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Original Research Article

Bioremediation of Low-Density Polyethylene (PE) by Bacteria

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Abstract: The necessity of having eco-friendly disposal policies designed for biodegradation of synthetic plastics is of utmost importance and needs attention in the present scenario, considering the serious impact of widely used packaging materials such as low-density polyethylene (PE) on the environment. Our study aimed to explore the degradation of PE by three different bacterial species. *Klebsiella*, *Escherichia coli*, and Pseudomonas aeruginos. The extent of biodegradation was evaluated by weight loss of the PE samples. The weight of the plastic sample before degradation was 1 gr and after the degradation, the sample weight of polyethylene (PE) was (0.64, ,.0.686,,0.923,) gr respectively. Scanning electron microscope (SEM) analysis revealed that morphological changes in the surface of PE were observed while FTIR images showed functional groups changes after 90 days incubation period. The formation of biofilms on the surface of polyethylene and the adhesion capabilities of bacteria are seen as the first step in the biodegradation process. These results. can colonize, modify and utilize PE as the sole carbon source, water. This manuscript also paves the way for future studies on biodegradation to solve the global problem.

Keywords: Plastic, Biodegradation, Bacteria, Polyethylene, Problem.

1-INTRODUCTION

Plastic is defined as a polymer of organic components such as cellulose and inorganic components such as carbon, hydrogen, nitrogen, sulfur, plastic is slow to decompose, strong, durable and lightweight made from petroleum derivatives. Plastic has been with use since the beginning of the twentieth century. It is considered an affordable, versatile and durable material. Plastic materials have gained widespread use due to their use in many industries including clothing, food, transportation, construction, medical industries, leather industries and others. Consequently, the global production of plastics is still on the rise. Over the past ten years, global production of plastics has increased by 97 million tons to reach 367 million tons (Sridharan, 2021).

Plastic plays an important role in many “short live” applications like packaging and these represent the main part of plastic wastes. Because of its propagation in our environment, several communities are now more sensitive to the impact of discarded plastic on the environment, including deleterious effects on wildlife and on theaesthetic qualities of cities and forest (Abbas and Abdulhay, 2024). **Bioremediation**: is the technique of using microorganism and plants for environmental cleanup of both organic and inorganic xenobiotics (Abatenh *et al*., 2017; Mohammed *et al*., 2019).

Since bioremediation eliminates, degrades, detoxifies, and immobilizes dangerous wastes and pollutants, microorganisms are crucial. Understanding the whole range of physiological, microbiological, ecological, biochemical, and molecular processes involved in pollutant transformation's essential for successful bioremediation employing microorganism (Nayak and Solanki, 2022; Qassim and Hind, 2024).

Ex situ or in situ bioremediation can be utilized, depending on a variety of criteria, such as cost, pollutant kinds, and concentration. Bioremediation is cheaper than incineration, and certain contaminants may be handled on site, minimizing exposure hazards for clean-up workers or broader exposure from transportation accidents (Qassim *et al*., 2023). Bioremediation carried out in situ, including bioventing, bespangling, and bioaugmentation, decontaminates without removing soil

from the site while ex situ treatments (such as land farming, biopiling, composting, bioreactors, and electrodialysis) treat the soil that is unearthed at the location (Parween, *et al*., 2018; Qassim and Mohammed, 2019).

