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Field Implementation of *Pseudomonas fluorescens* for Bioremediation of Single-Use Plastic Waste: A Biolayer in a Landfill Liner System

S. Sangeetha¹ and P. Hari Krishna²¹Department of Civil Engineering, VNR Vignana Jyothi Institute of Engineering and Technology, Vignana Jyothi Nagar, Pragathi Nagar, Nizampet S.O., Hyderabad-500118, Telangana, India²Department of Civil Engineering, National Institute of Technology, Warangal-506004, Telangana, India

†Corresponding author: S. Sangeetha; mailsangeethasundar@gmail.com

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ABSTRACT

Single-use plastic materials are widely used for packaging daily essentials and sanitary products. Fifteen such packaging materials were identified and collected. Twelve batches of samples were buried in twelve pits. *Pseudomonas fluorescens* in dextrose medium was applied to six pits, and six pits were subjected to natural degradation to serve as a control. The study was conducted for a period of six months, and samples were recovered after each month. Recovered samples were subjected to a weight-loss study and Fourier transform infrared (FTIR) spectroscopy. FTIR analysis indicated shifts of peaks toward lower wavenumbers, disappearance of peaks, and reductions in bond energy in all samples bioremediated with microbial solutions. The reduction in spectral energy associated with shifts in characteristic FTIR peaks was approximately 0.15–45%. Compared with the weight-loss technique, FTIR analysis was effective in understanding biodegradation phenomena. CHNS analysis of soil recovered from the pit revealed that the bioaugmentation technique increased the C/N ratio of the surrounding soil in the pit to 4.5%, which was absent initially. The results indicated that this technique could enhance the bioremediation of non-recoverable plastic waste.

1. INTRODUCTION

Many non-recycled plastic products, after their useful life, ultimately end up in soil or water as waste. Hundreds of microbes have been identified to degrade plastic polymers (Arutchelvi et al. 2008, Shah et al. 2008, Kumar Sen & Raut 2015, Montazer et al. 2018, Fesseha & Abebe 2019, Bahl et al. 2020, Bardají et al. 2020, Merchant et al. 2025). Microbes use plastic polymers as a source of carbon and mineralize them to carbon dioxide and water (Albertsson & Karlsson 1990, Reddy et al. 2009, Pérez-García et al. 2025). The success of any scientific finding lies in its field-scale implementation. Earlier bioremediation studies were conducted on a laboratory scale in a controlled environment. Plastic-degrading microbes were isolated from soil and water samples collected from dumpsites (Dubey & Thalla 2025, Iqbal et al. 2025, Turkey & Upadhyay 2025). Field bioremediation studies were limited to the soil burial technique. These field studies were conducted by burying plastic materials directly in soil without introducing potential bacterial strains (Orhan et al. 2004, Vijaya & Reddy 2008, Shovitri et al. 2017, Tai et al. 2019). The microbial species must thrive under the existing environmental conditions along with native microbial species. In the present study, field-scale bioaugmentation using *Pseudomonas fluorescens* to accelerate plastic biodegradation was investigated. The microbes were introduced

into the field, where 15 different plastic packaging materials were buried. Based on the effectiveness of the field-scale study, a biolayer is proposed for inclusion in the design of an engineered landfill system to enhance plastic-waste biodegradation.

2. MATERIALS AND METHODS

The plastic materials considered for the degradation study are generally used for packaging and are made of high-density polyethylene (HDPE), low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), polypropylene (PP), and polylactic acid (PLA). Fifteen such wrapping materials from products used in daily life were randomly selected. Details of these materials are provided in Table 1. Information on the polymer origin was obtained from the resin identification code (RIC) printed by the manufacturer. Each plastic material was washed with isopropyl alcohol, dried, and cut into 2 cm × 2 cm pieces. These pieces were weighed to an accuracy of 0.1 mg and attached to a mesh to facilitate sample recovery at the end of the biodegradation study. The study was conducted at VNR VJIET College, Hyderabad, Telangana, India. Initial characterization of soil collected from the field was performed. In the field, 12 pits, each 0.5 m in diameter and 0.7 m deep, were dug. In each pit, meshes with attached plastic pieces were buried in triplicate at different depths to verify the repeatability of the procedure. A schematic layout of the test pits used in the study is shown in Fig. 1.

Six of these pits were left for natural degradation. Because the major objective of the study was to compare

Table 1: Different types of single-use plastic materials subjected to field degradation.

Label	Description	Type of plastic
1F	Detergent cover	HDPE
2F	Cloth-like white bag	PP
3F	Transparent orange-colored cover	LDPE
4F	Black and white parcel packaging	PP
5F	Transparent cover	HDPE
6F	Aerated beverage label	PP
7F	Empty milk packet	LLDPE
8F	Packaging cover	HDPE
9F	Carry bags from textile shop	PP
10F	Food packing silver wrapper	HDPE
11F	Empty oil packet	HDPE
12F	File cover	PP
13F	Shampoo cover	HDPE
14F	Biodegradable white carry bag	Polylactic acid
15F	Instant noodles wrapper	HDPE

the performance of the field bioaugmentation technique with the natural degradation process, these pits were maintained as controls rather than sterile controls. The other six pits were bioaugmented with *Pseudomonas fluorescens* (PF). A total of 60 g of *Pseudomonas fluorescens* in dextrose medium (powder form), supplied by M/S SOM Phytopharma (India) Ltd., Hyderabad, was dissolved in 6 L of water. For each treatment pit, 1 L of the prepared bacterial solution

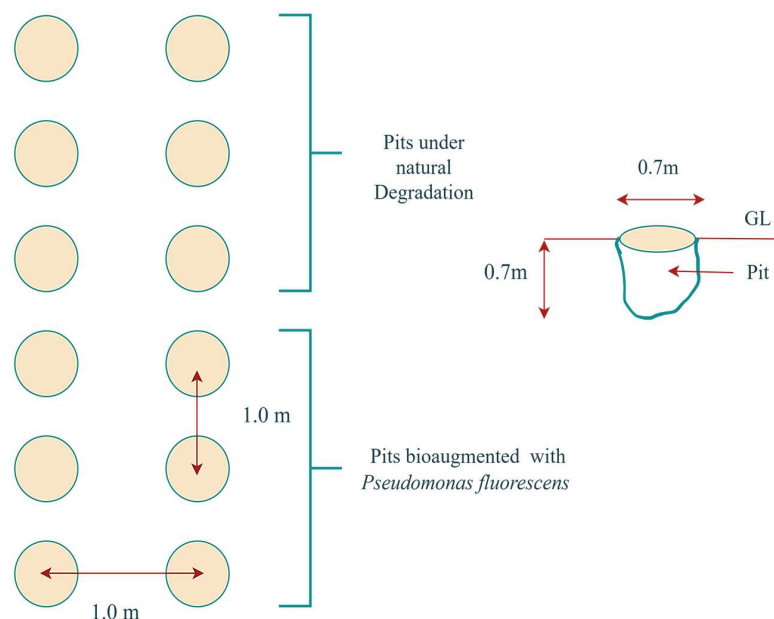


Fig. 1: Schematic representation of test pits for the field biodegradation study.

