Biodegradation of Polyethylene Using *Bacillus tropicus* Isolated from Sewage Wastewater Treatment Plant

Isha Isha , Shakir Ali , Young-Cheol Chang *

Posted Date: 23 October 2024

doi: 10.20944/preprints202410.1796.v1

Keywords: plastic pollution; polyethylene; biodegradation; microbes; *Bacillus tropicus*; FTIR; GC-MS; SEM

Article

Biodegradation of Polyethylene Using *Bacillus tropicus* Isolated from Sewage Wastewater Treatment Plant

Isha Isha[1], Shakir Ali [2] and Young-Cheol Chang [3,*]

[1] Department of Zoology, Government College University Lahore, Lahore 54000, Pakistan
[2] Advanced Sustainable and Environmental Engineering, Muroran Institute of Technology, Hokkaido 050-8585, Japan
[3] Course of Chemical and Biological Engineering, Muroran Institute of Technology, Hokkaido 050-8585, Japan
* Correspondence: ychang@muroran-it.ac.jp

Abstract: One of the most pressing environmental problems contemporary civilizations confront is the ever-increasing amount of plastic waste. Because of their impact on every living thing, these wastes are seen as a major issue on a global scale. To counteract the harmful environmental effects caused by conventional disposal methods, it is critical to show that eco-friendly alternatives are viable. Biodegradation is one of the best eco-friendly methods for removing plastic waste. In this study, we aimed to identify bacteria from sewage wastewater treatment plants (SWW) that could degrade polyethylene. The bacterial strains isolated from sewerage wastewater were incubated for 120 days in 50 ml of minimal salt media (MSM) containing 60mg polyethylene. After four months, our research revealed that *Bacillus tropicus* (SH4) demonstrated significant potential, degrading polyethylene up to 21.6%. We observed the changes after biodegradation using FTIR, GC-MS, and SEM analysis. In conclusion, microorganisms extracted from sewage wastewater possess the ability to mitigate plastic contamination in aquatic ecosystems. Future proteomics and genome investigations are necessary to elucidate the enzymes and metabolic processes implicated in plastic breakdown.

Keywords: plastic pollution; polyethylene; biodegradation; microbes; *Bacillus tropicus*; FTIR; GC-MS; SEM

1. Introduction

Plastic pollution has become one of our most urgent environmental issues. Due to its escalating manufacturing and widespread application in numerous industries, plastic waste has permeated practically every region of our world, from the deepest oceans to the most isolated wilderness areas. The ramifications of this development are extensive, profoundly affecting ecosystems, human health, and the overall welfare of our planet [1]. Recognizing the necessity of solving this global issue, academics and environmentalists have been aggressively pursuing creative solutions to mitigate plastic pollution.

Plastics also referred to as organic polymers, consist of elongated carbon chains that form the foundation of their molecular architecture. These synthetic materials are mostly sourced from fossil fuels and comprise carbon, hydrogen, nitrogen, and sulfur, along with different inorganic and organic chemicals [2].

Plastics are categorized into various types: natural, semi-synthetic, synthetic, thermoplastics, and thermosetting plastics. Plastic mass production commenced in the 1950s, with the majority of polymers first engineered for single-use purposes [3].

Although beneficial in numerous industrial and consumer applications, the durability and versatility of plastics have prompted environmental concerns. Non-biodegradable plastics can

 doi:10.20944/preprints202410.1796.v1

endure in the environment for millennia, substantially exacerbating global garbage accumulation. In 2010, China generated 8.8 million tonnes of plastic garbage annually, representing 27% of global production [4]. Indonesia generated 3.2 million tons of plastic garbage annually, accounting for 10% of the global total. Plastic has emerged as a substantial element of Indonesia's everyday refuse, comprising roughly 15% of its municipal waste [5]. In 2018, the European plastics industry indicated that global plastic production reached 335 million tons, with Europe accounting for 60 million tons of this significant figure. Furthermore, these output figures are anticipated to rise significantly in the forthcoming decades [6].

Although plastic is now essential to our daily existence, its durability and prevalence provides a significant environmental hazard. Due to their remarkable endurance, plastics remain insoluble for years, causing significant environmental damage as they gradually decompose into tiny particles. This persistent problem has led to an increase in plastic trash that jeopardizes numerous animals, including humans. In addition to contaminating our landscapes and waterways, plastic garbage imposes considerable environmental expenses and public health risks through incineration. Incinerating plastic trash emits toxic chemicals, such as carbon dioxide and dioxins, which are associated with respiratory disorders and cancer [7].

Plastic pollution, a widespread problem that crosses borders and cultures, requires efficient waste management and mitigation techniques. Although minimizing, recycling, and reusing plastics have become prevalent strategies for addressing the issue, there is still a necessity for more effective techniques, especially for mixed plastic trash. Plastic garbage disposal in landfills or incinerators occupies considerable space and poses the risk of releasing toxic gases into the environment. Consequently, it is essential to devise recycling technologies that are both efficient and environmentally sustainable. Biodegradation has surfaced as a viable and economical solution to this global issue [8].

As almost all waste materials flow through the sewage, sewage wastewater contains bacterial strains that have the potential to degrade plastic—the current study aimed to isolate polyethylene-degrading bacteria from a sewage wastewater treatment plant. This enzymatic method of plastic biodegradation is economical, environmentally friendly, and sustainable.

2. Materials and Methods

2.1. Sample Collection

We collected bacterial samples from the sewage wastewater treatment plant (Township, Lahore). The samples were stored in a bottle and taken to the laboratory. Wastewater was spread on Petri plates containing nutrient agar to get colonies.

