Studying the Effect of Glycerol and UV irradiation on Microbial Degradation of LDPE

Ms. K. Shivaleela.[1], Prof. Manjula Bhanoori[*]

[1]*Research scholar, Department of Biochemistry, Osmania University, Hyderabad*
***Corresponding Author**
Prof. Manjula Bhanoori
Department of Biochemistry, Osmania University, Hyderabad, Telangana, India

KEYWORDS
Glycerol, UV Irradiation, Microbial Plastic degradation, Environment Friendly, Plasticizer

ABSTRACT
The degradation of plastic using microbes has emerged as a key environmental friendly and cost-effective approach. Besides utilizing specific microbial strains, the breakdown of plastic can be further enhanced by incorporating degradation enhancers and pre-treating the plastic before degradation. Both methods are anticipated to significantly improve degradation rates. This study focused on examining the effects of these two factors on the microbial degradation of low-density polyethylene (LDPE). Pre-treatment with Glycerol, in varying concentrations and UV exposure at different time intervals was assessed for the impact on LDPE degradation Following these treatments, all plastic samples were collected from the media and tested for degradation improvement compared to the untreated control sample. FTIR, FESEM and SEM-EDS analysis showed oxidative degradation of LDPE. The results will be useful to develop a standard protocol and media composition to maximize plastic degradation with minimal resources and time. Once optimal conditions and media composition are established, a large-scale degradation setup can be implemented to help reduce plastic pollution in the environment.

INTRODUCTION:

Plastic is used in many everyday items because it is waterproof, lightweight, and convenient. We find it in things like buckets, milk packets, doorknobs, and carry bags. However, plastic has a major downside: it does not break down naturally, causing serious environmental pollution. Even though there are rules and campaigns to reduce plastic use, it is hard to stop using it completely because it is so useful, and people are used to it[1, 2]. A possible solution to this problem is to use microbes, like bacteria and fungi, to break down plastic. These microbes can digest plastic through a natural process that does not harm the environment[3]. Some fungi and bacteria are good at this, and fungi often do a better job because they can stick to the plastic's surface and use it as food[4]. In addition to using these microbes, there are ways to make the plastic break down even faster. One way is by adding substances like glycerol, which can help microbes degrade plastic more effectively[5]. Another way is by treating the plastic with UV light before letting the microbes work on it[6, 7].This study aims to see how well these methods work on a type of plastic called low-density polyethylene (LDPE). By finding the best conditions and materials, we can create a standard process for breaking down plastic quickly and efficiently, which can help reduce plastic pollution on a larger scale.

MATERIAL AND METHODS:

- **Selection and Activation of Microbial Culture**

Fungi and Bacteria were selected for LDPE degradation under specified conditions. The organisms used in the study were Fusarium oxysporum, Aspergillus niger, Bacillus subtilis, and Micrococcus luteus isolated from soil and effluent samples collected from Hayathnagar in Hyderabad. Their ability to degrade the LDPE and genomic identification was confirmed in our previous study [8]. Cultures were activated using PDA broth for fungi and Nutrient Agar Media for Bacteria. These cultures were then used for LDPE plastic degradation [9, 10].

- **Preparation of Medium for LDPE Degradation**

The basic medium for LDPE degradation consisted of broth with essential micro and macro nutrients along with LDPE. The media composition for both fungi and bacteria was same, with differences in incubation conditions and pH. Fungi were incubated at 25°C with pH 5.0 while Bacteria were incubated at 37°C with pH 7.0.

Standard Media Composition for LDPE Degradation is:

Mineral salt Media

$NaNO_3$ -2g

$MgSO_4$ -0.5g

KCl -0.5g

$Fe_2(SO_4)_3$ -0.01g

KH_2PO4 -0.14g

K_2HPO_4 -1.2g

Yeast extract -0.02g

Distilled water -1000

LDPE-0.5%

Testing the Role of UV Irradiation on Plastic Degradation

Media flasks were prepared, and LDPE pieces were subjected to UV irradiation for different durations (0 sec, 15 sec, 30 sec, 45 sec and 60 sec) before being added to the flasks. After inoculation and incubation for 60 days [11], results were analyzed. Media flask (without microorganism) served as control group. The experiment was conducted in triplicate (Figure 1).

- **Testing the Role of Glycerol concentration on Plastic Degradation**

The standard media composition was altered by adding different concentrations of glycerol (0%, 0.5%, 1%, 1.5% and 2%) to enhance LDPE degradation. Media were prepared, glycerol was added before inoculation, and then incubated with respective cultures for 60 days[9] media flask (without microorganism) served as control group. The experiment was conducted in triplicate.

