The Role of Bacterial Plasmids in the Biodegradation of Low-Density Polyethylene in Mitigating Climate Change

Sarah Oluwaseun Julius [1], Olusola Adeoye Oluwole [2], Emediong Peter Akpan [3], Christiana Amarachi Nwachukwu [4], Ogechi Judith Madukwe [5], Elizabeth Omowumi Adegoke [6], Olabisi Michael Femi [7], Echesirim Bright Emmanuel [8], Oluwasogo Isaac Adedokun [9], Olaitan Faith Jesupemi [10]

[1] *University of Ibadan*
Oduduwa Road, 200132, Ibadan, Nigeria

[2] *Ekiti State University*
Ado Ekiti, 362103, Ekiti, Nigeria

[3] *Manipal University Dubai*
G04, PO Box 345050, Dubai, UAE

[4] *National Open University of Nigeria*
Plot 91, Cadastral Zone, Nnamdi Azikwe Expressway, Jabi, Abuja, Nigeria

[5] *Florida Agricultural and Mechanical University*
1601 S. Martin Luther King Jr Blvd, Tallahassee, Florida, 32307, USA

[6] *The Polytechnic Ibadan*
P. M. B. 22, Ibadan, Oyo State, Nigeria

[7] *Federal University of Technology, Minna*
Gidan Kwanu, P. M. B. 65, Minna, Niger State, Nigeria

[8] *University of Port Harcourt*
P. M. B. 5323, Choba, Rivers State, Nigeria

[9] *Obafemi Awolowo University*
P. M. B. 13, Osun, 220282, Nigeria

[10] *Thomas Adewumi University*
University Drive, Off Idofin Road, Oko-Irese, Kwara State

DOI: 10.22178/pos.107-4

LCC Subject Category: QH1-(199.5)

Received 25.07.2024
Accepted 28.08.2024
Published online 31.08.2024

Corresponding Author:
Sarah Oluwaseun Julius
sarahjuliusoluwaseun@gmail.com

Abstract. Polyethene causes pollution and global warming not only because of the increase in the problem of waste disposal and landfilling but also because it releases carbon(iv)oxide and dioxins due to burning, which hurts the climate. Recent studies have shown that biodegradation using plasmid processing bacteria (PPB) could help prevent environmental damage from pollution and reduce carbon footprint and greenhouse gas emissions from polyethene usage. This study assayed the low-density polyethylene degrading potential of PPB assayed from polyethylene-polluted sites. Soil samples and waste sachet water bags, popularly called 'pure water', were collected from a polyethene dumpsite near Ekiti State University. These samples were analysed for bacterial loads and polyethene degrading PPB using nutrient agar and mineral salt medium, respectively.

The biodegradation of low-density polyethene using sachet water films was observed spectrophotometrically using the broth culture of the bacterial isolates for 30 days on a mineral salt medium. The bacteria were identified based on molecular characterisation using 16S RNA sequencing. Six bacterial isolates identified from polyethene polluted sites include: *Lysinibacillus xylanilyticus* strain BN-13 (S6),

Rhodopseudomonas palustris strain KRPR02 (W5), *Pseudomonasaeruginosa* strain JAY (N2), *Stenotrophomonas maltophilia* strain T7D7, *Pseudomonas aeruginosa* strain SMVIT-1 (S1) and *Achromobacter xylosoxidans* YEB (W11), and four isolates were observed to have the presence of plasmid after analysis.

The four isolates were used to degrade the PE films, which peaked at degradation on the 21 days, followed by a gradual declination on the 28 days. *L. xylanilyticus* strain BN-13(S6) exhibited the highest degradation of 0.898 nm, and *A. xylosoxidans* strain YEB (W11) exhibited a minor degradation of 0.788 nm. The result revealed that PPBs are competent biodegrades of polyethene wastes and can be used as a better approach to restoring polyethene and mitigating climate change.

Keywords: Biodegradation; Plasmid possessing bacteria; Polyethylene; Spectrophotometer.

INTRODUCTION

Plastic pollution, driven by the continued presence of low-density polyethene (LDPE) waste in the environment, has become an urgent global concern [4]. LDPE, a ubiquitous polymer used in various forms of packaging [7], contributes significantly to environmental degradation, posing a threat to ecosystems, wildlife and human health [12].

In response to this environmental crisis, innovative solutions have been explored, with a promising route being the use of plasmid-possessing bacteria (PPB) to degrade these environmental pollutants [35]. Some bacteria, especially those with extrachromosomal genetic elements called plasmids, have been shown to degrade low-density polyethene by exploiting the power of these plasmid-encoded genes, which carry enzymes such as oxidases, hydrolases, cutinises, etc. [25].