2.2. Applying Stress to Bacteria to Use Plastic as a Carbon Source

We incubated the obtained bacteria colonies with 60 mg commercially available polyethylene balloon pieces (Brand: Bouiexye) for 120 days in 50 ml minimal salt media (MSM). The flasks were placed in a shaking incubator at 37˚C.

2.2.1. Minimal Salt Media (MSM) Preparation

500ml minimal salt media was prepared in an autoclaved flask by adding salts given in 500ml distilled water (Table 1).

Table 1. indicates the ingredients and quantity used to make MSM media.

Serial No.	Compound	Quantity/500ml
1	K_2HPO_4	2.27g
2	Na_2HPO_4	5.97g
3	NH_4Cl	0.5g
4	$MgSO_4$	0.25g

 doi:10.20944/preprints202410.1796.v1

5	$CaCl_2$	0.0025g
6	$FeSO_4$	0.001g
7	$MnSO_4$	0.0005g
8	$ZnSO_4$	0.001g

2.3. Molecular Identification of Bacteria

The sample from the trial flask was streaked onto a nutrient agar plate for further screening after 120 days. The molecular-level characterization of the strain was preceded by gram staining. Thermo Scientific's geneJET Genomic DNA purification KIT (#K0721, #K0722) was used to isolate bacterial genomic DNA. The 16S rRNA gene was amplified using PCR with universal primers RS1 (5′AAACTCAATGAATTGACGG 3′) and RS3 (5′ACGGGCGGTGTGTA 3′). The ingredients for one 50 µl reaction mixture were 25 µl master mix, 1.5 µl primers, 10 µl template, and 12 µl nuclease-free water. Denaturation at 94 °C for 5 min was the first step in the PCR process. There were 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 45 sec, and extension at 72 °C for 5 min. Following that, 1% of the agarose gel was used to visualize the final products [9].

To purify the 16S rRNA gene, we employed the Gene JETTM PCR purification kit. The DNA and binding buffer were added to a reaction mixture before being passed through a purification column. Eliminating contaminants is as easy as washing. Following the removal of the DNA from the column by means of the elution buffer, it was submitted for sequencing [9]. After gene cleanup, the isolated bacteria's nucleotide sequences were analyzed using the NCBI BLAST database as part of the ribotyping procedure. Multiple sequence alignments were conducted using CLUSTAL W, which uncovered the commonalities [10]. The sequencing of all samples identified a novel strain, SH4.

Saitou and Nei (1987) utilized the Neighbor-Joining method to infer evolutionary history. Each branch is accompanied by the frequency of occurrences of the associated species clustered in the bootstrap test (1000 replicates) [11,12]. The Maximum Composite Likelihood method was employed to compute evolutionary distances, represented as the mean number of base substitutions per site. The MEGA11 software was utilized to perform evolutionary studies [13,14].

2.4. Weight Loss Experiment

Plastic pieces were removed, dried, and weighed. The % weight loss was measured by using the formula.

Weight loss = $\frac{Iw-Fw}{Iw} \times 100$

I_W= Initial weight

F_W= Final weight

2.5. Scanning Electron Microscopy (SEM) of Degraded Plastic Pieces

To get a highly magnified pictorial view, the physical change that appeared in the plastic pieces was further examined under SEM. Samples were sent to LCWU (Lahore College for Women University) for analysis.

2.6. Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

Following a four-month period in the incubator, the flasks were extracted. The plastic components were extracted from the flask. The culture was centrifuged at 6500 RPM for 10 minutes to eliminate cell debris, and GC-MS analyzed the supernatant in the TTI (Textile Testing) Lab in Lahore.

2.7. Fourier Transform Infrared Spectroscopy (FTIR)

The plastic fragments were dispatched to LCWU (Lahore College for Women University) for FTIR analysis to assess alterations in chemical bonds resulting from the enzymatic activity of bacteria.