Table 2: Basic properties of soil collected from the study location.

Properties	Standard Codes	Results/Values
Field dry density	IS: 2720 (Part 29): 1975	1.6 t m ⁻³
Water content	IS: 2720 (Part 2): 1973	7.4%
Specific gravity	IS:2720 (Part 3):1980	2.66
Grain size distribution: Sieve and Hydrometer Analysis	IS: 2720 (Part 4): 1985	Gravel: 5.6%; Sand: 89.7% Silt: 3.3%; Clay: 1.4%
Soil classification	IS: 1498: 1970	Poorly graded sand (SP)
pH	IS: 2720 (Part 26): 1987	8.82 at 28.6°C
Electrical Conductivity	IS: 14767: 1973	0.589 mS at 28.6°C
Salinity	Probe method	0.286 ppt at 28.6°C

was applied uniformly throughout the depth. The moisture content of each pit was maintained constant by supplying water periodically. To avoid disturbance, at the end of each month, one pit subjected to natural degradation and one pit subjected to bioaugmentation were excavated. After recovery, the plastic film samples were cleaned with isopropyl alcohol. Percentage weight loss was calculated by comparing the initial weight and the weight of the sample after recovery, as given in Eq. (1). For each sample, the maximum percentage weight loss observed during the six-month study period was considered.

$$\text{Percentage of weight loss} = \frac{w_a - w_b}{w_a} \times 100\% \quad \dots(1)$$

Here,

w_a and w_b are the weights of a plastic film before placing it inside the pit and after recovery, respectively.

FTIR analysis was then performed on samples recovered after 6 months of the study. FTIR analysis was performed directly on the plastic films. CHNS analysis was performed on

soil samples collected from the field before commencement of the study. After 6 months, soil samples from the control pit and the bioaugmented pit near the mesh were also subjected to CHNS analysis to assess carbon levels. The samples were tested by preparing KBr pellets.

3. RESULTS AND DISCUSSION

3.1. Basic Characteristics of Soil

Soil collected from the field location was tested to understand its basic properties. The properties of the soil are given in Table 2.

3.2. Field Weight Loss Study

Physical disintegration, expressed as percentage weight loss, was measured for all 15 samples at specified time intervals up to 6 months. The maximum percentage weight loss observed during the study period for all 15 samples subjected to natural and bioaugmented degradation is shown in Fig. 2. Of the 15

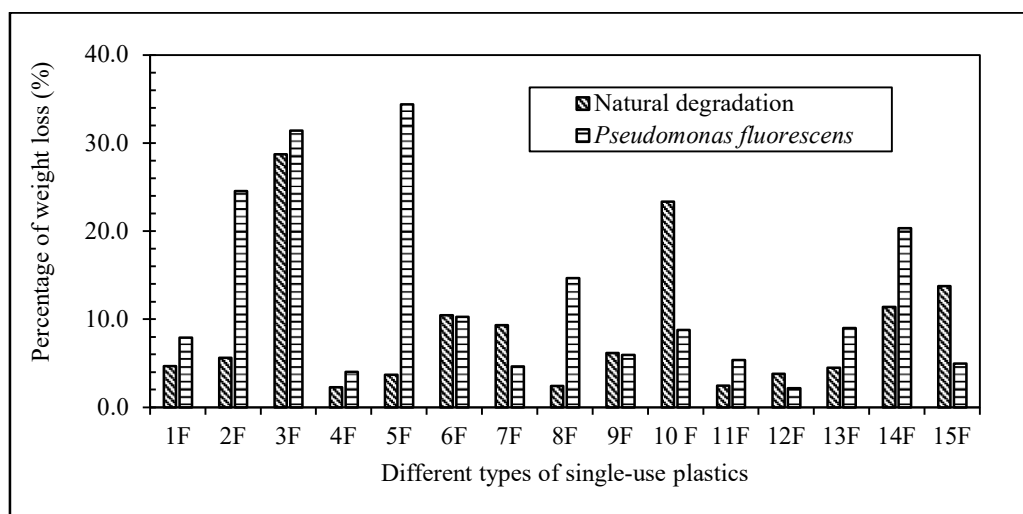


Fig. 2: Percentage of weight loss observed in the field degradation study.

samples, 9 showed greater degradation in the presence of *Pseudomonas fluorescens*. A maximum degradation of 34% was observed with the bioaugmentation technique for the transparent HDPE cover. Two samples showed equivalent degradation under both processes (6F and 9F). Samples 7F, 10F, 12F, and 15F exhibited greater weight loss during natural degradation. These samples were made from LLDPE, PP, and HDPE. The results of the field weight-loss study demonstrate the efficiency of *Pseudomonas fluorescens* in biodegradation under field conditions. However, a weight-loss study may not provide an accurate picture of plastic biodegradation. The samples were attached to the mesh using staples. Physical damage to the material was observed upon recovery due to staple holes and sharp particles in the soil.

3.3. FTIR Analysis

To investigate changes in the chemical bonds of polymeric hydrocarbon samples subjected to field bioaugmentation and natural degradation, FTIR analysis was performed on samples recovered from the field after 6 months of degradation. FTIR spectra of all 15 samples with prominent peaks are shown in Figs. 3–17. In the FTIR spectra of samples subjected to bioaugmentation, shifts of peaks toward lower wavenumbers and the disappearance of certain peaks were observed compared with samples subjected to natural degradation. In all single-use plastic samples, peaks corresponding to C–H stretching of the plastic polymer were observed in the 3000–2850 cm^{-1} region. Peaks of C–H deformation were observed around 1500–600 cm^{-1} . Minor peaks due to carbonyl bonds were observed in some samples at 1900–1500 cm^{-1} . Various peaks were observed in the spectra of all samples except 6F and 9F. However, these two samples also showed shifts toward lower wavenumbers.