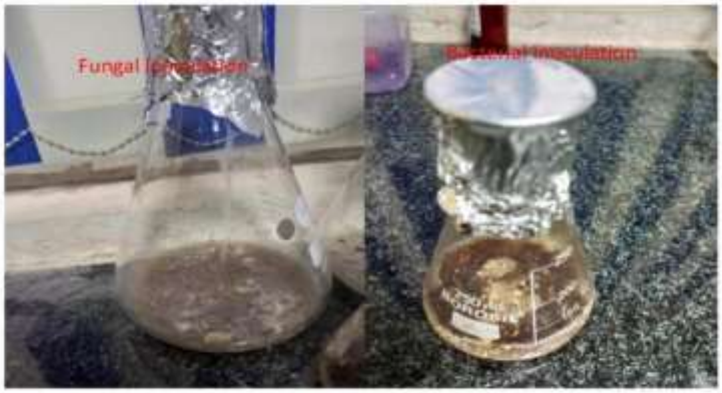


Figure 1: Different media flasks with plastic for degradation

The above picture shows media flasks with different glycerol concentrations of fungal and Bacterial inoculation.

Methods used for Testing the Efficacy of Degradation:

After 60 days of incubation, broth was filtered, and LDPE remnants were collected and washed. Quantitative assessment was done by weighing the plastic, and qualitative assessment was based on texture and weight loss. Results were analyzed using techniques like FTIR and FE-SEM [9, 12].

Fourier Transform Infrared Spectroscopy (FT-IR):

Fourier Transform Infrared Spectroscopy is an analytical technique that assists in understanding the molecular structure and composition of a material by studying the influence of infrared (IR) radiation on it. One gram weight of dried cell free extract was mixed with 2 mg of dry potassium bromide in a mortar and pestle (KBr). The powder was placed in a 2 mm diameter micro-cup and loaded onto an FTIR at 26**°C**. Using a Fourier Transform Infrared Spectrometer (SHIMADZU). the samples were scanned in the infrared range (wavenumber) of 4000 cm-1 to 400 cm-1.The functional groups present in the sample were identified using spectral data.

Field Emission Scanning Electron Microscopy (FE-SEM):

Field Emission Scanning Electron Microscopy (FE-SEM) is a high-resolution imaging technique that uses a focused beam of electrons emitted from a field emission source to scan the surface of a sample. It provides detailed images with nanometer-level resolution, making it ideal for analyzing surface morphology and fine structures. FESEM when coupled alongside EDS (Energy Dispersive X-ray Spectroscopy) (Zeiss) will identify the elemental composition of the sample by detecting X-rays emitted when the electron beam interacts with the sample. The sample is prepared by ensuring it is dry and conductive (non-conductive materials are coated with a thin layer of conductive material like gold or carbon). The sample was mounted on a holder using adhesives compatible with the SEM vacuum. The prepared sample was placed inside the SEM vacuum chamber to avoid interference from air molecules. SEM scans the surface to capture detailed images of the sample morphology. The EDS detector will be activated to collect X-rays emitted from the sample. The electron beam can be focused on specific regions of interest for elemental analysis. EDS generates an energy spectrum where each peak corresponds to a specific element. SEM images and EDS spectra will be combined to correlate morphological features with elemental composition.

RESULTS AND DISCUSSION:

Effect of glycerol and UV treatment was analysed in all 4 test organisms for their ability to degrade LDPE. Two fungi and two bacteria were tested with 4 different time exposures to UV radiation and four different concentrations of glycerol. Incubation was maintained for 60 days in all the test broths and later subjected to plastic separation and cleaning. Plastic pieces were measured for weight reduction and the texture of the plastic was also checked. Controls with untreated (unexposed to UV) and no added glycerol were used in the tests for comparative analysis. The results of UV exposure are furnished in Table 1. Significant effect was observed with *Aspergillus niger* compared to other microbes. The effect of glycerol on degradation is furnished in Table 2. *Micrococcus luteus* showed better degradation when compared to other microbes. These results help to select a better organism to cause maximum degradation under selected exposure conditions. The graphical illustration of the treatment of UV irradiation and glycerol are given in Figures 2 and 3 respectively.