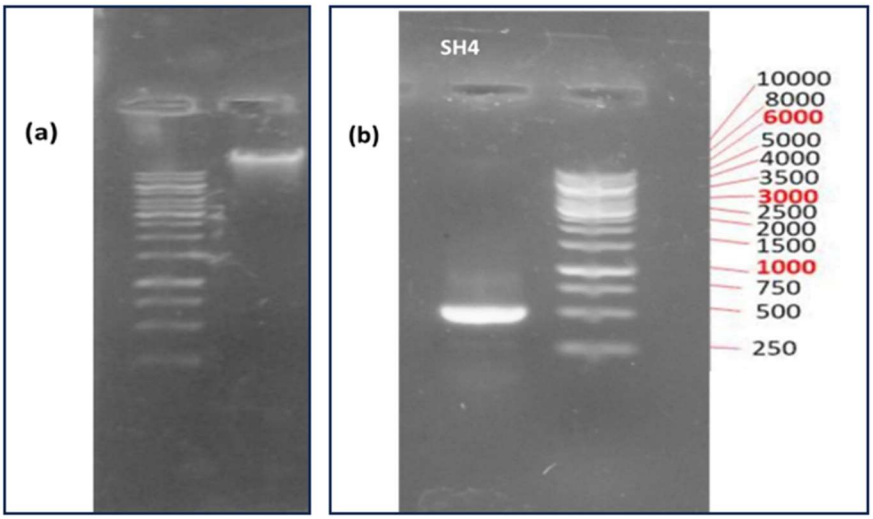
 doi:10.20944/preprints202410.1796.v1

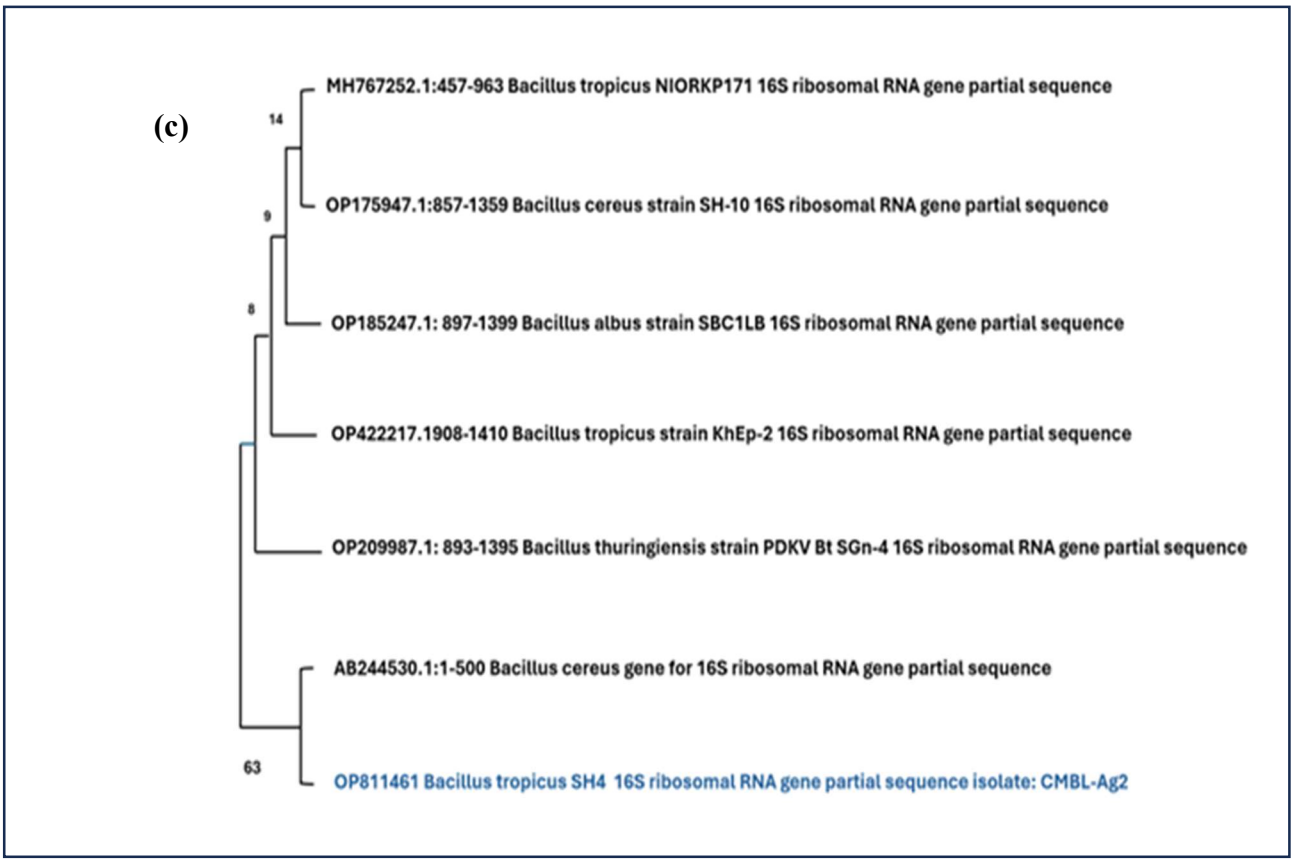
3. Results

3.1. Molecular Identification

Polyethylene waste has emerged as a significant environmental concern due to its inert chemical properties. Biodegrading and eradicating plastic waste from the ecosystem is a significant problem. Microbes are crucial for the biodegradation of synthetic polymers, including low-density polyethylene. The isolation of pure plastic-degrading strains requires careful cultivation and sub-culturing of the strain on media where plastic is the sole carbon and energy source [15].

In the present study, Bacterial strains for polyethylene were collected from sewage wastewater treatment plants. After 16S rRNA gene sequencing, the sample SH4 was identified as *Bacillus tropicus*, which showed 21.6% biodegradation [Figure 1]. The PE-degrading bacteria that have been reported so far belong to *Pseudomonas* sp.[16], *Bacillus* sp. [17], *Mycobacterium* sp. [18], and *Nocardia* sp. [19].





 doi:10.20944/preprints202410.1796.v1

Figure 1. Indicates visualized DNA on 1% agarose gel (a) isolated DNA of strain (b) 16S rRNA gene amplification by PCR (c) a Dendrogram of *Bacillus tropicus* strain SH4 showing resemblance with other bacterial strains.

3.2. Weight Reduction

To observe the potential of our isolated strain, the weight loss of polyethylene was observed over a period of 120 days. With passing days, polyethylene weight decreased, and after 120 days, we observed a maximum weight loss of 21.6% [Figure 2].