Figure 1 - Plastic-polluted sites posing threats to the environment

Scientists use genetic engineering techniques to introduce plasmids carrying specific polyethene degradation genes into the bacteria. Degradable plasmids, among other types, carry genes necessary for biodegradation of various environmental pollutants, including plastics [3, 25]. This genetic modification allows the bacteria to produce enzymes capable of breaking down the complex structure of polyethene [39]. This corresponds to the principles of green technology [1]. Green technologies prioritise sustainability and respect for the environment [16] and reduce ecological impact, making it an exceptional approach [21].

The biodegradation of polyethene represents a complex process, and at its heart are bacteria possessing by plasmids, which serve as drivers of this transformation mechanism [29]. Plasmids, small circular DNA structures independent of bacterial chromosomal DNA, play a crucial role in transporting and expressing genes that encode enzymes capable of cleaving elastic polyethene chains [13].

Here, we review key aspects of the plasmid possessing involvement of bacteria in polyethene biodegradation.

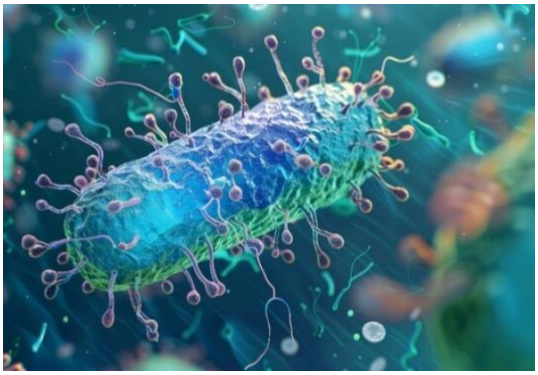

Figure 2 – Plasmids as genetic tools for plastic biodegradation

1. Role of Plasmids in Bacteria. Plasmids are extrachromosomal genetic elements of bacteria that can multiply independently. They often carry genes that give the bacteria favourable characteristics. In the biodegradation of polyethene, plasmids play an essential role by carrying genes that encode enzymes that initiate and facilitate the degradation of polyethene polymers [13]. Genetic engineering for polyethene biodegradation: Scientists use genetic engineering techniques to introduce plasmids carrying specific polyethene degradation genes into bacteria [2]. This genetic modification allows the bacteria to produce enzymes capable of breaking down the complex structure of polyethene, thus starting the biodegradation process.

Several bacteria have been identified and engineered for their ability to biodegrade polyethene via plasmids [20]. Examples include *Ideonella sakaiensis*, which produces enzymes capable of breaking down PET (polyethene terephthalate), and Pseudomonas species, known for their versatility in breaking down various environmental pollutants, including polyethene. Escherichia coli. It is designed to produce multiple enzymes that target different bonds in LDPE [13]. Understanding the complex relationship between plasmid possessing bacteria and polyethene biodegradation is essential to understanding the potential of this technology. Genetic manipulation, driven by plasmids, represents a promising cutting-edge approach for mitigating plastic pollution and promoting ecologically sustainable practices [2].

2. Application of plasmid possessing bacteria in the environment

a) In-situ bioremediation: One approach is in situ bioremediation, which involves directly applying plasmid possessing bacteria to contaminated sites. This can be achieved by spraying or injecting bacterial solutions in areas with high concentrations of plastic waste, such as marine environments or contaminated land [36].

b) Ex-situ bioremediation: Another method is ex-situ bioremediation, which involves processing accumulated plastic waste in controlled environments. Process plastic waste collected in controlled environments such as bioreactors or composting plants [9]. Example: Bioreactors designed to optimise conditions for bacterial degradation of LDPE.

c) Integration into waste management systems: Plasmid bacteria can also be integrated into existing systems. By including these bacteria in recycling plants or landfills, we can improve the biodegradation of LDPE and the overall waste treatment efficiency [19].

d) Monitoring and regulation: Establishing protocols for safely using genetically modified bacteria in the environment is essential. This includes monitoring their impact on native ecosystems and ensuring they do not cause unwanted damage [33]. Regulations and guidelines help manage the implementation and operation of these bioremediation techniques.

3. Plasmid possessing enzymes produced by bacteria. Enzymes are biological catalysts that accelerate the degradation of LDPE. Plasmid possessing bacteria have been engineered to produce specific enzymes that target plastic polymers [34]. The main enzymes involved are:

a) Laccases: Authors [32] catalyse the oxidation of phenolic substrates and participate in the degradation of complex polymers.

b) Hydrolases break the ester bonds in polymers, facilitating the breakdown of LDPE into smaller molecules [17].

c) Oxidases: Catalyze redox reactions, thus contributing to the breakdown of plastic molecules [1].

d) Esterase: Hydrolyses ester bonds, common in synthetic polymers such as LDPE [26].