The initial and final weight of plastic were calculated and % of plastic degradation was calculated using the formula:

$$\boldsymbol{Weight\ loss}\ (\%) = \frac{(\boldsymbol{Initial\ weight\ - Final\ weight})}{\boldsymbol{Intial\ weight}} \times \mathbf{100}$$

Table 1: Percentage weight loss of LDPE film exposure to UV rays for Different Time Intervals

SI. No	Identified Isolates	Pre-wight and post-weight in mg with UV exposure Time in seconds					Percentage of weight loss
		0	15	30	45	60	
1	*Fusarium oxysporum*	96.6± 0.72	96.0± 0.56	95.4± 0.72	95.0±1.06	94.6±1.04	2%

2	*Aspergillus niger*	94.0± 0.90	93.2± 0.61	92.8± 0.44	92.2±1.15	91.7±0.44	2.4%
3	*Bacillus subtilis*	97.5± 0.95	97.0± 0.95	96.6± 0.46	96.2±0.85	96.0±0.66	1.3%
4	*Micrococcus luteus*	94.7± 0.75	94.2± 0.87	93.7±1.25	93.1±0.66	92.8±0.85	2%

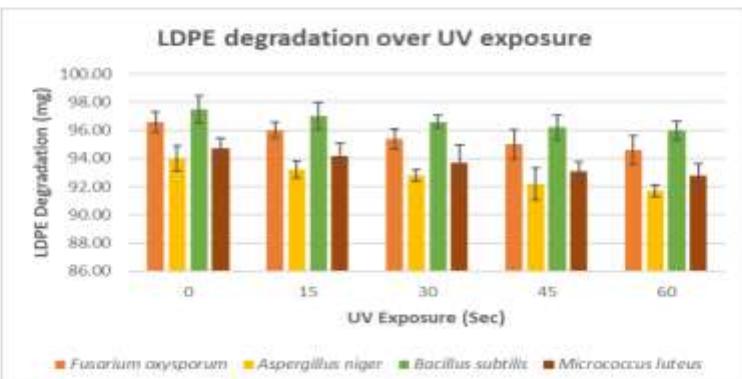


Figure 2: weight loss of LDPE film exposure to UV rays

Table 2: Percentage weight loss of LDPE film upon treatment with glycerol

Sl. No	Identified Isolates	Pre-weight and post-weight in mg					Percentage of weight loss
		Glycerol concentration 0%	Glycerol concentration 0.5%	Glycerol concentration 1%	Glycerol concentration 1.5%	Glycerol concentration 2%	
1	***Fusarium oxysporum***	**97.6±0.75**	**97.1±0.92**	**96.6±0.79**	**96.0±1.06**	**95.7±0.72**	**1.9%**
2	***Aspergillus niger***	**95.3±0.87**	**94.9±0.36**	**94.0±1.01**	**93.2±0.66**	**92.7±0.87**	**2.7%**
3	***Bacillus subtilis***	**96.5±0.61**	**96.1±1.00**	**95.4±1.21**	**94.7±1.11**	**93.8±0.62**	**2.8%**
4	***Micrococcus luteus***	**97.2±0.98**	**96.8±0.85**	**95.0±1.15**	**94.2±1.10**	**93.5±0.92**	**3.8%**