In the bioaugmented samples, narrowing of broad peaks and disappearance of peaks in the C–H region (3000–2850 cm^{-1}), relative to natural degradation, were observed in samples 1F, 2F, 12F, 13F, and 15F. For analysis, the plastic film samples were categorized into groups based on the origin of the polymeric material from which each sample was manufactured. The peaks were identified based on standard FTIR spectroscopy literature (Coates 2000, Stuart 2004, Silverstein et al. 2005, Smith 2011). The changes in spectral peaks are described below.

3.3.1. FTIR Spectra of HDPE Samples

HDPE has a linear structure, which accounts for its higher density. The material is generally stiff, tough, and opaque (Crawford 2002, Emblem 2012). Samples 1F, 5F, 8F, 10F, 11F, 13F, and 15F are films made from HDPE polymer, and the spectral peaks after 6 months of bioremediation under natural and PF-bioaugmented conditions are shown in Figs. 3–9. Characteristic spectral peaks of virgin HDPE film include double peaks between 2950–2800 cm^{-1} representing CH_2 asymmetric and symmetric stretching, a pair of split peaks at 1400–1300 cm^{-1} due to C– CH_3 bending, and another set of split peaks around 730–700 cm^{-1} , as reported in the literature. After 6 months of bioremediation, many additional peaks were also observed in Figs. 3–17. This is because the samples considered in the present study are commercial wrappers that contain antioxidants, surface coatings, additives, color dyes, and other composite substances. Hence, to investigate microbial activity relative to natural degradation, all spectra from the bioaugmented batch were compared with those of samples subjected to natural degradation.

In Fig. 3, for sample 1F, a detergent wrapper, the peak wavenumbers shifted toward lower values, the double peaks

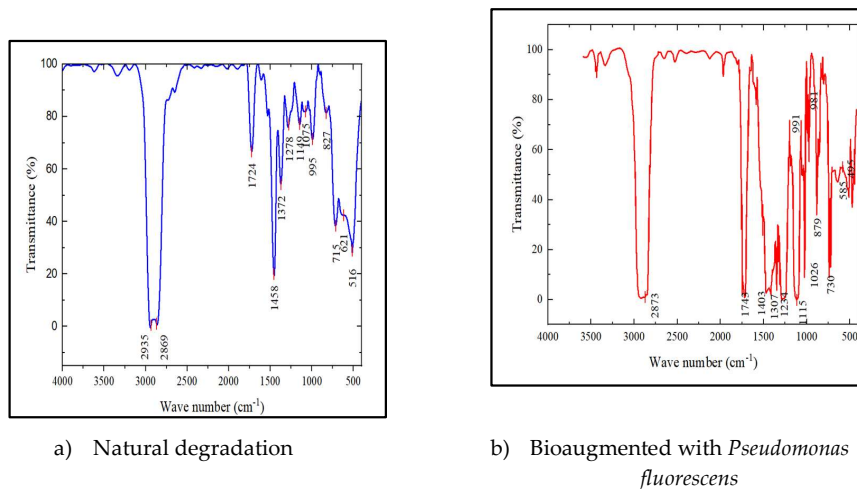


Fig. 3: FTIR spectrum of field sample 1F.

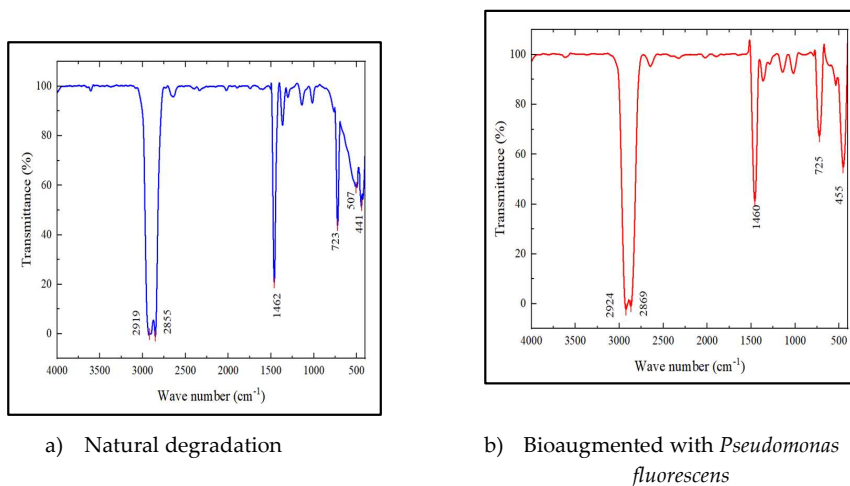


Fig. 4: FTIR spectrum of field sample 5F.

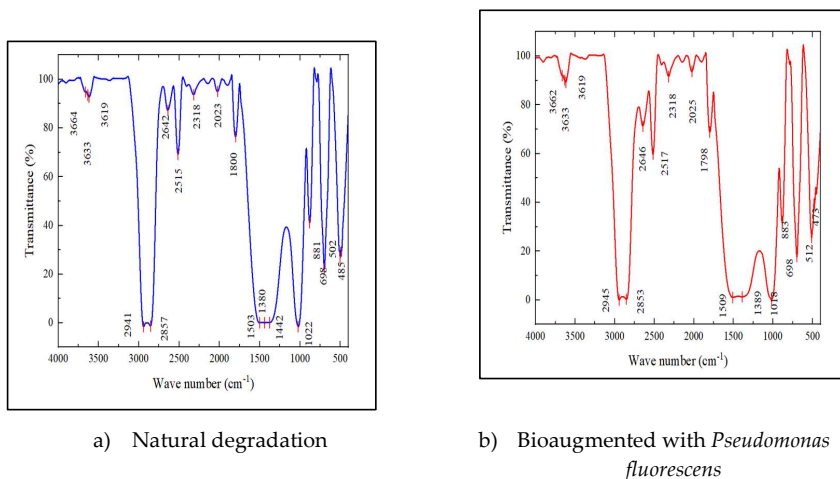


Fig. 5: FTIR spectrum of field sample 8F.

in the C–H region became blunt, and a single significant peak appeared. A new peak at approximately 981 cm^{-1} corresponding to HDPE oxidation was observed (Stuart 2004). Peaks shown in Fig. 4 for sample 5F are identical to those reported by various researchers (Gulmine et al. 2002, Smith 2011, Smith 2021, Chaudhary & Vijayakumar 2020). A weak band observed at 507 cm^{-1} in Fig. 4a under natural degradation was absent in the bioaugmented sample due to microbial activity. In Fig. 5, in addition to CH_2 stretching and C– CH_3 bending, peaks corresponding to the OH bond and weak bands representing carbon triple and double bonds are present. This indicates that the material contains HDPE along with other polymeric units. As a result of bioaugmentation, the broad band at $1500\text{--}1440\text{ cm}^{-1}$ with a triple peak narrowed to two prominent peaks, and a reduction in peak wavenumbers was observed.