4. Plasmid possessing post-degradation processes of polyethene by bacteria. After degradation, it is essential to carry out regular environmental monitoring to ensure that the degradation process is complete and that no harmful residues exist. Ecological impact studies help assess long-term effects on local ecosystems and biodiversity [8]. When plasmid possessing bacteria degrade polyethene, the plastic breaks down into smaller molecules such as monomers (ethylene) and oligomers. In addition, microbial activity can produce by-products such as CO2, water and biomass [27].

In addition, the degradation of polyethene significantly reduces plastic pollution, thus improving the quality of soil and water in the treated areas [30]. This contributes to overall health and environmental sustainability. The microbial biomass created during the degradation process can serve as a source of nutrients for other microorganisms, thus promoting the ecological balance [33].

Also, degradation products can improve soil fertility and structure, acting as natural soil amendments. Continuous research is essential to improve biodegradation efficiency further. This includes developing improved biodegradation techniques and integrating these methods with other sustainable waste management practices to create a holistic approach to plastic pollution [15].

Polyethylene, with its strong molecular structure, presents a significant challenge to the environmental ecosystem due to its slow degradation and persistence in the environment [14]. However, introducing plasmid possessing bacteria and their ability to biodegrade polyethene offers hope [29]. In this section, we examine the environmental impact of this biodegradation process and its potential to pave the way for sustainable waste management practices.

The biodegradation of polyethene results in the breakdown of large polyethene molecules into more minor, more manageable compounds. The enzymatic activity of the bacteria facilitated by the plasmids reduces polyethene waste, thus reducing plastic pollution in different environmental compartments [28].

Contribution to sustainable waste management: The ability of plasmid possessing bacteria to degrade polyethene is consistent with the principles of sustainable waste management. Transforming polyethene waste into ecological by-products represents an ecological approach to waste disposal and recycling [22].

Positive effects on ecosystems: The accumulation of polyethene waste concerns terrestrial and aquatic ecosystems, affecting flora and fauna [13]. Polyethylene biodegradation, orchestrated by plasmid possessing bacteria, can relieve ecosystems' stress, promoting healthier and more balanced environmental conditions.

Polyethylene biodegradation, orchestrated by plasmid possessing bacteria, not only helps combat plastic pollution but also has implications for climate resilience.

Biodegradation of polyethene as a green technology. The use of plasmid possessing bacteria for the biodegradation of polyethene conforms to the principles of green technology [23]. Green technologies prioritise sustainability, respect for the environment and reduction of the ecological impact, making biodegradation of polyethene an exceptional approach.

Mitigation of the impacts of climate change. The biodegradation of polyethene helps mitigate climate change's impacts by addressing an important source of environmental pollution. Reducing plastic waste through biodegradation eases the environmental burden of producing and disposing of petroleum-based plastics, indirectly mitigating climate change [5].

Adaptation to environmental challenges. Sustainable practices, including the biodegradation of polyethene, contribute to the overall resilience of ecosystems and communities that face environmental challenges. Promoting environmentally friendly waste management and the biodegradation of polyethene helps communities adapt to changing environmental conditions and possible climate events [35].