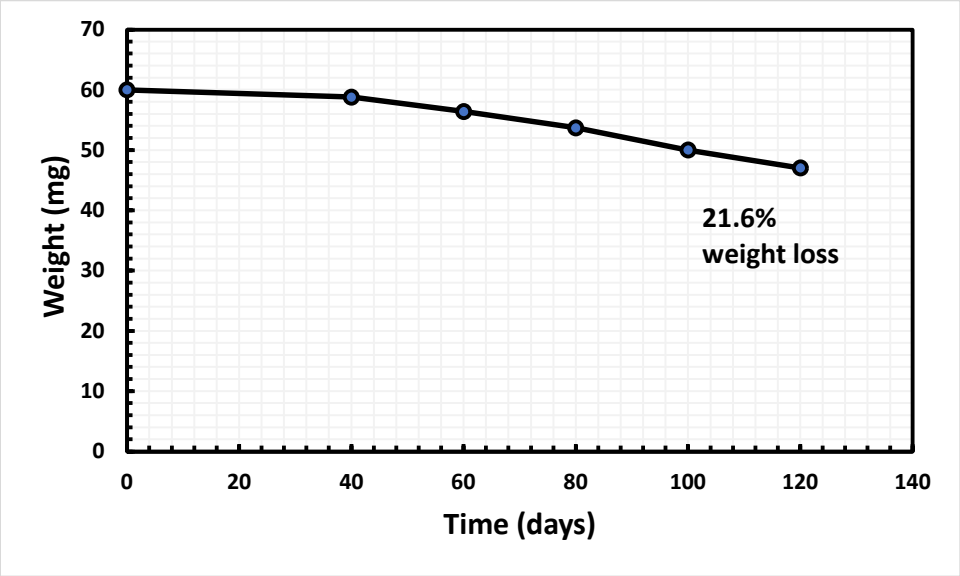


Figure 2. Weight reduction of polyethylene with time by action of *Bacillus tropicus.*

The present study utilized 60mg of plastic pieces to examine the degradation capacity of *B. tropicus* obtained from a sewage wastewater treatment plant. *B. tropicus* exhibited a 21.6% reduction in the weight of polyethylene fragments from the original mass. LDPE films subjected to treatment with *Pseudomonas knackmussii* N1-2 and *Pseudomonas aeruginosa* RD1 for a duration of 8 weeks exhibited weight reductions of 5.95% and 3.62%, respectively [20].

3.3. GC_MS Analysis

As a result of the activity of the strain *B. tropicus* SH4, [Figure 3] Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl 1,3,5-Benzetriol, 3TMS derivative Cyclotrisiloxane, hexamethyl Arsenous acid, tris(trimethylsilyl) ester Tris(tert-butyldimethylsilyloxy)arsane Cyclopentasiloxane, decamethyl-Cyclohexasiloxane, dodecamethyl 3-Amino-2-phenazinol ditms Benzeneethanamine, N-[(pentafluorophenyl)methylene]-.beta.,3,4-tris[(trimethylsilyl)oxy] Benzamide, 4-ethyl-N-benzyl-N-propyl trans-(2-Chlorovinyl)dimethylethoxysilane Ethanone, 2-(4-hydroxy-5,6-dimethylthieno [2,3-d]pyrimidin-2-ylthio)-1-(4-ethylphenyl) Cyclotetrasiloxane, octamethyl 1,4-Benzenedimethanethiol, 2TBDM 2,6-Dihydroxybenzoic acid, 3TMS Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl were detected in the supernatant [20,21] [Table 2].

 doi:10.20944/preprints202410.1796.v1

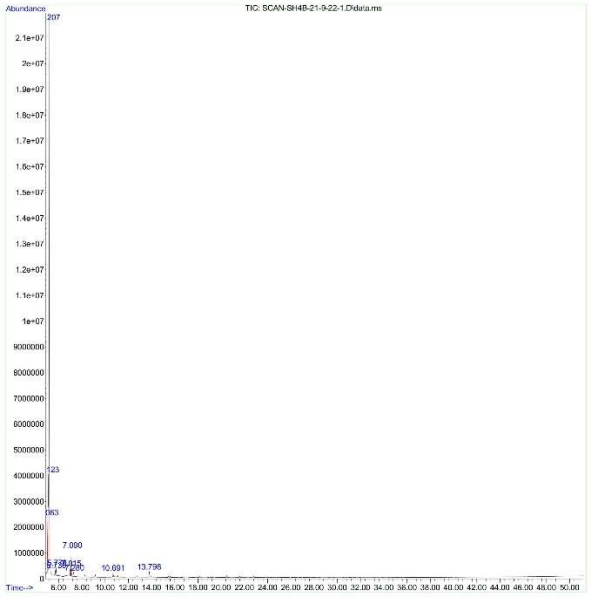


Figure 3. shows a GC-MS analysis of products produced as a result of biodegradation by SH4 (*Bacillus tropicus*).

Table 2. In GC-MS analysis, the biodegradation of *B. tropicus* SH4 results in the production of the chemicals listed.

Bacteria	Compounds
Bacillus tropicus	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl
	1,3,5-Benzetriol, 3TMS derivative
	Cyclotrisiloxane, hexamethyl
	Arsenous acid, tris(trimethylsilyl) ester
	Tris(tert-butyldimethylsilyloxy)arsane
	Cyclopentasiloxane, decamethyl-
	Cyclohexasiloxane, dodecamethyl
	3-Amino-2-phenazinol ditms
	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-.beta.,3,4-tris[(trimethylsilyl)oxy]
	Benzamide, 4-ethyl-N-benzyl-N-propyl
	trans-(2-Chlorovinyl)dimethylethoxysilane
	Ethanone, 2-(4-hydroxy-5,6-dimethylthieno [2,3-d]pyrimidin-2-ylthio)-1-(4-ethylphenyl)
	Cyclotetrasiloxane, octamethyl
	1,4-Benzenedimethanethiol, 2TBDM
	2,6-Dihydroxybenzoic acid, 3TMS
	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl