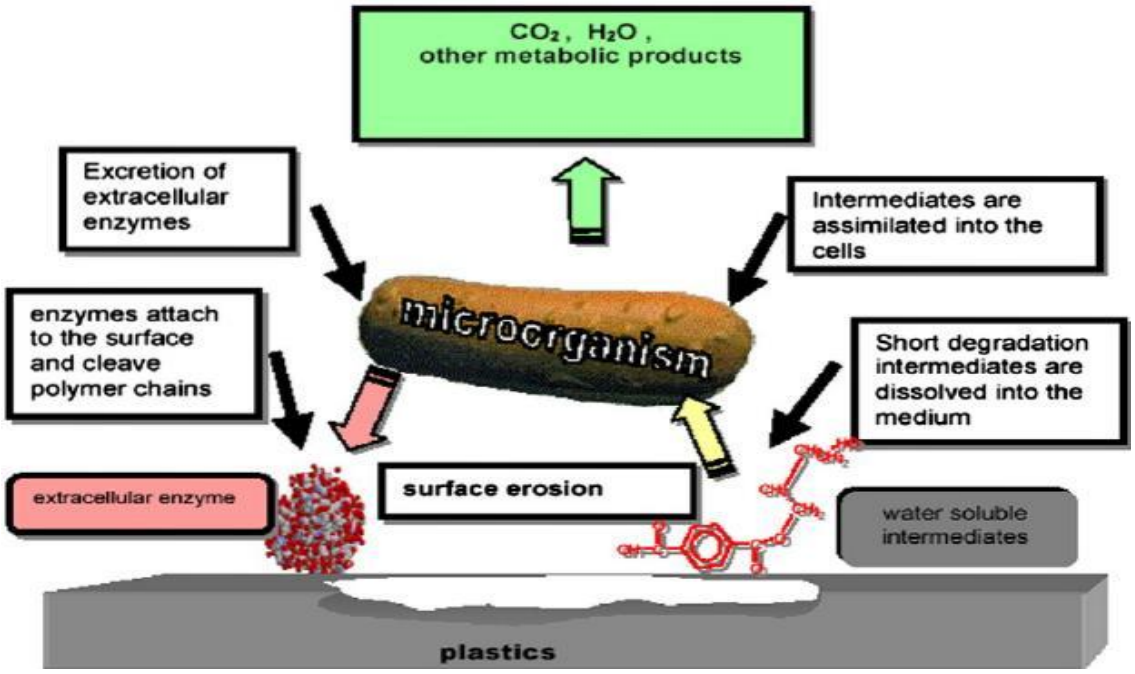


Figure 1: Bioremediation of Plastics (Elahi, Amina *et al*., 2021)

MATERIAL AND METHODS

2.1 Collection of samples

Samples of soil contaminated with plastic waste were collected in several different areas in Diwaniyah Governorate according to Table 1. The method of (Amal *et al.*, 2018) was followed for the purpose of collecting soil samples. The surface soil was removed to a depth of (5-15cm). 100 g of soil was taken using a spoon and placed in clean plastic bags. It was transferred to the laboratory and stored in the refrigerator at a temperature of 4 °C until laboratory analyses were conducted on it**.**

Table 1: Four different soil samples were collected from different locations within the city of Diwaniyah

NO	Site of samples
1	Alfrat
2	Algeria
3	Arabism
4	Housing District

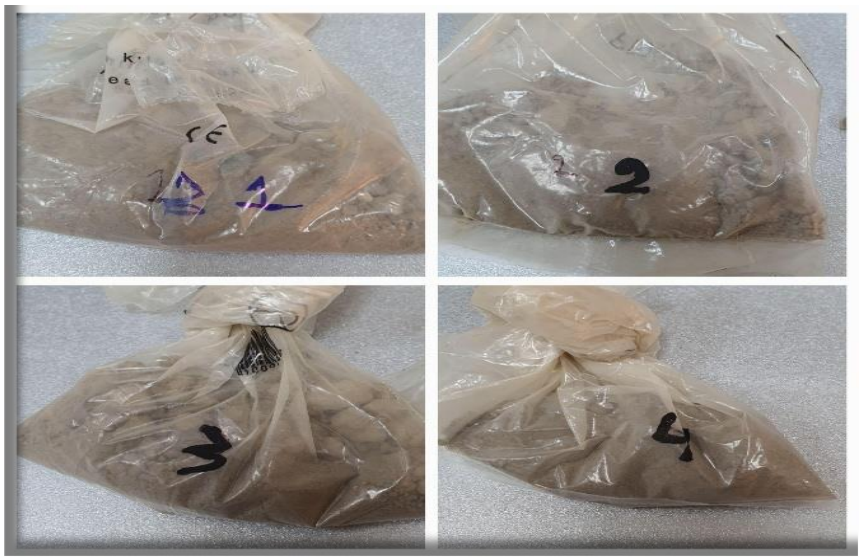


Figure 2: Contaminated soil sample

2.2: Isolation and identification of bacteria from contaminated soil

We take 1 gm of soil contaminated with microorganisms using a loop, and the petri dishes are numbered according to the number of soil samples, the dishes are planted with soil contaminated with agricultural media, Macconkey ager, Blood ager, and Nutrient ager.

