 120 rpm in shaking condition in 30 C. Two isolates namely PUA 11 and PUA 35 showed a highest growth in the initial stage (24 hour) compare to other isolates was 0.063 + 0.006 and 0.061 + 0.02 respectively (Figure 3). Upon completion of the incubation phase, the maximum growth was observed in PUA 35 (0.352 + 0.002), followed by PUA 20 (0.345 + 0.003), PUA 11 (0.336 + 0.002), and PUA 6 (0.310 + 0.002), respectively. In addition, to confirm the hexadecane degradation, the clear zone assay was carried out, and the isolates with the highest growth potential, such as PUA 35, PUA 20, PUA 11, and PUA 6, showed a zone of clearance on the MSM plate with hexadecane (Figure 4).

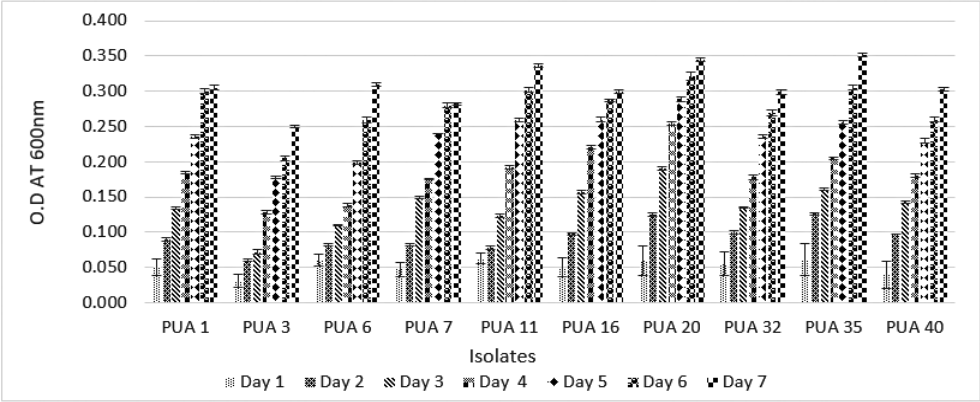


Fig. 3: Observation of Growth at 600 nm in 1% Hexadecane Containing Medium

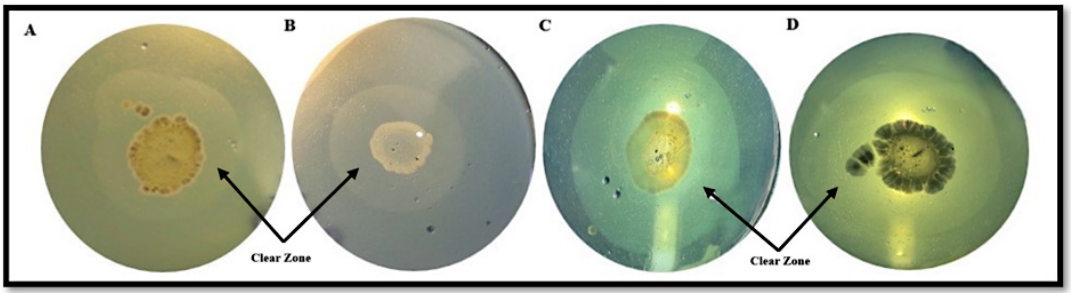


Fig. 4: Zone of clearance formed by isolates on MSM with 1% Hexadecane, A. PUA 6, B. PUA 11, C. PUA 20, D. PUA 35

Lab Scale Degradation LDPE Sheets

The actinomycete strains capable of decomposing LDPE powder were then tested for their capacity to degrade commercially available LDPE sheets, often used for packaging. To determine the level of degradation, a comparison was made between the control group (uninoculated LDPE sheets) and the test group (LDPE sheets inoculated with the isolates PUA 20, PUA 35, PUA 11, and PUA 6) after one month of incubation. The weight of the LDPE sheets in the inoculated samples was found to have significantly decreased compared to the control, indicating microbial degradation activity. The degradation performance of each potent isolate is presented in Figure 5. Among all the isolates, PUA 20 had the highest degrading efficiency, with a weight loss of

16.2% ± 0.2, followed by PUA 35 (15.5% ± 0.1), PUA 11 (14.6% ± 0.2), and PUA 6. These findings indicate that the selected actinomycete isolates have the potential to biodegrade LDPE materials.