3.4. FTIR Analysis

 doi:10.20944/preprints202410.1796.v1

We found that the O-H stretching of carboxylic acid caused a signal at 3270 cm^{-1} in the FTIR spectra of *Bacillus tropicus* SH4-treated polythene. At 3016, you can see the peak that represents the alkene's C-H stretching. The C-H stretching of the alkane group is shown by the peaks at 2910, 2946, and 2840. At the same time, the Peak at 1729 corresponds to the C-H bending of aromatic compound. C=C stretching of conjugated alkene is demonstrated by the peak at 1642. The peak at 1536 shows the N-O bond stretching of the nitro compound. The peak at 1365 is attributed to the C-H bond bending of alkane (gem dimethyl). The C-O stretching of the alkyl aryl ether is seen by the peak at 1209. Peaks at 1088 and 1027 indicate C-N stretching of the amine group [Figure 4]. According to Samanta et al. (2020), the ester group's C-O stretching and the alkene group's C=C bending are indicated by the peaks at 1027 and 960, respectively [22].

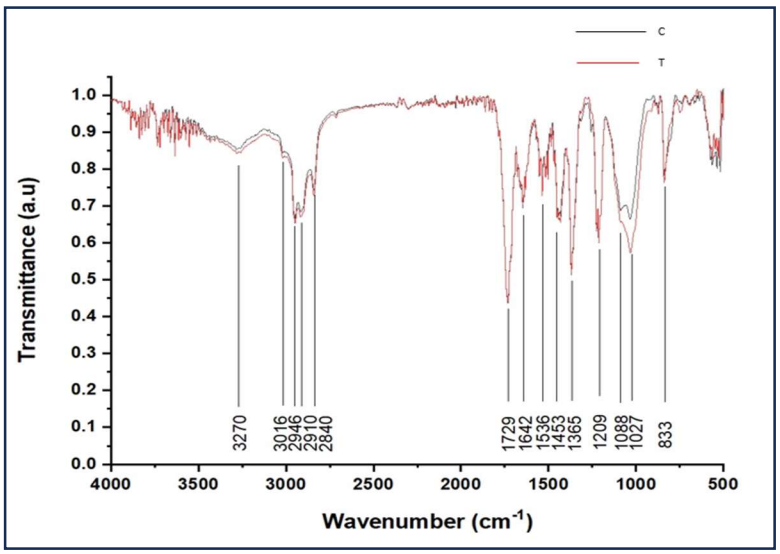
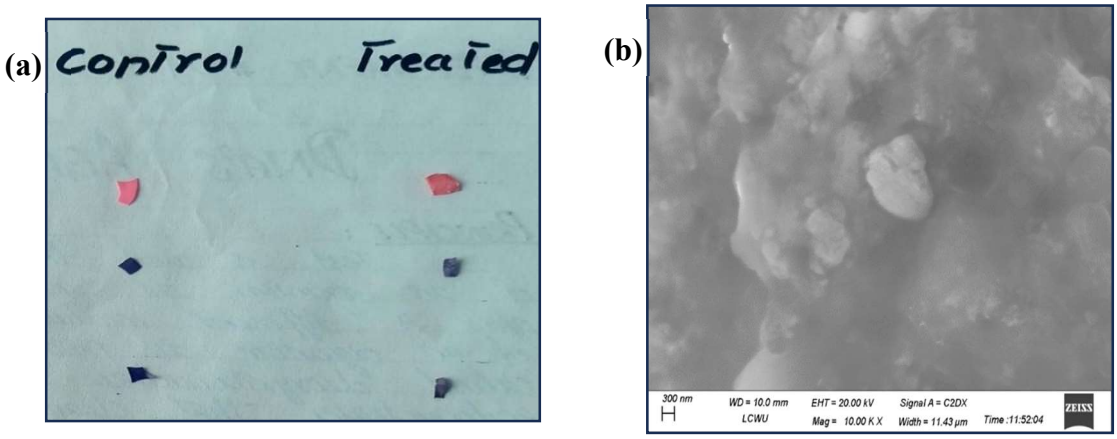


Figure 4. FTIR spectra of polyethylene treated with *B. tropicus*.

3.5. SEM Analysis for Physical Change in Plastic Pieces

After 120 days, significant changes were observed in the physical characteristics of plastic pieces. In comparison with the control, the treated pieces were thin and porous. SEM analysis assessed the LDPE surface alterations [Figure 5], with analogous investigations documented in prior literature. These findings robustly corroborate the data given in the current study [20,23,24].