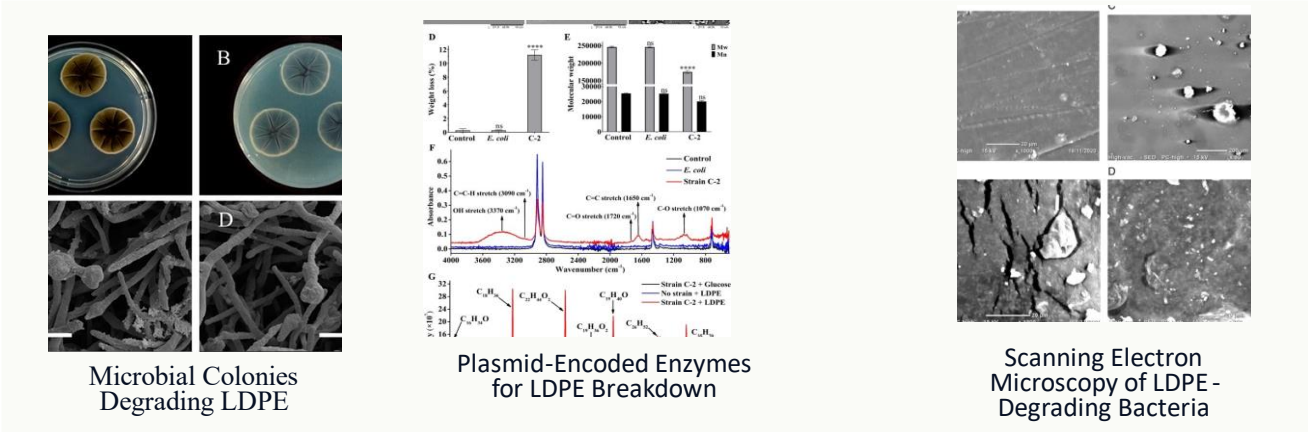

Figure 3 – Figure 3a shows a petri dish with bacterial colonies capable of breaking down low-density polyethylene (LDPE) plastic; Figure 3b Illustrates specific enzymes and metabolic pathways encoded on bacterial plasmids that facilitate the biodegradation of LDPE; Figure 3c shows a scanning electron microscope (SEM) image of bacterial cells adhering to and degrading the surface of LDPE [34]

As we delve into this exploration, it is essential to recognise the interconnectedness of environmental sustainability and climate resilience [31]. Our choices in addressing plastic pollution shape our planet's health and influence our capacity to adapt to the challenges posed by a changing climate. This study seeks to unravel the multifaceted dimensions of polyethene biodegradation, examining how the innovative use of plasmid possessing bacteria can catalyse transformative change [13].

METHODS

1. The isolates used in this study were obtained from previous research conducted on polyethene biodegradation [40]. They were identified as Lysinibacillus xylanilyticus strain BN-13 (S6), Rhodopseudomonas palustris strain KRPR02 (W5), Pseudomonas aeruginosa strain JAY (N2), Stenotrophomonas maltophilia strain T7D7, Pseudomonas aeruginosa strain SMVIT-1 (S1), and Achromobacter xylosoxidans YEB (W11). The isolates were confirmed to possess biodegradable capabilities. The low-density polyethene (LDPE) bag water films used were obtained from the Ekiti State University water conditioning plant.

2. Molecular characterisation of bacterial isolates. The molecular characterisation of the bacterial isolates was carried out using the method proposed [10]. The polymerase chain reaction (PCR) program included initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1.5 min and a final extension at 72°C for 15 min. The amplified product was resolved on a 1% (w/v) agarose gel. The PCR product was evaluated using the Wizard SV gel and PCR purification system. The nucleotide sequence was determined manually using the dioxide nucleotide chain termination method or automatically using the Big Dye Terminator Cycle Sequencing Kit v3.1 on the ABIPRISM 3100-Avant genetic analyser with universal primers and vice versa. DNA sequence analysis was performed using the Blast and Bio editing programs. Isolates were identified based on similarity scores, and sequences were submitted to the NCBI gene bank to obtain an accession number.

3. Isolation of plasmids and recovery of LDPE-degrading bacterial strains. Plasmids were isolated using the QIAGEN plasmid mini-purification kit. The plasmid extraction protocol was used to confirm the presence of plasmids. The extracted plasmids were analysed using gel to see if the isolates contained plasmids. The integrity of the extracted plasmid was checked on a 1% agarose gel to confirm amplification. The buffer (1xTAE buffer) was prepared and then used to prepare a 1% agarose gel. The suspension was microwaved for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with three µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits energy as visible orange light).

A comb was inserted into the holes of the casting table, and the molten agarose was poured into the table. The gel is allowed to solidify for 20 minutes to form wells. The 1xTAE buffer was poured into the gel reservoir to barely submerge the gel. Two microliters (2 l) of 10x blue gel filling dye (which gives colour and density to samples to facilitate loading into wells and monitor gel progress) was added to 10 µl of each PCR product and loaded into the wells after the DNA step of 100–3000 bp. was loaded into healthy 1. The gel was electrophoresed at 120 V for 45 min, visualised by UV transillumination, and photographed. The sizes of the PCR products were estimated by comparison with the molecular weight mobility scale performed with the experimental samples on the gel 600nm.

RESULTS AND DISCUSSION

Molecular characterisation of the isolates. Based on molecular characterisation using 16S rRNA sequencing, the six isolates were identified as follows: Isolate PE2 as *Lysinibacillus xylanilyticus* strain BN-13 16S, isolate PE4 as *Rhodopseudomonas palustris* strain KRPR02, Isolate S1 as *Pseudomonas aeruginosa* strain JAY2N, Isolate S6 as *Stenotrophomonas maltophilia* strain T7D7, Isolate W5 as *Pseudomonas aeruginosa* strain SMVIT-1, Isolate W11 as *Achromobacter xylosomonas.*

Isolation of plasmids and treatment of bacterial strains that degrade LDPE. Plasmid analysis of the six isolates revealed that only four isolates contained plasmids which include: *Lysinibacillus xylanilyticus* strain BN-13 16S, *Rhodopseudomonas palustris* strain KRPR02, *Pseudomonas aeruginosa* strain SMVIT-,*Achromobacter xylosomonas* however, *Stenotrophomonas maltophilia* strain T7D7 and *Pseudomonas aeruginosa* strain JAY2N, however, had no plasmids. The four isolates with

plasmids were exposed to 25 µl of Ethidium bromide and were found to lose their plasmids upon incubation overnight. The curing was confirmed by the loss of using low-density polyethene as a substrate.