The major spectral change observed for sample 10F, other than peak shifting, is the reduced intensity of the peak at 1526 cm^{-1} in the bioaugmented sample compared with the naturally degraded sample, as shown in Fig. 6.

In Fig. 7, the significant bands representing HDPE are the OH group at $3600\text{--}3300\text{ cm}^{-1}$, the aromatic C=C band at 1649 cm^{-1} , C–O stretching at 1269 and 1208 cm^{-1} , and C–O–C stretching at 1173 cm^{-1} . The major change resulting from bioaugmentation, as shown in Fig. 7b, is the absence of peaks at 1173 and 1208 cm^{-1} . The peak intensities in the functional-group region also decreased.

In Fig. 8, the prominent changes resulting from bioaugmentation are the disappearance of the C=C aromatic-ring stretching peaks at 1614 and 1528 cm^{-1} . The functional-group peaks in the fingerprint region ($1500\text{--}500\text{ cm}^{-1}$)

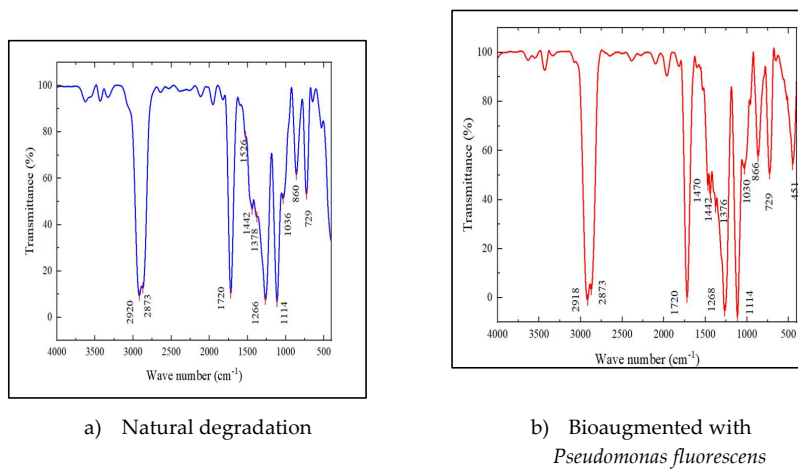


Fig. 6: FTIR spectrum of field sample 10F.

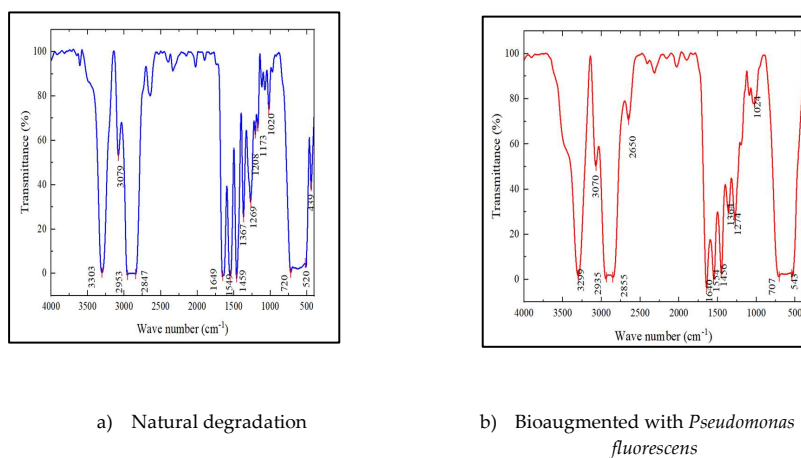


Fig. 7: FTIR spectrum of field sample 11F.

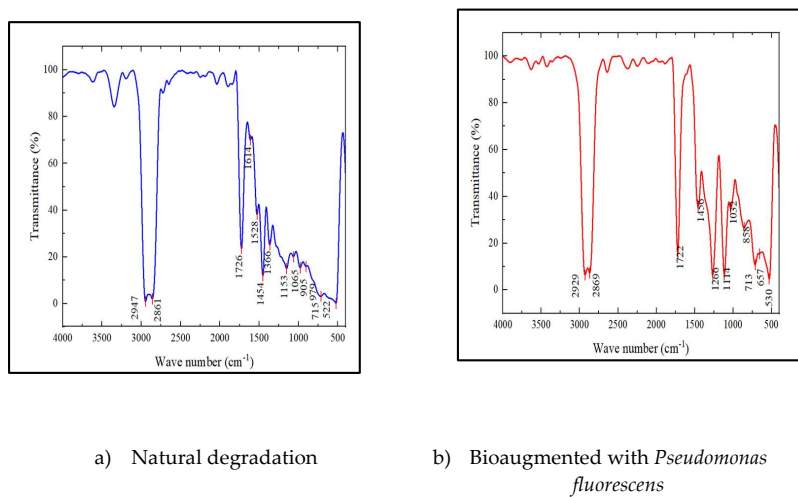


Fig. 8: FTIR spectrum of field sample 13F.