 doi:10.20944/preprints202410.1796.v1

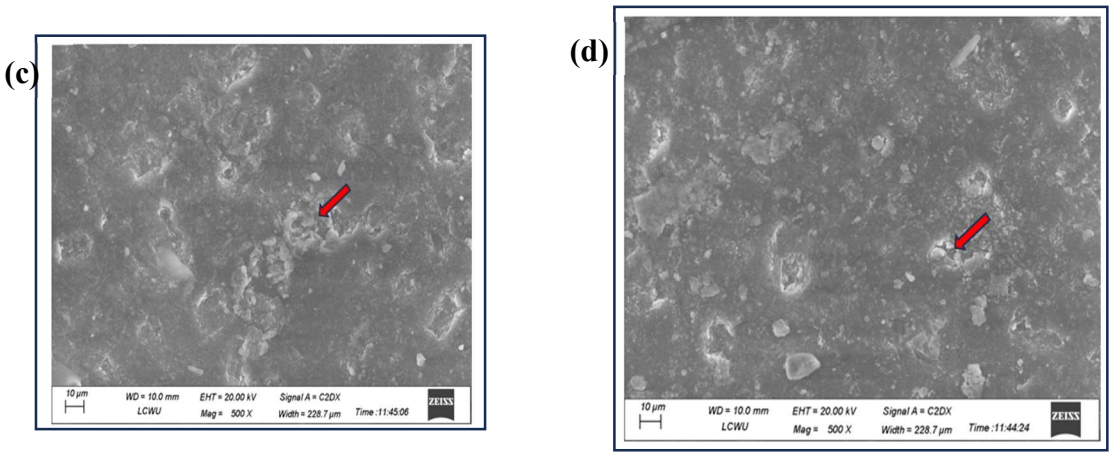


Figure 5. (a) Indicates the obvious changes observed in plastic pieces in comparison to the control. (b) SEM analysis of control. (c) and (d) indicating the SEM analysis of polyethylene degraded by *Bacillus tropicus*.

4. Discussion

When plastic pollution entering an area surpasses the rate of natural elimination processes or cleanup operations, plastic accumulates in the ecosystem. Natural degradation processes for plastics can take decades or even millennia [25]. There is an increasing problem with marine contamination due to solid waste on a global scale. This problem affects generations to come [26].

Marine litter is defined as any human-made solid waste in the ocean, whether on land or at sea. This includes materials transported to the ocean via rivers, drains, sewage, wind, or water systems but does not include organic materials like food and vegetable scraps [27].

The current study aimed to isolate and characterize polyethylene-degrading bacteria in sewage wastewater. This is because sewage is one of the biggest sources of natural water pollution and potential microbes for removing toxicants. We collected the sewage samples from the wastewater treatment plant to isolate plastic-degrading bacteria. After initial screening and 120 days of incubation with polyethylene, we found that sample SH4 reduced plastic by 21.6%. After 16S rRNA gene sequencing, this strain was detected *as Bacillus tropicus*.

The study by Mukhaifi et al. (2021) sought to isolate and characterize bacteria capable of degrading polyethylene terephthalate (PET) from Shatt al-Arab water and sewage in Basra, identifying the bacteria as *Klebsiella pneumoniae*. The results indicated a statistically significant difference in PET degradation, with a 24% reduction over 7 days, which grew to 46% after 4 weeks in comparison to the control group. These results are correlated with the current study [28].

Meng et al. (2024) identified a novel marine strain of *Pseudalkalibacillus* sp. MQ-1 is capable of degrading polyethylene (PE) up to 6.37% in 60 days. Scanning electron microscopy and water contact angle analyses demonstrated that MQ-1 may cling to polyethylene films, rendering them hydrophilic [29].

GC-MS analysis was used to determine which polyethylene products had deteriorated. They were found when the compounds that had the strongest correlation with the Wiley Library database and the MS fragmentation pattern were compared [Figure 3]. We performed the GC-MS analysis to detect compounds in MS media produced due to the biodegradation of polyethylene. Many new compounds were produced by *B. tropicus* activity [Table 2]. We neglected the compounds that were common with the control.

Plastic polymers' biodegradation byproducts following 140 days of incubation were investigated using gas chromatography-mass spectrometry. Three compounds were identified in this study: cis-2-chlorovinyl acetate (7.11 min), tri-decanoic acid (21.43 min), and octa-decanoic acid (22.46 min). The presence of tri-decanoic and octa-decanoic acids in the material under investigation implies the biodegradation process involves the creation of carbonyl groups. These groups are further oxidized to produce ketones and aldehydes, as confirmed by nuclear magnetic resonance (NMR)

 doi:10.20944/preprints202410.1796.v1

analysis. Thus, the current research shows that fatty acids and other metabolic intermediates are important for microbial consortiums to biodegrade plastic. It was determined that the end-products in this investigation did not pose any health risks [30].

Shahnawaz et al. (2016) identified 1-trimethylsilylmethanol, 1,2,3-trimethylbenzene, ethyl-3,5-dimethylbenzene, hexadecanoic acid, 1,4-dimethyl-2-ethylbenzene, and 1,2,3,4-tetramethylbenzene [31]. Roy et al. (2008) cultivated a consortium of *Bacillus pumilus, Bacillus halodenitrificans*, and *Bacillus cereus* on polyethylene particles, revealing the presence of both oxygenated chemicals and unoxidized low molecular weight hydrocarbons [32]. Roy et al. (2008) identified alkanes, fatty acids, and ester-containing compounds as products of biodegradation by *Pseudomonas putida, Pseudomonas syringae,* and *Pseudomonas aeruginosa* [32].

Our study also incorporated FTIR analysis to observe the changes in plastic chemical structure after degradation. FTIR spectra showed bond bending and bond stretching of the C-C bond of alkane, C=C of alkene, and N-O of the nitro compounds. In the study of Khandare et al. (2021), the chemical changes in the LDPE structure were investigated using FTIR. If you want to know what functional groups are in LDPE or any other biodegradable molecules, you can use the FTIR analysis to see changes in the carbon backbone [33].

Previous research has made heavy use of this method, as seen by the work of Nadeem et al. (2021), who demonstrated a decrease in transmittance between 1100 and 1150 cm^{-1}, suggesting the formation of new (-C-O-C) bonds due to the weakening of C-C bonds. Except for the untreated control, all LDPE film FTIR spectra exhibited the production of a characteristic carbonyl peak at 1,712 cm^{-1}, which was significantly diminished following 90 days of bacterial incubation. The process of plastic biodegradation can be better understood by observing the presence and disappearance of carbonyl peaks [34]. In another investigation, Selke et al. (2015) also noticed a reduction in the creation of a carbonyl peak at 1,712 cm^{-1} after 90 days of treatment with bacteria [35]. In order to understand the basic process of biodegradation, previous studies have shown that new functional groups can appear and disappear in both treated and control LDPE films, which supports the current study's conclusions [24,36,37].