Then incubate for 24 hours at 37℃ (Sarah, 2017) Three types of bacteria were identified based on morphological and morphological characteristics and the change in the color of the medium. *Klebsiella, Escherichia coli, Pseudomonas aeruginos*.

2.4 Pretreatment of polyethylene.

The polyethylene was cut into small and fine pieces washed with ethanol chemical to remove soil and dirt then, washed it with distilled water and dried (Khlaif and Abdulhay, 2023; Qassim *et al*., 2021).

2.5 polyethylene inoculation with bacteria

Nutrient basal media (NBM) was disinfected and transferred into the flasks. PE (1g) materials were cut into small parts (5 cm×2 cm), sterilized with 70% ethanol for 30 min and washed with sterile distilled water for 20 min. Materials of PE were placed in flasks containing the NBM (100ml) then they were inoculated with 1ml of activated isolated by *Pseudomonas, Klebsiella* and *Escherichia coli.* The flasks control and flask contain plastic were incubated in the incubator on a rotary shaker at 37°C. Results were observed after Three month (Kyaw *et al*., 2012).

Figure 3: Polyethylene inoculation with bacteria

2.6 Weight loss measurement of PE after bacterial Growth

PE were recovered after three months of incubation of culture, washed with methanol and finally washed with distilled water. The weight loss was measured according to the following equation (Gauri; 2016).

$$weight\ loos\ = \frac{(Initial\ weight - final\ weight \times 100\%)}{Initial\ weight}$$

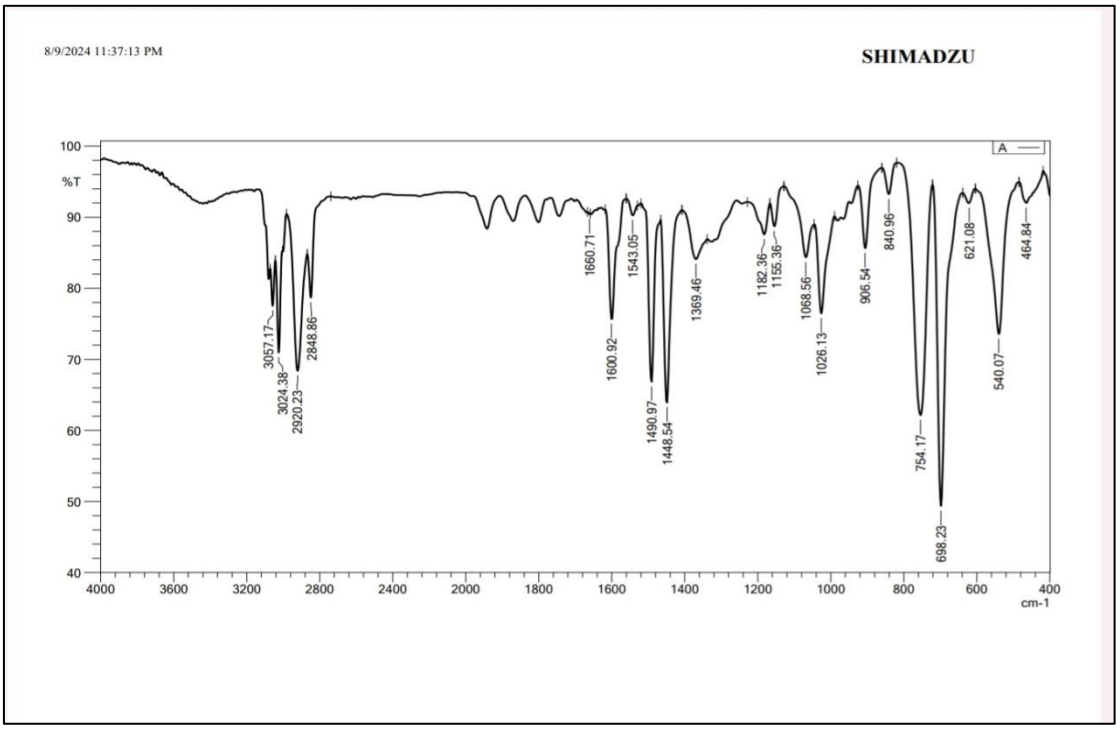
Table 2: Polyethylene weight loss after three months with isolated bacteria *Klebsiella, Escherichia coli* and *Pseudomonas*