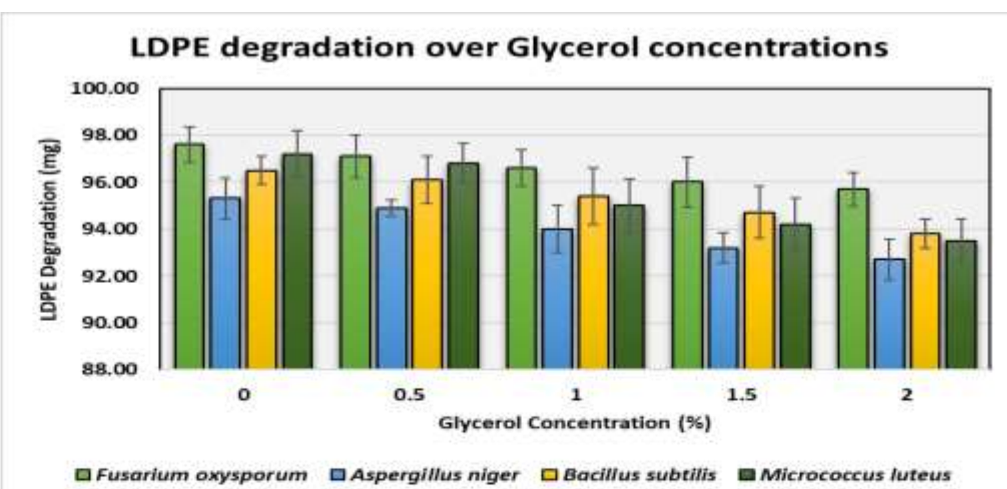
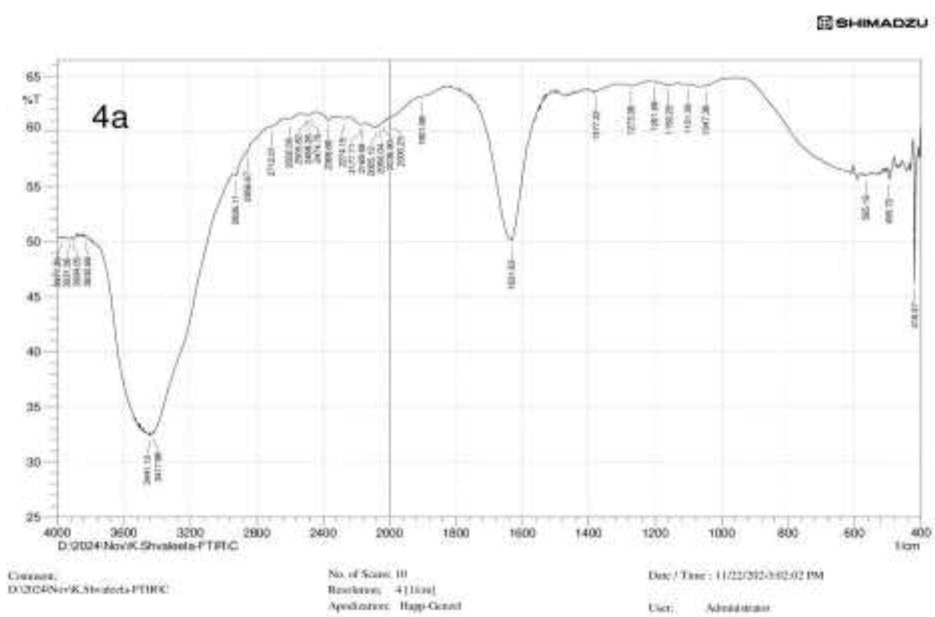


Figure 3: Weight loss of LDPE film upon treatment with Different Concentrations of glycerol

In the results of UV and glycerol treatment, Micrococcus luteus showed high degradation of LPDE with 2% weight loss in UV treatment and 3.8% with glycerol treatment. Micrococcus luteus, grown in 2% glycerol medium, degraded LDPE sheets, were further analyzed using FTIR and SEM, LDPE sheets treated with only glycerol without Micrococcus luteus served as control.

FTIR analysis:

In the present study, FTIR spectroscopy analysis was performed for LDPE treated with glycerol without inoculation of Micrococcus luteus and inoculated LDPE treated with glycerol. We used the spectrum of a typical range of 4000-400 cm^{-1} to identify functional groups in organic compounds, including polymers like LDPE (Low-Density Polyethylene). Functional groups identified by FT-IR analysis are shown in Figures 4 and Table 3. The LDPE control film (with glycerol and without Micrococcus luteus) showed a peak at 2926 cm-1 while LDPE treated (with glycerol and Micrococcus luteus) indicated a shift and increase in the peak intensity at 2928cm-1. We observed shifts in the C-H stretching peaks of alkanes (2928 cm-1 to 2729 cm-1) and a new peak at 1321 was observed in micrococcus treated LDPE. Khandare et al. (2021) observed a peak intensity at 2854 cm^{-1} in their study on the biodegradation of LDPE using Cobetia species [13]. A previous study by Anudurga Gajendiran et al. (2016) reported a C=O stretching peak of the aldehyde group at 1735.93 cm^{-1} in LDPE films incubated for 90 days with Aspergillus clavatus [14]. We also observed a peak at1735.24 cm^{-1} in the Micrococcus degraded LDPE (Figure 4b) indicating presence of carbonyl groups which was not observed in control. Additionally, we observed an increase in hydrophilic functional groups, indicated by peaks in the 3200-3600 cm^{-1} regions, which correspond to -OH groups. This further emphasizes the role of glycerol as a plasticizer which enhances degradation by increasing the accessibility of polymer matrix to microbial enzymes. Glycerol also acts as a compatibilizer in various applications[15]. In conclusion the obtained results indicate oxidative degradation of LDPE polymer.