The SEM examination allowed us to observe the surface morphological changes on the LDPE film following 120 days of treatment with sewage bacteria. The scanning electron micrographs (SEMs) revealed surface degradation, fragility, damaged layers, cracks, and scratching in the LDPE film treated with any of the four marine bacteria compared to the control film, which remained smooth, undamaged, and clear. Sanin et al. (2003) found that FE-SEM images of synthetic polymer (LDPE) fragmented into monomeric forms when incubated with certain bacterial strains. These strains included *Rhodococcus corallinus* strain 11, *Pseudomonas* sp. strain A, and *Pseudomonas* sp. strain D. Using AFM images, it was also noted that all LDPE films treated with bacterial isolates developed cracks, grooves, and roughness after the same incubation condition, whereas the control film, which was not supplemented with any bacteria, remained smooth and intact. This confirms that the degradation is caused by enzyme activity. Results from scanning electron microscopy (SEM) and atomic force microscopy (AFM) demonstrate that, under low-nutrient conditions, bacterial isolates cling to surfaces and use C-source from LDPE substrates [33,38].

4. Conclusions

In conclusion, plastic pollution is an emerging global problem that needs some sustainable, economical, and eco-friendly approaches for mitigation. Conventional methods, such as the incineration of plastic, can be toxic to the environment. Biodegradation is an environmentally friendly method for plastic removal. Sewage wastewater can be a good source of potential bacteria that can degrade significantly. This study found *Bacillus tropicus* from the sewage wastewater treatment plant that degraded up to 21.3% of polyethylene. FTIR analysis indicated significant changes in the plastic structure after biodegradation. GC-MS analysis indicated new compounds produced as a result of *B. tropicus* activity. SEM analysis showed the deterioration of the plastic surface. So, we can infer that this bacterium has significant potential in polyethylene reduction in sewage. Further proteomics and metagenomics studies are needed to learn more about the plastic breakdown pathways these bacteria

 doi:10.20944/preprints202410.1796.v1

use. Future studies should focus on finding the role of bacterial community of sewage in environmental sustainability and world economy.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, S.A. and I.; methodology, S.A.; software, S.A.; validation, Y.-C.C., S.A. and I.; formal analysis, Y.-C.C.; investigation, S.I.; resources, S.A.; data curation, I.; writing—original draft preparation, I.; writing—review and editing, I.; visualization, I.; supervision, Y.-C.C.; project administration, S.A.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Data Availability Statement: Data will be provided on demand.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sonke, J. E., Koenig, A. M., Yakovenko, N., Hagelskjær, O., Margenat, H., Hansson, S. V., ... & Thomas, J. L. (2022). A mass budget and box model of global plastics cycling, degradation and dispersal in the land-ocean-atmosphere system. Microplastics and Nanoplastics, 2(1), 28.
2. Schirmeister, C. G., & Mülhaupt, R. (2022). Closing the carbon loop in the circular plastics economy. Macromolecular rapid communications, 43(13), 2200247.
3. Mohamadi, M. (2023). Plastic Types and Applications. Plastic Waste Treatment and Management: Gasification Processes, 1-19.
4. Cudjoe, D., Brahim, T., & Zhu, B. (2023). Assessing the economic and ecological viability of generating electricity from oil derived from pyrolysis of plastic waste in China. Waste Management, 168, 354-365.
5. Arisman, & Fatimah, Y. A. (2023). Waste management in indonesia: strategies and implementation of sustainable development goals (sdgs) and circular economy. In Circular Economy Adoption: Catalysing Decarbonisation Through Policy Instruments (pp. 131-157). Singapore: Springer Nature Singapore.
6. Drzyzga, O., & Prieto, A. (2019). Plastic waste management, a matter for the 'community'. Microbial biotechnology, 12(1), 66.
7. Ali, S., Isha, & Chang, Y. C. (2023). Ecotoxicological Impact of Bioplastics Biodegradation: A Comprehensive Review. Processes, 11(12), 3445.
8. Kibria, M. G., Masuk, N. I., Safayet, R., Nguyen, H. Q., & Mourshed, M. (2023). Plastic waste: challenges and opportunities to mitigate pollution and effective management. International Journal of Environmental Research, 17(1), 20.
9. Bakht, A., Rasool, N., & Iftikhar, S. (2020). Characterization of plastic degrading bacteria isolated from landfill sites. International journal of clinical microbiology and biochemical technology, 3(1), 030-035.
10. Korotkov, E. V., Suvorova, Y. M., Kostenko, D. O., & Korotkova, M. A. (2021). Multiple alignment of promoter sequences from the Arabidopsis thaliana l. Genome. Genes, 12(2), 135.
11. Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406-425.
12. Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. evolution, 39(4), 783-791.
13. Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences, 101(30), 11030-11035.
14. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular biology and evolution, 38(7), 3022-3027.
15. Gao, L., & Gu, J. D. (2021). A new unified conceptual framework involving maintenance energy, metabolism and toxicity for research on degradation of organic pollutants. International Biodeterioration & Biodegradation, 162, 105253.
16. Tamnou, E. B. M., Arfao, A. T., Nougang, M. E., Metsopkeng, C. S., Ewoti, O. V. N., Moungang, L. M., ... & Nola, M. (2021). Biodegradation of polyethylene by the bacterium Pseudomonas aeruginosa in acidic aquatic microcosm and effect of the environmental temperature. Environmental Challenges, 3, 100056.
17. Yao, Z., Seong, H. J., & Jang, Y. S. (2022). Degradation of low density polyethylene by Bacillus species. Applied Biological Chemistry, 65(1), 84.