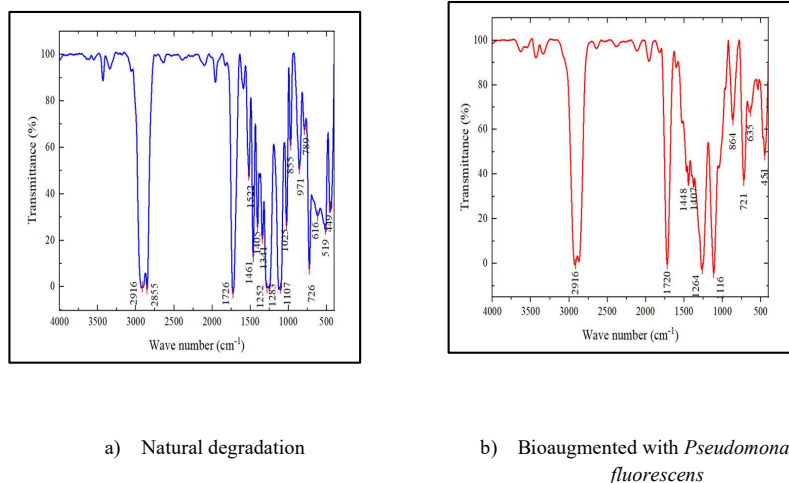


Fig. 9: FTIR spectrum of field sample 15F.

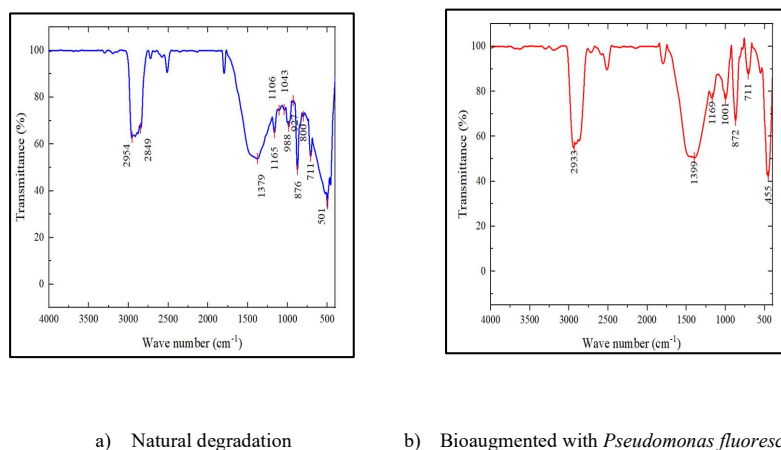


Fig. 10: FTIR spectrum of field sample 2F.

shifted predominantly. Sample 15F also showed similar changes in the fingerprint region, as depicted in Fig. 9, due to biodegradation of the polymeric material. The number of peaks also decreased.

3.3.2. FTIR Spectra of Polypropylene Samples

Sample 2F is made of a cloth-like nonwoven polypropylene material. Bags made from these materials are used as replacements for polyethylene carry bags after plastic bans in many locations. This material is also a synthetic polymer and is similarly nondegradable under natural conditions. In Fig. 10, after biodegradation, the peak at 2849 cm^{-1} corresponding to symmetric CH_2 stretching became less intense.

Fewer peaks are observed in the fingerprint region (1500–500 cm^{-1}) in the polypropylene samples. In Fig. 11, the overall spectrum shows a reduction in peak wavenumbers. Peaks in the low-wavenumber region at 663 and 440 cm^{-1}

were absent in the bioaugmented samples. The spectral peaks of samples 6F and 9F showed only a reduction in peak wavenumbers at the end of the bioaugmentation study, as shown in Figs. 12 and 13.

Sample 12F is comparatively harder than the other polymers considered in the present study, and its spectral variations are shown in Fig. 14. Similar to samples 13F and 15F, the number of prominent peaks in the fingerprint region decreased. A side peak at 2670 cm^{-1} in the sample subjected to natural degradation was attributed to the presence of impurities and can be disregarded.

3.3.3. FTIR Spectrum of LDPE Sample

The structure of LDPE contains a long chain of carbon atoms with complex side chains (Crawford 2002, Montazer et al. 2020). This results in the lower density of the polymeric material. Sample 3F is a transparent thin carry bag made of

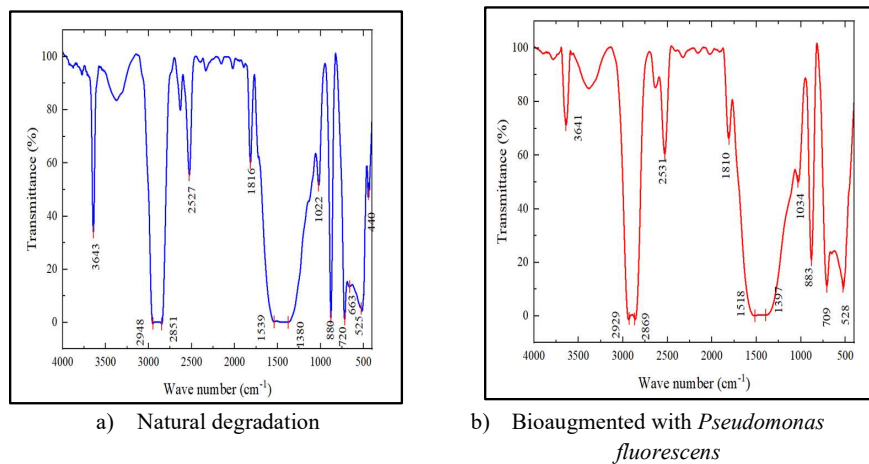


Fig. 11: FTIR spectrum of field sample 4F.

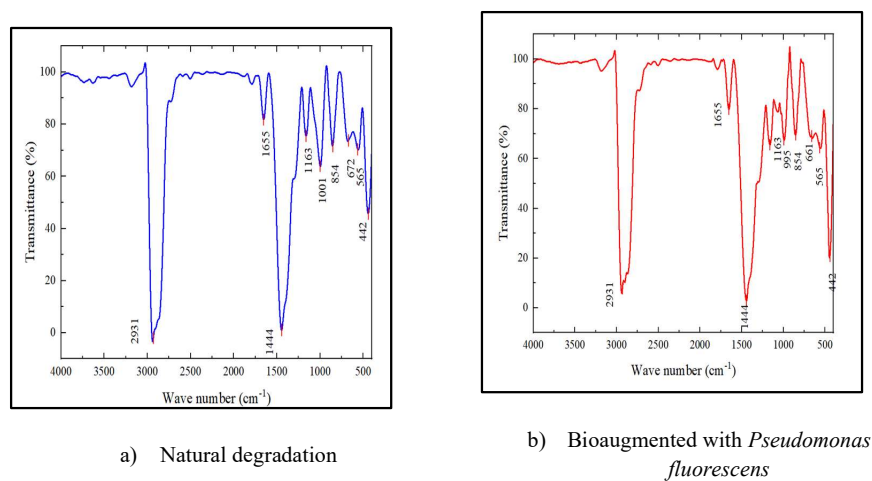


Fig. 12: FTIR spectrum of field sample 6F.