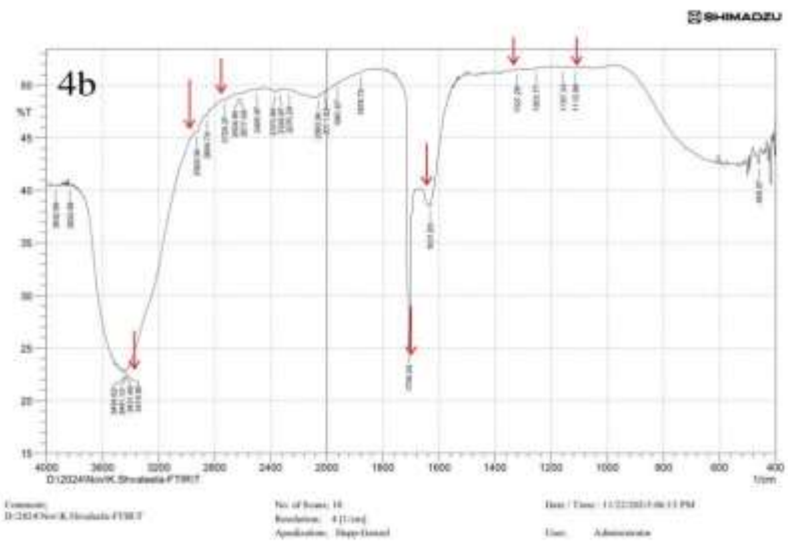


Figure 4.FTIR Analysis of LDPE degradation: Glycerol treated LDPE(4a) glycerol and Micrococcus luteus and glycerol treated LDPE(4b)

Table 3: Major IR absorption band and band shifts possible assignment in the typical FTIR spectrum of LDPE Sheets treated with only Glycerol and Micrococcus luteus with Glycerol:

Frequency cm^{-1} (Assignment)	Absorption Bands in LDPE film with Glycerol	Absorption Bands in *Micrococcus luteus* treated LDPE film with Glycerol
2928-2729 (C-H stretching for alkanes)	Observed stretch at 2926.11,2856.67,2712.01	Observed stretch at 2928.04,2854.74,2729.37
3419-(O-H stretch for hydroxyl)	_	Observed stretch at3419.90
1735(-C=O stretch for carbonyl)	_	Observed stretch at1735.2
1631(-C=C- stretch for alkenes)	Observed stretch at1631.83	Observed stretch at1631.83

1377(C-H bending for alkanes)	**Observed bending at 1377.22**	**_**
1321-1112(-CO-stretching vibrations)	**Observed stretch at 1273.06,1201.69,1156.20**	**Observed stretch at1321.28,1253.71,1157.33,1112.96**

FE-SEM Analysis:

To ascertain the modifications brought about in their morphological structure and elemental composition, only Glycerol treated LDPE (control) and Glycerol treated LDPE inoculated with Micrococcus luteus were examined using SEM and EDS. The following SEM and EDS analysis results provide evidence of further degradation of LDPE in microbial treated LDPE films (Figures 5 and 6)

1. Surface Morphology Analysis (SEM Images)

•**Micro-cracks and Pitting:** SEM images reveal increased surface irregularities, including micro-cracks, pits, and a rough texture, compared to control. This indicates that the Micrococucus luteus exposure has broken down the polymer matrix, contributing to physical degradation.

•**Fragmentation:** Small fragments or particles visible on the sample surface suggest that polymer chains are breaking down, possibly due to oxidation. This fragmentation indicates colonization of microorganisms and that LDPE break down has started.

•**Surface Roughness:** The overall surface appeared significantly rougher and more porous. These features may result from glycerol interaction and Micrococucus action, leading to increased brittleness and breakdown of polymer structure.

Rough, pitted textures, cracks, and irregularities suggest ongoing breakdown due to microbial activity in comparsion to Control film of LDPE with glycerol (without Micrococcus luteus) which remained smooth and clear (Figure 5). Previous studies by Khandare et al. 2021, obtained similar results in the SEM analysis of LDPE treated with marine bacteria (Cobetia species) which displayed surface degradation, fragility, damaged layers, cracks and scratches, whereas control films (without marine bacterial treatment) remained smooth, unbroken, and transparent. Similarly, Sanin et al. 2003 observed grooves and fissures in FE-SEM images, where bacterial strains (Pseudomonas sp. strain D, Pseudomonas sp. strain A, and Rhodococcus corallinus strain 11) degraded LDPE into monomeric forms [13].