Lane 1 100 BP marker

Lane 2 *Lysinibacillus xylanilyticus* strain BN-13 (S6)

LANE 3 *Pseudomonas aeruginosa* strain SMVIT-1 (S1)

LANE 4 *Rhodopseudomonas palustris* strain KRPR02 (W5)

LANE 5 *Achromobacter xylosoxidans* YEB (W11)

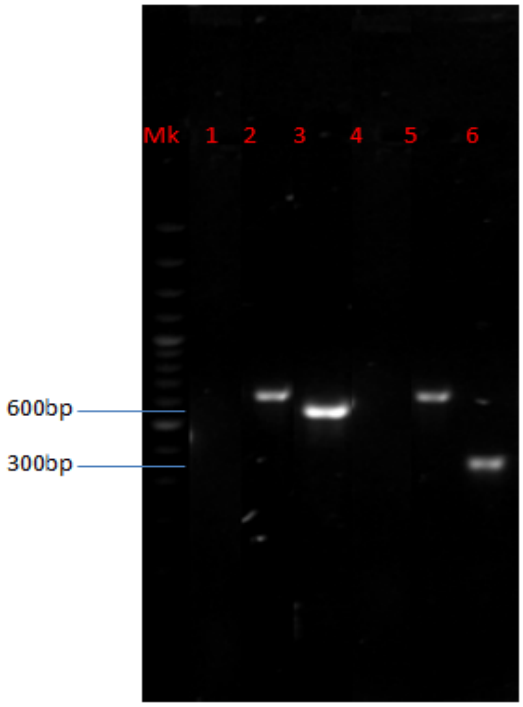

Figure 4 – The gel electrophoresis of plasmids of the degrading bacterial strain

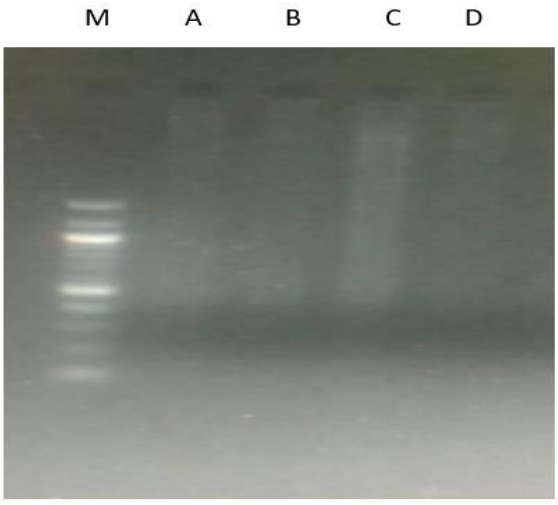

Figure 5 – Image of cured isolates after exposure to Ethidium bromide

Table 1 – Showing the four isolates that have the presence of plasmid

Bacterial Isolate	Plasmid Presence
Lysinibacillus xylanilyticus strain BN-13 (S6)	✓
Rhodopseudomonas palustris strain KRPR02 (W5)	✓
Pseudomonas aeruginosa strain SMVIT-1 (S1)	✓
Achromobacter xylosoxidans strain YEB (W11)	✓

*Adapted from the research article: The Role of Plasmid Mediated Bacteria in the Biodegradation of Low-Density Polyethylene in Mitigating Climate Change

Table 2 – Optical density of LDPE degradation using bacterial isolates with plasmids

Isolates	Day 1 (nm)	Day 7 (nm)	Day 14 (nm)	Day 21 (nm)	Day 28 (nm)
L. xylanilyticus strain BN-13 (S6)	0.853	0.867	0.888	0.898	0.518
A. xylosoxidans strain YEB (W11)	0.603	0.662	0.736	0.788	0.748
P. aeruginosa strain SMVIT-1 (S1)	0.728	0.863	0.636	0.820	0.501
R. palustris strain KRPR02 (W5)	0.824	0.830	0.842	0.887	0.873

*Data from the provided context

Table 3 – Optical density of LDPE degradation using bacterial isolates without plasmids (Control)