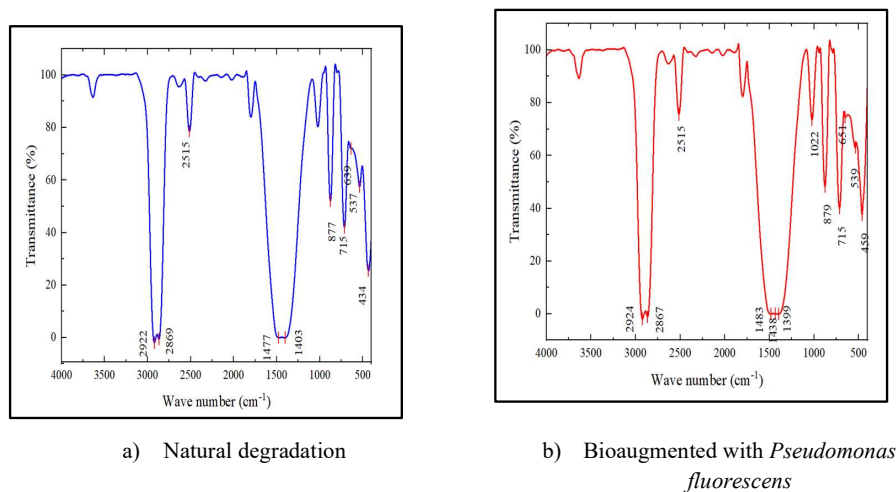


Fig. 13: FTIR spectrum of field sample 9F.

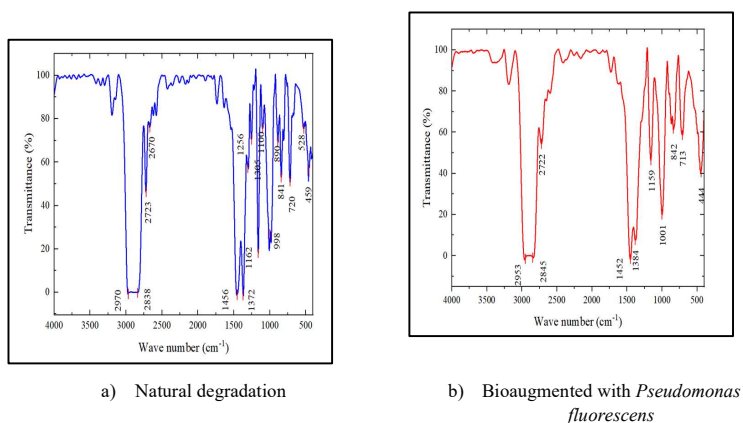


Fig. 14: FTIR spectrum of field sample 12F.

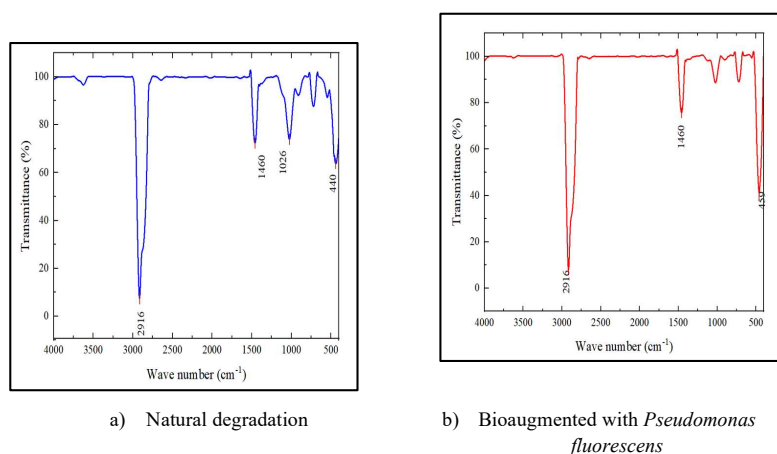


Fig. 15: FTIR spectrum of field sample 3F.

LDPE, and its spectral variations due to bioaugmentation are shown in Fig. 15. A peak at 1026 cm^{-1} corresponding to C–C skeletal vibrations became comparatively less intense in the bioaugmented sample.

3.3.4. FTIR Spectrum of LLDPE Sample

Compared with LDPE, the side chains of LLDPE are short, and its density is slightly greater than that of LDPE. Comparison of the FTIR spectra in Fig. 16 after

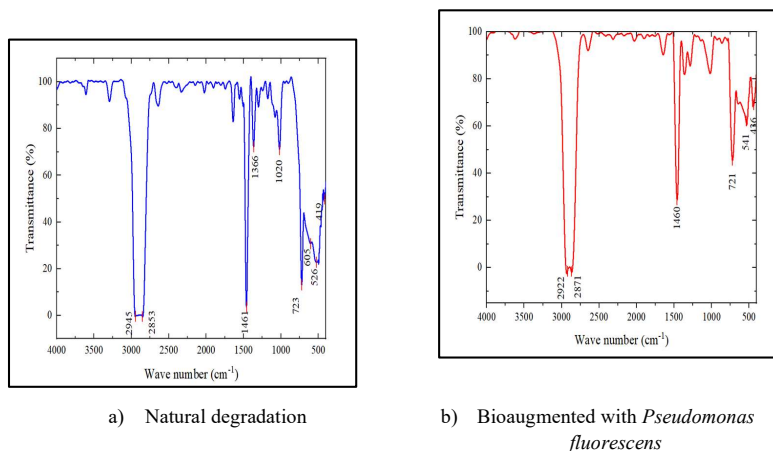


Fig. 16: FTIR spectrum of field sample 7F.

the degradation study showed that the peak due to CH₂ bending at 1366 cm⁻¹ and the peak at 1020 cm⁻¹ due to C–C skeletal vibrations became less intense in the bioaugmented sample.

3.3.5. FTIR Spectrum of Polylactic Acid Sample

Polylactic acid is a biodegradable polymer derived from

natural substances and contains lactic acid (Shah et al. 2008, Bahl et al. 2020, Chamas et al. 2020, Wei & Wierckx 2021). A considerable reduction in overall spectral peak intensity was observed in the bioaugmented sample compared with the sample subjected to natural degradation, as shown in Fig. 17, due to a reduction in film thickness. The sample is biodegradable under natural conditions.