 doi:10.20944/preprints202410.1796.v1

18. Sun, X., Chen, Z., Kong, T., Chen, Z., Dong, Y., Kolton, M., ... & Sun, W. (2022). Mycobacteriaceae mineralizes micropolyethylene in riverine ecosystems. Environmental Science & Technology, 56(22), 15705-15717.
19. Abraham, J., Ghosh, E., Mukherjee, P., & Gajendiran, A. (2017). Microbial degradation of low density polyethylene. Environmental Progress & Sustainable Energy, 36(1), 147-154.
20. Hou, L., Xi, J., Liu, J., Wang, P., Xu, T., Liu, T., ... & Lin, Y. B. (2022). Biodegradability of polyethylene mulching film by two Pseudomonas bacteria and their potential degradation mechanism. Chemosphere, 286, 131758.
21. Kavitha, R., & Bhuvaneswari, V. (2021). Assessment of polyethylene degradation by biosurfactant producing ligninolytic bacterium. Biodegradation, 32(5), 531-549.
22. Samanta, S., Datta, D., & Halder, G. (2020). Biodegradation efficacy of soil inherent novel sp. Bacillus tropicus (MK318648) onto low-density polyethylene matrix. Journal of Polymer Research, 27(10), 1-16.
23. Arkatkar, A., Juwarkar, A. A., Bhaduri, S., Uppara, P. V., & Doble, M. (2010). Growth of Pseudomonas and Bacillus biofilms on pretreated polypropylene surface. International Biodeterioration & Biodegradation, 64(6), 530-536.
24. Gupta, K. K., & Devi, D. (2020). Characteristics investigation on biofilm formation and biodegradation activities of Pseudomonas aeruginosa strain ISJ14 colonizing low density polyethylene (LDPE) surface. Heliyon, 6(7).
25. MacLeod, M., Arp, H. P. H., Tekman, M. B., & Jahnke, A. (2021). The global threat from plastic pollution. Science, 373(6550), 61-65.
26. Li, X., Tian, Y., Xu, C., & Cheng, B. (2019). The impact of marine pollution control on the output value of marine fisheries based on the spatial econometric model. Journal of Coastal Research, 98(SI), 381-384.
27. Velis, C. A. (2014). Plastic waste in marine litter: Action now and at the source. Waste Management & Research, 32(4), 251-253.
28. Mukhaifi, E. A., Al-Atbi, H. S., & Ali, S. F. (2023). Isolation and Identification of Polyethylene Terephthalate Degrading Bacteria from Shatt Al-Arab and Sewage Water of Basrah City. Baghdad Science Journal, 20(5 (Suppl.)).
29. Meng, Q., Yi, X., Zhou, H., Song, H., Liu, Y., Zhan, J., & Pan, H. (2024). Isolation of marine polyethylene (PE)-degrading bacteria and its potential degradation mechanisms. Marine Pollution Bulletin, 207, 116875.
30. Skariyachan, S., Patil, A. A., Shankar, A., Manjunath, M., Bachappanavar, N., & Kiran, S. (2018). Enhanced polymer degradation of polyethylene and polypropylene by novel thermophilic consortia of Brevibacillus sps. and Aneurinibacillus sp. screened from waste management landfills and sewage treatment plants. Polymer Degradation and Stability, 149, 52-68.
31. Shahnawaz, M., Sangale, M. K., & Ade, A. B. (2016). Bacteria-based polythene degradation products: GC-MS analysis and toxicity testing. Environmental Science and Pollution Research, 23(11), 10733-10741.
32. Roy, P. K., Titus, S., Surekha, P., Tulsi, E., Deshmukh, C., & Rajagopal, C. (2008). Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. Polymer degradation and stability, 93(10), 1917-1922.
33. Khandare, S. D., Chaudhary, D. R., & Jha, B. (2021). Marine bacterial biodegradation of low-density polyethylene (LDPE) plastic. Biodegradation, 32(2), 127-143.
34. Nadeem, H., Alia, K. B., Muneer, F., Rasul, I., Siddique, M. H., Azeem, F., & Zubair, M. (2021). Isolation and identification of low-density polyethylene degrading novel bacterial strains. Archives of Microbiology, 203(9), 5417-5423.
35. Selke, S., Auras, R., Nguyen, T. A., Castro Aguirre, E., Cheruvathur, R., & Liu, Y. (2015). Evaluation of biodegradation-promoting additives for plastics. Environmental Science & Technology, 49(6), 3769-3777.
36. Shah, A. A., Hasan, F., Akhter, J. I., Hameed, A., & Ahmed, S. (2008). Degradation of polyurethane by novel bacterial consortium isolated from soil. Annals of microbiology, 58, 381-386.

 doi:10.20944/preprints202410.1796.v1

37. Dwicania, E., Rinanti, A., & Fachrul, M. F. (2019, December). Biodegradation of LLDPE plastic by mixed bacteria culture of Pseudomonas aeruginosa and Brevibacterium sp. In Journal of Physics: Conference Series (Vol. 1402, No. 2, p. 022105). IOP Publishing.
38. Sanin, S. L., Sanin, F. D., & Bryers, J. D. (2003). Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. Process biochemistry, 38(6), 909-914.