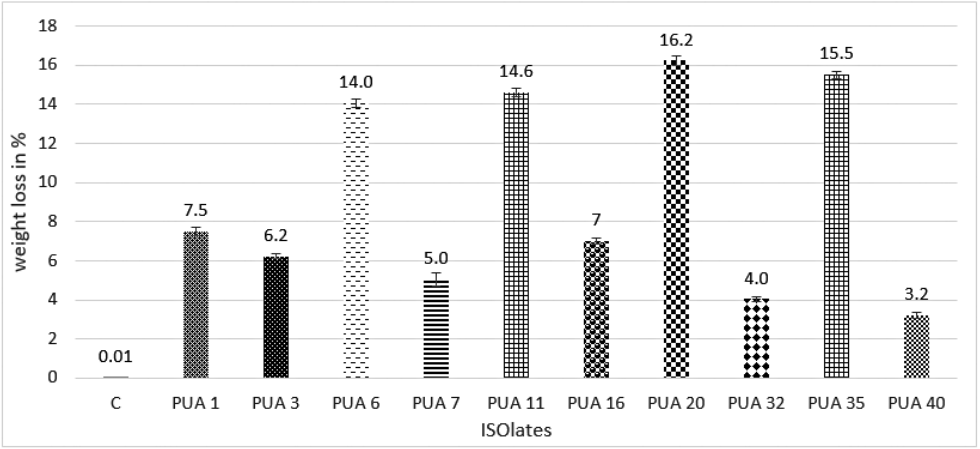
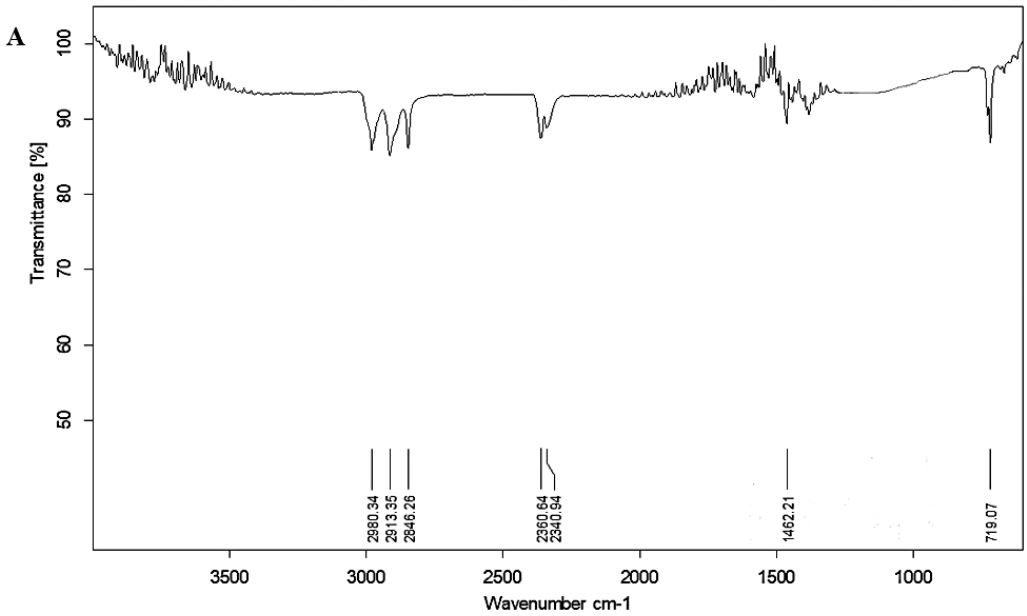


Fig. 5: Percentage of LDPE sheet weight loss after 30 days of incubation

FTIR Analysis of LDPE Sheets

The FTIR spectroscopy was used to confirm that the chemical modification occurs on the LDPE sheets by the isolated actinomycetes. The FTIR spectrum of control sheet showed the absorbance peaks at 719.07 cm-1 cm^{-1} (C-H rock), 1462.21 cm^{-1} (-C-H blending), and peaks at 2846.26 and 2913.35 cm^{-1}, 2980.35 cm-1 indicates - C-H stretch (figure 6). which are typical for polyethylene and indicate the integrity of its aliphatic hydrocarbon backbone (Figure 6).