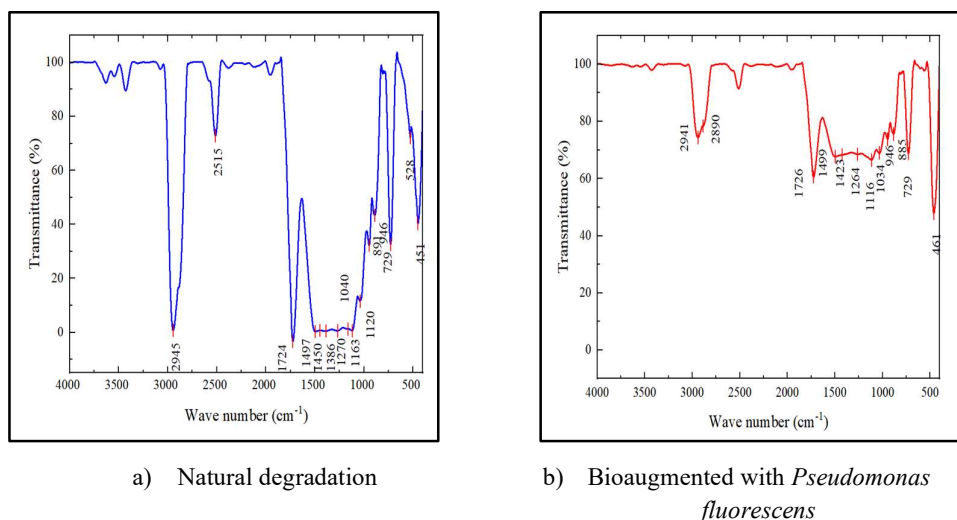


Fig. 17: FTIR spectrum of field sample 14F.

Table 3: Summary of FTIR analysis.

Sample ID	Major FTIR peak changes after bioaugmentation	Key spectral observation
1F	Shift of peaks to lower wavenumbers; double C–H peaks became blunt; new peak at ~981 cm ⁻¹	Evidence of HDPE oxidation and weakening of C–H bonds
2F	Reduced intensity of CH ₂ symmetric stretching peak at 2849 cm ⁻¹ ; fewer peaks in fingerprint region	Partial degradation of polypropylene
3F	Reduced intensity of C–C skeletal vibration peak at 1026 cm ⁻¹	Structural weakening of LDPE chains
4F	Overall reduction in peak wavenumbers; disappearance of weak bands at 663 and 440 cm ⁻¹	Loss of weaker functional groups due to biodegradation
5F	Weak band at 507 cm ⁻¹ disappeared in treated sample	Microbial activity altered HDPE-associated bands
6F	Only peak shifting toward lower wavenumbers observed	Limited but detectable molecular alterations
7F	CH ₂ bending and C–C skeletal vibration peaks became less intense	Reduction in polymer chain integrity
8F	Broad triple peak in 1500–1440 cm ⁻¹ narrowed to two peaks; reduced peak positions	Significant alteration in methyl-related bands
9F	Only reduction in peak wavenumbers observed	Minor spectral changes indicating slow degradation
10F	Disappearance of peak at 1526 cm ⁻¹	Decrease in functional group intensity
11F	Peaks at 1173 and 1208 cm ⁻¹ disappeared; reduced intensity in fingerprint region	Loss of C–O–C and C–O stretching bands
12F	Reduced number of peaks in fingerprint region; peak at 2670 cm ⁻¹ absent	Structural simplification after biodegradation
13F	C=C peaks at 1614 and 1528 cm ⁻¹ disappeared	Major changes in fingerprint region and aromatic structures
14F	Overall reduction in spectral intensity; degradation of peaks at 1386 and 528 cm ⁻¹	Natural biodegradability enhanced under bioaugmentation
15F	Disappearance of peaks in fingerprint region; reduced number of peaks	Overall weakening of polymeric material

According to Singla et al. (2012), the material has several closely spaced peaks in the fingerprint region. The peak at 1386 cm^{-1} , indicating CH_2 rocking, and another peak in the low-wavenumber region at 528 cm^{-1} were reduced in the bioaugmented sample.

The major changes in the FTIR spectral peaks of bioaugmented and naturally degraded samples are given in Table 3.

3.3.6. Bond Energy Change of Field Samples

The energy of an organic bond is directly proportional to its wavenumber. The higher the wavenumber, the stronger the bond. From the spectral analysis of samples subjected to natural degradation and bioaugmentation, each material exhibited variations across different peaks. Commercially utilized polymeric packaging materials contain various chemical bonds in addition to virgin polymeric bonds. To account for changes across the entire spectra of samples subjected to natural degradation and bioaugmentation with PF, the bond energy change of each spectrum was calculated using the peak wavenumbers above the threshold height and Eq. (2) (Smith 2011), and the results are shown in Fig. 18.

$$E = hc\nu \quad \dots(2)$$

where E is the energy change in joules, h is Planck's constant ($6.626 \times 10^{-34}\text{ J s}$), c is the speed of light ($3 \times 10^8\text{ m s}^{-1}$), and ν is the wavenumber (m^{-1}).

The change in bond energy for all bioaugmented samples was lower than that for samples subjected to natural degradation. This indicates the efficacy of the technique under field conditions. Sample 8F had the highest bond energy due to the presence of a greater number of methyl-related bands in the high-frequency region. The largest

decrease in bond energy relative to the natural degradation process was observed in sample 15F, at approximately 45%. This sample was made of HDPE. The reduction in bond energy change relative to the natural degradation process ranged from 0.85–45%, 0.15–44%, 17%, 24%, and 9% for HDPE, PP, LDPE, LLDPE, and PLA samples, respectively. Samples made of the same polymeric material showed different degrees of decrease in bond energy. This is attributed to the fact that each sample was manufactured differently for various commercial wrapping purposes, with different thicknesses, color prints, and surface coatings.