Isolates	Day 1 (nm)	Day 7 (nm)	Day 14 (nm)	Day 21 (nm)	Day 28 (nm)
L. xylanilyticus strain BN-13 (S6)	0.485	0.598	0.693	0.826	0.806
A. xylosoxidans strain YEB (W11)	0.517	0.617	0.644	0.710	0.508
P. aeruginosa strain SMVIT-1 (S1)	0.550	0.635	0.852	0.820	0.619
R. palustris strain KRPR02 (W5)	0.608	0. 673	0.734	0.876	0.607

*Data from the provided context

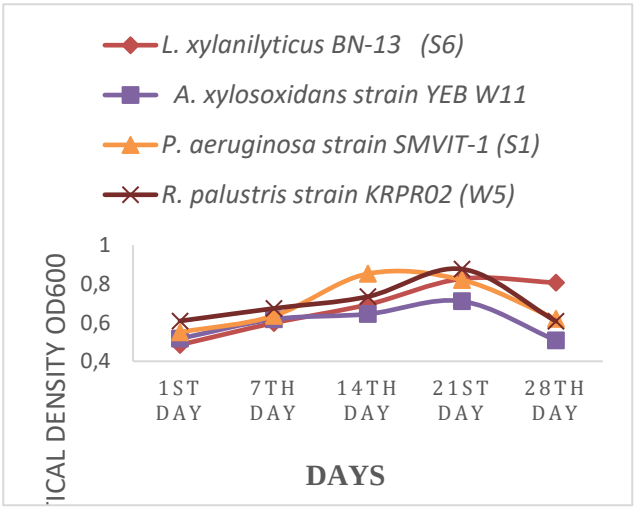

Figure 6 – Growth pattern of LDPE degradation using plasmid possessing bacteria

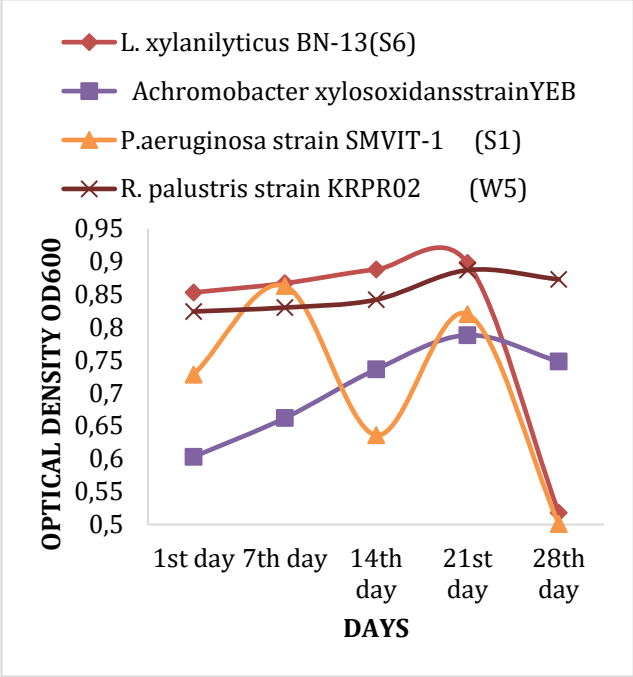

Figure 7 – Growth pattern of LDPE degradation using bacterial isolates without plasmids

CONCLUSIONS

It can be concluded that biodegradation using bacterial isolates has proven effective through the utilisation of plasmids in the degradation of polyethene. This method offers a viable solution to the problem of polyethene accumulation in the environment, which contributes to releasing methane, excessive carbon, dioxins during combustion, and other harmful substances that deplete the ozone layer, lead to global warming, and ultimately contribute to climate change.

To address the challenges posed by polyethene waste, it is recommended that appropriate disposal strategies, such as biodegradation using plasmid-possessing microbes, be further developed and implemented at regional levels rather than relying on incineration, which has harmful environmental effects. Additionally, raising awareness about polyethene pollution at the local, regional, and global levels is essential. Encouraging the use of PPB biodegradation offers a sustainable and effective solution to LDPE pollution, potentially significantly reducing its environmental and health impacts. This approach is critical in creating a cleaner, pollution-free environment and mitigating climate change.

REFERENCES

1. Allen, D. T., Shonnard, D. R. (2001). *Green engineering: designing environmentally friendly chemical processes*. Pearson Education.