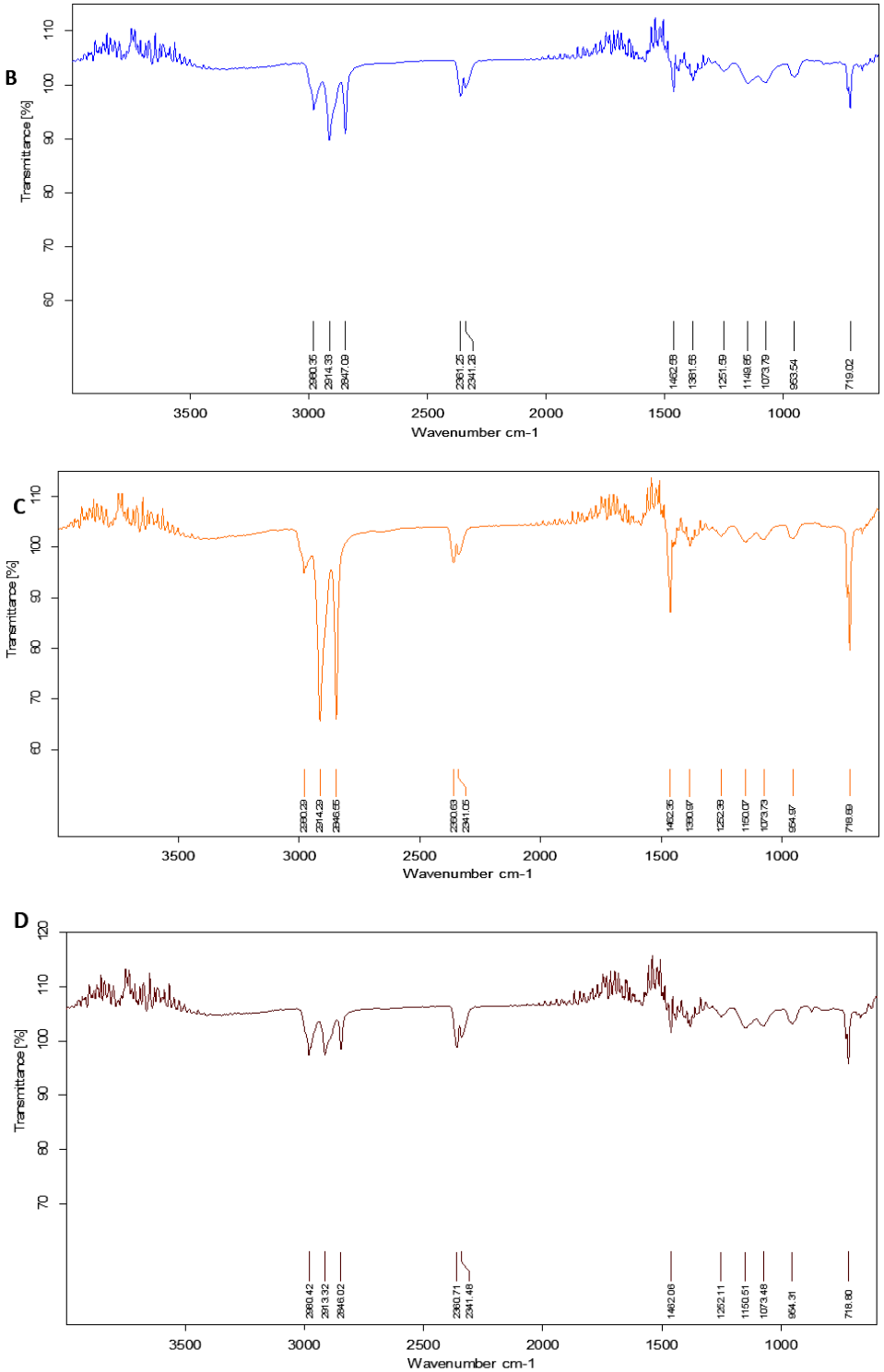


Fig. 6: The FTIR Spectrum of the LDPE sheet after 30 days of incubation, inoculated with test A. Control, B. PUA 11, C. PUA 20, D. PUA 35

In comparison with the control, there were modifications in the absorbance peaks of the LDPE sheets inoculated with the isolates, which is shown in Figure 6. There was a presence of new absorbance peaks in PUA 20, PUA 35, and PUA 11, which were at 953.54 cm-1, 1073.79 cm-1, 1149.35 cm-1, and 1251.59 cm^{-1} (Figure 6). These additional peaks indicate the development of novel functional groups, including ether (C-O-C), carbonyl (C=O), and hydroxyl (-OH) moieties, indicating oxidative or hydrolytic breakdown of the polymer matrix. The appearance of these functional groups offers support to the theory that actinomycetes aided in the partial disintegration of the polyethylene structure by enzymatic activity, presumably involving oxidative enzymes such as laccases, peroxidases, or oxygenases. The observed changes in the FTIR spectra give molecular-level evidence of structural disruption and chemical transformation in the polymer chains, confirming the biodegradation capacity of the selected actinomycete isolates.