The smaller decrease in the biodegradable PLA sample is due to the sample having undergone considerable changes during natural degradation, with relatively fewer variations observed under bioaugmented conditions. It should also be noted that the bond energy change of biodegradable plastic is greater than that of other synthetic polymers. Hence, the change in bond energy does not indicate whether a material is degradable. It only compares changes and loss of bond strength in a material before and after degradation. As the weight-loss technique accounts only for physical changes, only 9 samples showed greater degradation than under the natural process. In FTIR analysis, internal changes at the molecular level were investigated. Hence, FTIR provides a better representation of the bioremediation process. Because the FTIR spectra of samples recovered after 6 months were compared in Fig. 18, it may not be meaningful to include error bars.

3.4. CHNS Analysis

Because the soil obtained from the site is homogeneous, CHNS analysis was performed directly on the oven-dried

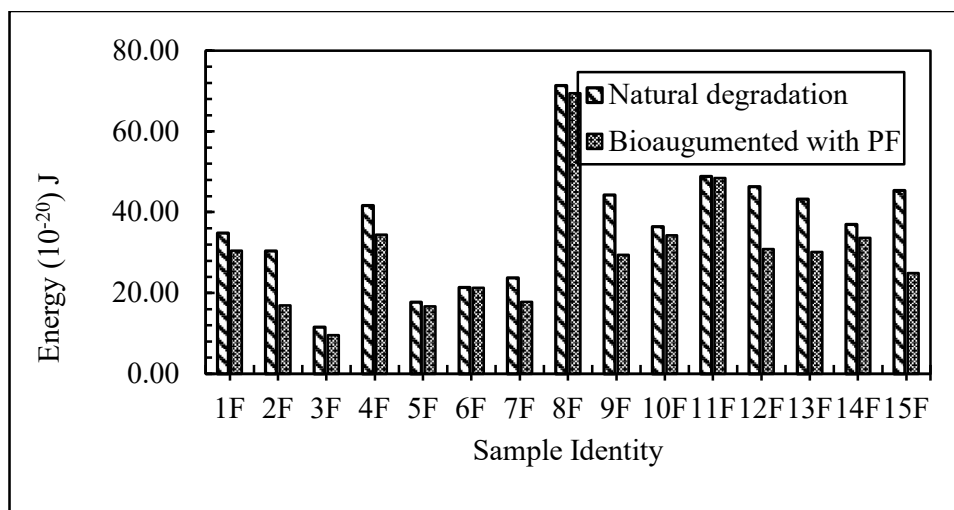


Fig. 18: Bond energy change of field samples from the FTIR spectral peaks.

Table 4: CHNS analysis of field samples.

S. No.	Soil Samples	N (%)	C (%)	H (%)	S (%)
1	Before treatment	0.49	0	0.73	0.42
2	Control pit after 6 months	0.56	0	0.81	0.23
3	PF-treated pit after 6 months	0.43	1.92	0.64	0.15

samples. CHNS results for samples collected before the study, from the control pit, and from the PF-bioaugmented pit after 6 months are given in Table 4. Initially, the soil collected at a depth of 0.7 m before commencement of the study contained no traces of carbon. No carbon was observed in the control pit even after 6 months of the study. The carbon content of the pit bioaugmented with PF was 1.92%. This can be attributed to biomass deposited by microbial activity. The presence of biomass resulting from biodegradation was also reported by Ardisson et al. (2014).

In the field study, the soil had no carbon content before the commencement of the study. Soil samples collected after 6 months of bioaugmentation were tested for carbon after the buried plastic films were removed and showed increased carbon levels attributable to biomass deposited during bioaugmentation. Due to microbial activity in the bioaugmented pit, the C/N ratio of the sample increased to 4.5%, which was initially absent.

4. PROPOSAL OF BIOLAYER IN LANDFILL LINER SYSTEM

Fig. 19 shows a schematic diagram of the biolayer proposed for the landfill liner system. In an engineered landfill, unrecoverable waste is disposed of daily, forming a daily cell. The daily cell is covered with a soil-like fraction present in the landfill, referred to as daily cover (Warmadewanthi et al. 2021). This daily cover can be bioaugmented with potential plastic-degrading microbes, forming a biolayer. In the present study, bioaugmentation with *Pseudomonas fluorescens* was

found to be effective in the bioremediation of plastic waste. Many such plastic-degrading microbes classified at biosafety level 1 must be studied under landfill conditions. The technique can then be implemented extensively in engineered landfills on a large scale. However, challenges associated with large-scale implementation, including environmental variability, microbial survivability, long-term monitoring, and operational feasibility, must be further studied.

5. CONCLUSIONS

The study involved a preliminary investigation of a field-scale bioaugmentation technique using FTIR analysis. The following conclusions were drawn from the present study.

- Bioaugmentation using *Pseudomonas fluorescens* enhanced the degradation of polypropylene material by a maximum of 4.3 times and that of polyethylene-based material by 9.3 times in terms of percentage weight loss.
- It was also observed that the FTIR study provides a better representation of the bioremediation process than the field weight-loss study. FTIR spectra of bioaugmented field samples indicate the absence of C–H peaks and a shift of major peaks toward lower wavenumbers.
- For the same type of base polymer, degradation rates vary among different samples of the same classification in both the weight-loss study and FTIR bond-energy calculations.
- After field bioaugmentation using *Pseudomonas fluorescens*, the carbon level and C/N ratio of the soil increased to 4.5%, which was absent before treatment.

The success of the bioaugmentation process in the field under open atmospheric conditions indicates that this technique can be used to design a biolayer in an engineered landfill system. The field-scale bioaugmentation technique can be further investigated using CO₂ evolution studies,

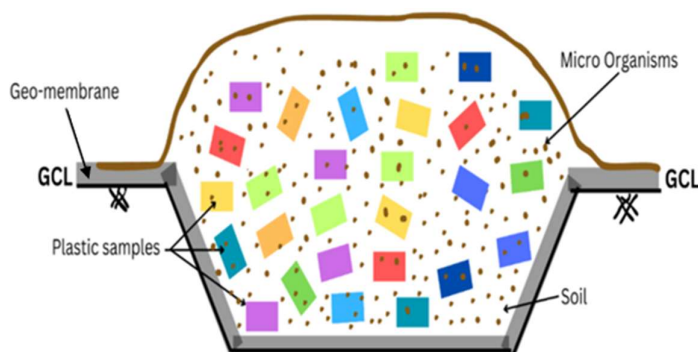


Fig. 19: Proposal of a biolayer in a landfill liner system.

thermal characterization techniques, molecular-weight measurement, and scanning electron microscopy (SEM) analysis.

6. ACKNOWLEDGEMENT

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