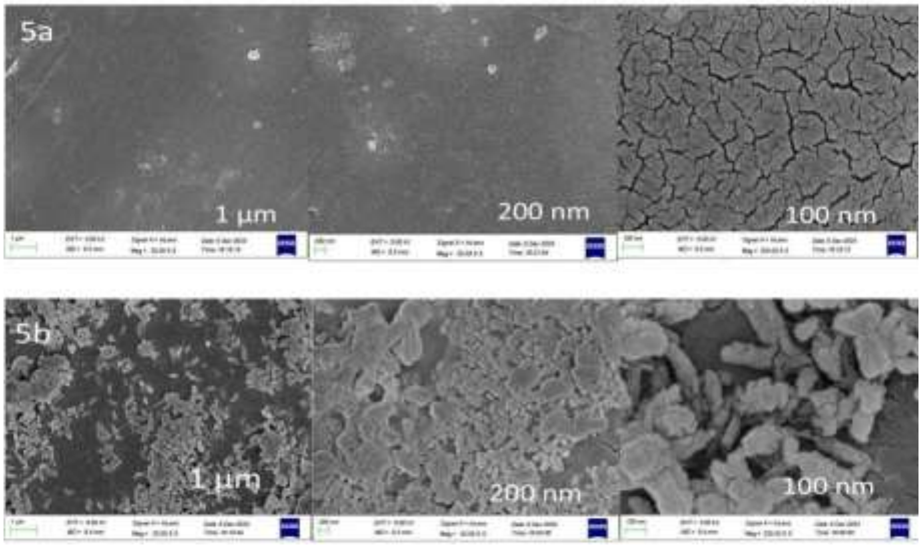


Figure 5: FE-SEM Analysis of LDPE degradation: Glycerol treated LDPE(5a) glycerol and Micrococcus luteus treated LDPE(5b).

2.Elemental Composition Analysis (EDS Data)

The results of Elemental Changes Over Time are furnished in Figure 6 The results shows evidence of oxidation.

• Oxygen (O):

The glycerol treated LDPE inoculated with Micrococcus luteus sample had far higher oxygen content (33.19%) than the only glycerol treated LDPE (17.02%). This rise in oxygen is probably caused by the creation of functional groups that contain oxygen, like hydroxyls and carbonyls (FTIR data also supports this result). According to published studies glycerol aids in the adhesion of microorganism to LDPE (Saadan et al.2017). The microbial attack at polymer surface is called as oxidation production stage which leads to oxygen enhancement (16).

•Carbon (C):

The carbon content in the glycerol treated LDPE sample was around 82.98% and significantly reduced in Micrococcus luteus treated LDPE to 64.3%. This shift suggests ongoing degradation due to photooxidative processes. A reduction in the carbon weight percentage may indicate polymer chain scission. The carbon reduction could also signify the loss of volatile degradation products that contain carbon (Figure 6).

Presence of Other Elements

• Calcium (Ca):

Trace amount of calcium was observed only in glycerol and Micrococcus treated LDPE, possibly due to residuals of Mineral Salt Medium from the initial glycerol pretreated stage. This element might also suggest some chemical interaction with the added glycerol and microbial degradation (Figure 6b).

3. Spectrum Analysis (EDS Spectra)

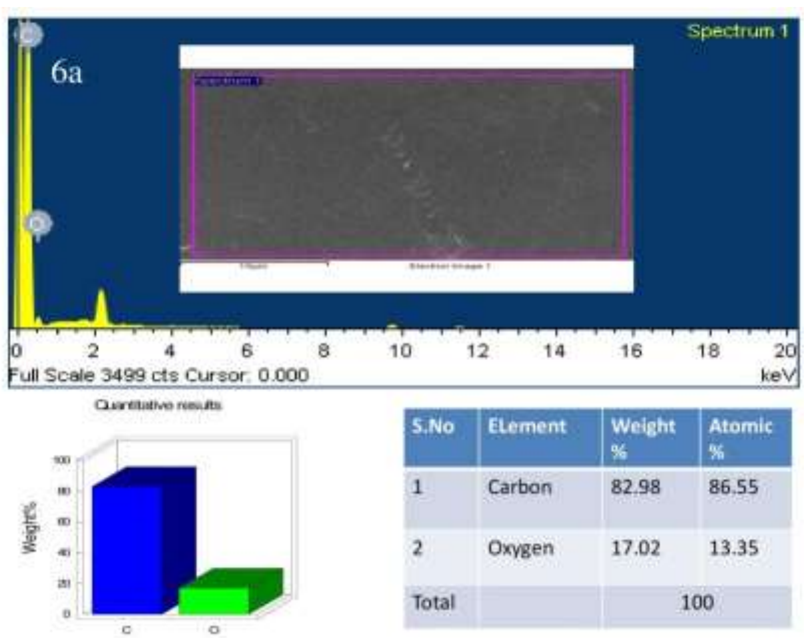
•Peaks for Oxygen and Carbon:

The EDS spectra showed prominent peaks for both oxygen and carbon. The relative intensities of these peaks changed after microbial degradation, with oxygen peaks increasing, which correlates with the oxidation process expected in photo-degraded LDPE. The carbon peak showed a reduction in intensity, aligning with the EDS compositional analysis, which indicates a reduction in carbon content due to bond scission and possible loss of small carbon compounds.