Discussion

Plastic dumping sites or garbage dump sites are well studied for the understanding of plastic degradation mechanisms in earlier research and described as a major source of obtaining microbes with plastic-degrading abilities.[27] The dump site soils are rich in nutrients and hence they harbour diverse microbiota with potential for biodegradation and bioremediation of waste.[28] Polyethylene bags and plastic bottles were degraded by microbes isolated from plastic dumping sites, including *Bacillus cereus*, *Cellulosimicrobium spp*., *Streptomyces weraensis*, and *Streptomyces rochei*.[29] In this study total of 50 actinomycetes were isolated from various plastic dump sites of Rajkot. These isolates were screened for LDPE degradation by observing their growth in MSM along with LDPE powder, which confirms that the isolated actinomycetes were capable of using LDPE as a source of carbon. The similar methods were used by Hussein *et al*. (2015)[16] and Soud (2019)[30] For the screening of LDPE degradation by *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Acinetobacter ursingii*, and *Streptomyces spp*.. The clear zone assay on polyethylene glycol was earlier used by Rana and Rana (2020)[31] For the screening of LDPE-degrading bacteria isolated from Himachal Pradesh, India. The zone of clearance confirms the LDPE degrading ability of isolates by the production of hydrolytic enzymes.[21] The growth of isolates PUA 20, PUA 35, PUA 11, and PUA 6 in the presence of 1% hexadecane in MSM confirms the ability to digest oil or hydrocarbon.[25] Similar results were observed by Ahmed *et al*.(2021)[32] Where the LDPE degrading bacteria *Enterococcus cloacae*, *Pseudomonas putida*, and *Ralstonia pickettii*, *were able to grow when hydrocarbons were present.* The formation of a clear zone on the MSM plate containing hexadecane confirms the degradation by isolated actinomycetes.

The lab-scale LDPE sheet degradation was confirmed by determination of weight loss after 1 month of incubation time. The weight loss determination is the simplest technique frequently used for the confirmation of LDPE degradation.[33] In past, Soud (2019)[30] isolated streptomyces has shown 15 % weight loss of LDPE strips by isolate SSP 14, similar to that we were also able to obtained a LDPE sheet degradation rate of 16.2 % + 0.2 and 15.5 + 0.1 by PUA 20 and PUA 35 respectively which was slightly higher compare to Soud (2019).[30] Biodegradation of LDPE sheets was carried out by Pramila & Ramesh (2015)[34] using *Bacillus cereus* and *Pseudomonas* putida, confirming the changes in surface and tensile strength of the used LDPE sheets and reporting the 2.80 % weight loss.[34] Abraham *et al*. (2016)[35] compared the biodegradation of LDPE by fungus species *Aspergillus* nomius (4.9%) and *actinomycetes Streptomyces* sp. (5.2 %) for 90 days and confirmed the higher degradation of LDPE by actinomycetes.[35] The chemical changes of the degraded LDPE sheets in comparison with the control sample were done using FTIR spectroscopy. Formation of new narrow peaks at 953.31-953.54 cm^{-1} and between 1000 to 1200 cm^{-1} in the test samples of PUA 20, PUA 35, and PUA 11 indicates the presence of phenolic peak of C-O, which confirms the presence of primary and secondary alcohol groups during the process of degradation.[36] Moreover, the main bands of the LDPE sheets are a band at about 2900 cm^{-1} that is asymmetrically stretched by CH2, a band at about 1461–1466 cm^{-1} that exhibits bending deformation, and a band at 718–720 cm^{-1} that exhibits rocking deformation.[33,37] This may provide the interlink between the pathway used by microbes to degraded complex polymer and further lead to identification of common gene that can target multiple contaminants at once in natural habitats

so the pollution can be removed. Furthermore, this study also aligns with Sustainable Development Goals 12, 14, and 15, which focus on reducing waste and harmful chemicals to protect both marine and terrestrial ecosystems.

Conclusion

The present study emphasizes the promising capability of actinomycetes in the simultaneous biodegradation of low-density polyethylene (LDPE) and hydrocarbons, two major environmental pollutants. Among the 50 isolates screened from plastic dumping sites in Rajkot, Gujarat, several strains demonstrated notable efficacy in degrading LDPE sheets, with a maximum weight loss of 16.2% observed within a one-month incubation period. The ability of selected isolates to grow on hexadecane further confirmed their hydrocarbon-degrading capabilities. FTIR analysis provided strong evidence of polymer structural breakdown, revealing the formation of new functional groups indicative of LDPE degradation. These findings suggest that actinomycetes, with their versatile metabolic capabilities, hold significant promise as eco-friendly agents for bioremediation. Harnessing such microbial systems could contribute to integrated waste management strategies aimed at reducing plastic and hydrocarbon pollution, paving the way for more sustainable environmental practices.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript includes all datasets generated or examined during this research investigation.

Ethics Statement

This study did not include human participants, animal subjects, or any material requiring ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from Other Sources

All the figures, tables, and material in this research are original.

Author Contributions

- **Unnati Bhaveshbhai Yagnik:** Conceptualization, Data Collection, Analysis, Writing original draft, Revision
- **Mousumi Bijoykumar Das:** Supervision, Review, Revision

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