2. Rosander, A., Connolly, E., & Roos, S. (2008). Removal of Antibiotic Resistance Gene-Carrying Plasmids from Lactobacillus reuteri ATCC 55730 and Characterisation of the Resulting Daughter Strain, L. Reuteri DSM 17938. *Applied and Environmental Microbiology, 74*(19), 6032–6040. doi: 10.1128/AEM.00991-08

3. Bayley, S. A., Morris, D. W., & Broda, P. (1979). The relationship of degradative and resistance plasmids of Pseudomonas belonging to the same incompatibility group. *Nature, 280*(5720), 338–339. doi: 10.1038/280338a0

4. Borrelle, S. B., Ringma, J., Law, K. L., Monnahan, C. C., Lebreton, L., McGivern, A., Murphy, E., Jambeck, J., Leonard, G. H., Hilleary, M. A., Eriksen, M., Possingham, H. P., De Frond, H., Gerber, L. R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M., & Rochman, C. M. (2020). Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science, 369*(6510), 1515–1518. doi: 10.1126/science.aba3656

5. Briggs, H. (2017, April 24). *Plastic-eating caterpillar could munch waste, scientists say*. Retrieved from https://www.bbc.com/news/science-environment-39694553

6. IUCN. (N. d). *Plastic pollution*. Retrieved from https://iucn.org/resources/issues-brief/plastic-pollution

7. Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances, 3*(7). doi: 10.1126/sciadv.1700782

8. Gu, J.-D. (2020). Biodegradability of plastics: the issues, recent advances, and future perspectives. *Environmental Science and Pollution Research, 28*(2), 1278–1282. doi: 10.1007/s11356-020-11501-9

9. Bisht, S., Pandey, P., Bhargava, B., Sharma, S., Kumar, V., & Sharma, K. D. (2015). Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. *Brazilian Journal of Microbiology, 46*(1), 7–21. doi: 10.1590/s1517-838246120131354

10. G. Kulkarni, A., & Kaliwal, B. B. (2014). Bioremediation of Methomyl by Soil Isolate – Pseudomonas Aeruginosa. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 8*(12), 01–10. doi: 10.9790/2402-081240110

11. Sharma, S., & Adholeya, A. (2011). Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by Paecilomyces lilacinus fungi. *International Biodeterioration & Biodegradation, 65*(2), 309–317. doi: 10.1016/j.ibiod.2010.12.003

12. Kaseem, M., Hamad, K., & Deri, F. (2012). Thermoplastic starch blends: A review of recent works. *Polymer Science Series A, 54*(2), 165–176. doi: 10.1134/s0965545x1202006x

13. Guha, A., Kumari, B., Bora, T. C., & Roy, M. K. (1997). Possible involvement of plasmids in degradation of malathion and chlorpyriphos by Micrococcus sp. *Folia Microbiologica, 42*(6), 574–576. doi: 10.1007/bf02815468

14. Ruan, M.-Y., Liang, B., Mbadinga, S. M., Zhou, L., Wang, L.-Y., Liu, J.-F., Gu, J.-D., & Mu, B.-Z. (2016). Molecular diversity of bacterial bamA gene involved in anaerobic degradation of aromatic hydrocarbons in mesophilic petroleum reservoirs. *International Biodeterioration & Biodegradation, 114*, 122–128. doi: 10.1016/j.ibiod.2016.06.005

15. Lorenz, P., & Eck, J. (2005). Metagenomics and industrial applications. *Nature Reviews Microbiology, 3*(6), 510–516. doi: 10.1038/nrmicro1161

16. McDonough, W. (2002). Design for the Triple Top Line: New Tools for Sustainable Commerce. *Corporate Environmental Strategy, 9*(3), 251–258. doi: 10.1016/s1066-7938(02)00069-6

17. Müller, R., Schrader, H., Profe, J., Dresler, K., & Deckwer, W. (2005). Enzymatic Degradation of Poly(ethylene terephthalate): Rapid Hydrolyse using a Hydrolase from T. fusca. *Macromolecular Rapid Communications, 26*(17), 1400–1405. doi: 10.1002/marc.200500410

18. Ojha, N., Pradhan, N., Singh, S., Barla, A., Shrivastava, A., Khatua, P., Rai, V., & Bose, S. (2017). Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimisation. *Scientific Reports, 7*(1). doi: 10.1038/srep39515

19. Pichtel, J. (2014). *Waste management practices: municipal, hazardous and industrial* (2nd ed.). CRC Press.

20. Kleindienst, S., Seidel, M., Ziervogel, K., Grim, S., Loftis, K., Harrison, S., Malkin, S. Y., Perkins, M. J., Field, J., Sogin, M. L., Dittmar, T., Passow, U., Medeiros, P. M., & Joye, S. B. (2015). *Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. Proceedings of the National Academy of Sciences, 112*(48), 14900–14905. doi: 10.1073/pnas.1507380112

21. Clark, J., & Macquarrie, D. (2002). *Handbook of green chemistry and technology*. Wiley-Blackwell.

22. Fickling, D. (2004, April 30). Plastic pollution is a growing problem. Here are some ideas on how to solve it. Retrieved from https://www.japantimes.co.jp/commentary/2024/04/30/world/plastic-pollution-keeps-growing/