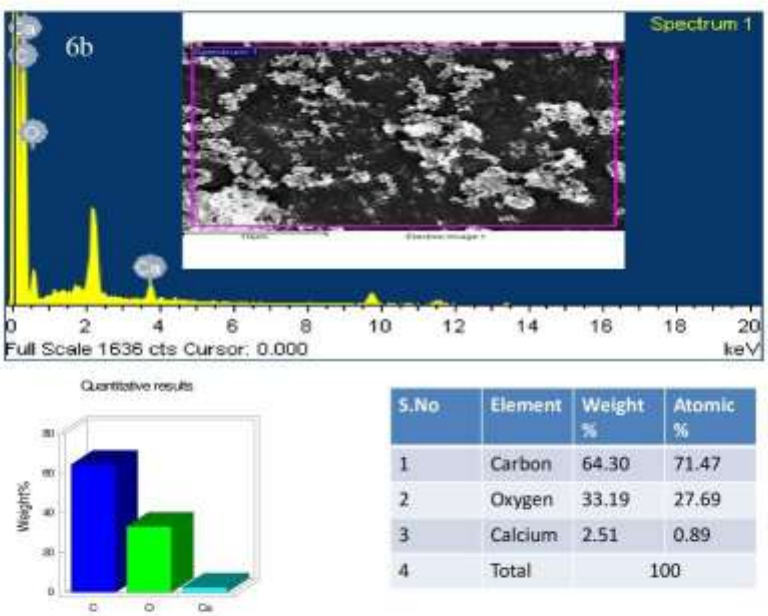
•Omission of Certain Peaks:

Specific peaks (like those around 1.650, 2.349, 9.698, and 11.474 keV) have been omitted in the analysis, as they do not significantly contribute to the primary elements of interest (C and O) or are outside the critical analysis range for degradation studies.

In conclusion SEM and EDS analysis indicates significant degradation of LDPE due to Micrococcus action. Surface roughness, cracks, and fragmentation observed in SEM images, indicating physical degradation of the LDPE matrix. Increased oxygen content, suggesting oxidative degradation and reduced carbon content, indicating polymer chain scission.



S.No	ELement	Weight %	Atomic %
1	Carbon	82.98	86.55
2	Oxygen	17.02	13.35
Total		100	



S.No	Element	Weight %	Atomic %
1	Carbon	64.30	71.47
2	Oxygen	33.19	27.69
3	Calcium	2.51	0.89
4	Total	100	

Figure 6: SEM EDS Analysis of LDPE degradation: Glycerol treated LDPE(6a) glycerol and Micrococcus luteus treated LDPE(6b)

CONCLUSION

In the current study, the role of UV irradiation and glycerol on LDPE degradation by various selected cultures was tested. Four organisms tested for degradation were two fungi and two bacteria namely Fusarium oxysporum, Aspergillus niger, Bacillus subtilis, and Micrococcus luteus. UV irradiation of LDPE before degradation was tested with variable exposure times: 0, 15, 30, 45, and 60 seconds, respectively. The effect of glycerol on LDPE degradation was studied at 4 different concentrations which include 0, 0.5, 1.0, 1.5, and 2.0 %, respectively. UV exposure to LDPE causes the initiation of structural damage and enhances the rate of degradation by microbes. Glycerol, which acts as a plasticizer, altered the structure of LDPE causing enhanced degradation. All the analyses revealed a direct and positive role of UV irradiation and glycerol addition on plastic degradation. Elevation in degradation was measured as percentage of degradation. The highest degradation was exhibited by Aspergillus niger (2.4%) with UV treatment and Micrococcus luteus (3.8%) with glycerol treatment. The quality of degradation was tested based on the changed texture of the LDPE after incubation. There was a visible change detected in the thickness and stiffness of the plastic. The FTIR data showed modifications in the peaks, especially in the carbonyl and hydroxyl regions indicating chemical changes brought on by biodegradation. We observed an important indicator of the polymer's oxidative breakdown in the carbonyl region, which is located about 1735 cm^{-1}. Analysis by FE-SEM and EDS showed the presence of wrinkles, cracks, and pitted structures indicated the partial degradation of the polymer. EDS analysis showed increased oxygen and decreased carbon levels. The results of the present study may be useful for obtaining a standard media composition and optimum degradation conditions which can be performed on a large scale to clean up plastic pollution and thereby protect the environment.