23. Royer, S.-J., Ferrón, S., Wilson, S. T., & Karl, D. M. (2018). Production of methane and ethylene from plastic in the environment. *PLOS ONE, 13*(8), e0200574. doi: 10.1371/journal.pone.0200574

24. Green, M., & Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Retrieved from https://www.cshlpress.com/pdf/sample/2013/MC4/MC4FM.pdf

25. Saxena, G., Kishor, R., Saratale, G. D., & Bharagava, R. N. (2019). Genetically Modified Organisms (GMOs) and Their Potential in Environmental Management: Constraints, Prospects, and Challenges. *Bioremediation of Industrial Waste for Environmental Safety*, 1–19. doi: 10.1007/978-981-13-3426-9_1

26. Gomez-Saez, G. V., Pop Ristova, P., Sievert, S. M., Elvert, M., Hinrichs, K.-U., & Bühring, S. I. (2017). Relative Importance of Chemoautotrophy for Primary Production in a Light Exposed Marine Shallow Hydrothermal System. *Frontiers in Microbiology, 8*. doi: 10.3389/fmicb.2017.00702

27. Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances, 26*(3), 246–265. doi: 10.1016/j.biotechadv.2007.12.005

28. Shukla, S. R., Harad, A. M., & Mahato, D. (2006). Depolymerisation of nylon 6 waste fibers. *Journal of Applied Polymer Science, 100*(1), 186–190. doi: 10.1002/app.22775

29. Shukla, S. R., Harad, A. M., & Mahato, D. (2006). Depolymerisation of nylon 6 waste fibers. *Journal of Applied Polymer Science, 100*(1), 186–190. doi: 10.1002/app.22775

30. Mishra, M., & Thakur, I. S. (2011). Purification, characterisation and mass spectroscopic analysis of thermo-alkalotolerant β-1, 4 endoxylanase from Bacillus sp. and its potential for dye decolorisation. *International Biodeterioration & Biodegradation, 65*(2), 301–308. doi: 10.1016/j.ibiod.2010.12.002

31. Ghatge, S., Yang, Y., Ahn, J.-H., & Hur, H.-G. (2020). Biodegradation of polyethylene: a brief review. *Applied Biological Chemistry, 63*(1). doi: 10.1186/s13765-020-00511-3

32. Soni, D. K., Singh, R. K., Singh, D. V., & Dubey, S. K. (2013). Characterisation of Listeria monocytogenes isolated from Ganges water, human clinical and milk samples at Varanasi, India. *Infection, Genetics and Evolution, 14*, 83–91. doi: 10.1016/j.meegid.2012.09.019

33. Carr, T. M., Wheaton, J. D., Houtz, G. M., & Ciofani, M. (2017). JunB promotes Th17 cell identity and restrains alternative CD4+ T-cell programs during inflammation. *Nature Communications, 8*(1). doi: 10.1038/s41467-017-00380-3

34. Aminian-Dehkordi, J., Rahimi, S., Golzar-Ahmadi, M., Singh, A., Lopez, J., Ledesma-Amaro, R., & Mijakovic, I. (2023). Synthetic biology tools for environmental protection. *Biotechnology Advances, 68*, 108239. doi: 10.1016/j.biotechadv.2023.108239

35. Tokiwa, Y., & Calabia, B. P. (2006). Biodegradability and biodegradation of poly(lactide). *Applied Microbiology and Biotechnology, 72*(2), 244–251. doi: 10.1007/s00253-006-0488-1

36. Vidali, M. (2001). Bioremediation. An overview. *Pure and Applied Chemistry, 73*(7), 1163–1172. doi: 10.1351/pac200173071163

37. Wilkes, R. A., & Aristilde, L. (2017). Degradation and metabolism of synthetic plastics and associated products byPseudomonassp.: capabilities and challenges. *Journal of Applied Microbiology, 123*(3), 582–593. Portico. doi: 10.1111/jam.13472

38. World Bank Group. (2018). *What Waste 2.0: A Global Overview of Solid Waste Management to 2050*. Retrieved from https://datatopics.worldbank.org/what-a-waste/

39. Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., & Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science, 351*(6278), 1196–1199. doi: 10.1126/science.aad6359

40. Oluwole, O. A., Oluyege, J. O. Olowomofe, T. O., Daniel, A., Adekunle, A., & Adegunle, O. (2018). Optimisation of culture conditions for the biodegradation of low-density polyethylene films by plastic waste dumpsite bacteria. *Animal Science Journal, 9*(1), 01–15.