Acknowledgements

The authors would like to acknowledge the OsmaniaUniversity, Hyderabad, and Telangana Social Welfare Residential Degree College, Mancherial for providing the lab oratorical support for the conduction of work. We also acknowledge Dr. Kuraganti Gunaswetha,Kakatiya University, and Jyosthna for helping consolidate the data.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

FUNDING SOURCE

The complete research is self -funded and do not have any funding source.

REFERENCE:

1. (Orr) IG, Hadar Y, Sivan A (2004) Colonization, biofilm formation and biodegradation of polyethylene by a strain of Rhodococcus ruber. Appl Microbiol Biotechnol 65:. https://doi.org/10.1007/s00253-004-1584-8
2. Danso D, Chow J, Streit WR (2019) Plastics: Environmental and Biotechnological Perspectives on Microbial Degradation. Appl Environ Microbiol 85:. https://doi.org/10.1128/AEM.01095-19
3. Sudhakar M, Doble M, Murthy PS, Venkatesan R (2008) Marine microbemediated biodegradation of low- and high-density polyethylenes. Int Biodeterior Biodegradation 61:203–213. https://doi.org/10.1016/j.ibiod.2007.07.011
4. Sivan A (2011) New perspectives in plastic biodegradation. Curr Opin Biotechnol 22:422–426. https://doi.org/10.1016/j.copbio.2011.01.013
5. Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62:1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030
6. Manzur A, Limón-González M, Favela-Torres E (2004) Biodegradation of physicochemically treated LDPE by a consortium of filamentous fungi. J Appl Polym Sci 92:265–271. https://doi.org/10.1002/app.13644
7. Kale G, Kijchavengkul T, Auras R, et al (2007) Compostability of Bioplastic Packaging Materials: An Overview. Macromol Biosci 7:255–277. https://doi.org/10.1002/mabi.200600168
8. Shivaleela K,Manjula B,(2024)Biodegradation of Low Density Polyethylene by Microbes An Experimental Study:Afr.J. Biomed.Res.27(6S): 344352.https://doi.org/10.53555/AJBR.v27i6S.6391
9. Kyaw BM, Champakalakshmi R, Sakharkar MK, et al (2012) Biodegradation of Low Density Polythene (LDPE) by Pseudomonas Species. Indian J Microbiol 52:411–419. https://doi.org/10.1007/s12088-012-0250-6
10. Jeon HJ, Kim MN (2013) Isolation of a thermophilic bacterium capable of lowmolecular-weight polyethylene degradation. Biodegradation 24:89–98. https://doi.org/10.1007/s10532-012-9560-y
11. Montazer Z, Habibi-Najafi MB, Mohebbi M, Oromiehei A (2018) Microbial Degradation of UV-Pretreated Low-Density Polyethylene Films by Novel Polyethylene-Degrading

Bacteria Isolated from Plastic-Dump Soil. J Polym Environ 26:3613–3625. https://doi.org/10.1007/s10924-018-1245-0

12. Tribedi P, Sil AK (2013) Low-density polyethylene degradation by Pseudomonas sp. AKS2 biofilm. Environ Sci Pollut Res 20:4146–4153. https://doi.org/10.1007/s11356-012-1378-y
13. Shrikant D. Khandare . Doongar R. Chaudhary . Bhavanath Jha(2021) Marine bacterial biodegradation of low-density polyethylene (LDPE) plastic. Biodegradation. https://doi.org/10.1007/s10532-021-09927-0
14. Gajendiran A, Krishnamoorthy S, Abraham J. Microbial degradation of low-density polyethylene (LDPE) by Aspergillus clavatus strain JASK1 isolated from landfill soil. 3 Biotech. 2016 Jun;6:1-6. DOI 10.1007/s13205-016-0394-x
15. Pushpadass, H. A., Bhandari, P., & Hanna, M. A. (2010). Effects of LDPE and glycerol contents and compounding on the microstructure and properties of starch composite films. Carbohydrate Polymers, 82(4), 1082-1089.https://doi.org/10.1016/j.carbpol.2010.06.032
16. Saadan, Mohd ZH, Zurina Mohamad, Mazura Jusoh, Rohah A. Majid, Nurfhairna Baharulrazi, and Siti HC Man. "Degradable Polyethylene/Rice Bran Film: Effect of Gylcerol." Chemical Engineering Transactions 56 (2017): 49-54.https://doi.org/10.3303/CET1756009