Weight of polyethylene (g)			
Isolated bacteria	**Initial weight**	**Final weight**	**Percentage%**
Pseudomonas	1	0.36	0.64%
Klebsiella	1	0.314	0.686%
Escherichia coli	1	0.077	0.923

2_7. Fourier transform infrared spectroscopy (FT-IR) analysis

FTIR spectrum of PE (without bacterial inoculation) treatments had peaks of C–H stretching and C–H blending (in the range of 2800–3000, 1400–1550, and 650–750 cm−1) which indicates functional groups of alkanes in the PE backbone (Fig 4a). The control samples of PE treatment exhibited the range of 3200–3400 cm−1which relates to the peak for OH group, whereas the samples of inoculated bacterial exhibited a reduction in the peak intensity in the same range of 3200–3400 cm−1. The bacteria treatment generated new functional groups in the nitro compound (–N=O stretch) range of 1550–1500 cm−1 and the alcohol, carboxylic

acid, esters, ethers compound (–C–O stretch) range of 1320–1000 cm−1 which was not observed in the control treatment. When the FTIR spectrum of cut polyethylene treated by *Pseudomonas* bacteria exhibits significant changes, surface interaction and limited degradation can be deduced. The broad peak at 3452.58 cm^{-1} corresponds to O-H stretching, indicating oxidation and interaction with water. The peak at 3159.40 cm^{-1} is N-H stretching, possibly due to possible amine group interaction. The bands at 3080.32 cm^{-1}, 2918.30 cm^{-1}, and 2845.00 cm^{-1} all represent C-H stretching, which shows the carbon backbone has remained intact. A peak at 1600.92 cm^{-1} is due to C=C stretching and probably comes from unsaturated degradation by-products. Peaks at 1454.33 cm^{-1} and 1328.95 cm^{-1} show C-H bending vibrations, indicating small changes in the structure. C-O stretching peaks at 1166.50 cm^{-1} and 1108.44 cm^{-1} are due to polymer oxidation, while the peaks at 1026.06 cm^{-1} and 904.61 cm^{-1} represent C-H bending vibrations. Finally, out-of-plane bending peaks at 750.31 cm^{-1} and 695.50 cm^{-1} confirm that some PE structure was maintained. The spectrum indicates that the surface underwent oxidative changes while the origin structure was kept intact (Khandare *et al*., 2021).



Figuer 4a: FTIR spectra of polyethylene (PE) before consumption by bacteria

The significant changes in the FTIR spectrum of polyethylene treated with bacteria indicate that the bacteria are putting compounds into and altering their surface. A broad peak at 3456.44 cm^{-1} is attributed to O-H stretching: oxidative changes occur. However, the peak at 3159.40 cm^{-1} corresponds to N-H stretching, indicating that interaction with bacterial proteins affects our results. The bands at 3080.32 cm^{-1}, 2922.16 cm^{-1}, and 2846.93 cm^{-1} represent C-H stretching vibrations, showing again that the structure of aliphatic hydrocarbons in this polymer is largely retained. However, the spectrum peaks at 1600.92 cm^{-1}, associated with C=C stretching, so it may come from the formation of unsaturated compounds or degradation products. Other peaks at 1553.34 cm^{-1} and 1450.47 cm^{-1} indicate C-H bending vibrations, therefore maintaining the polymer structure partially but with slight changes. The peaks at 1328.95 cm^{-1} and 1180.44 cm^{-1} come from C-O stretching, suggesting that the bacterial action has brought about an oxidative transformation in polymer chains. Further changes are detected in the peaks at 1028.06 cm^{-1} and 904.61 cm^{-1}, where C-H bending vibrations signify changes in polymer structure. Finally, the peaks at 754.17 cm^{-1} and 696.30 cm^{-1} indicate out-of-plane bending vibrations: this data suggests the presence of a structure like aromatic-like or partial degradation. Consequently, although surface degradation and oxidative changes show up in the spectrum, the core structure of polyethylene is largely preserved (Shraddha Awasthi *et al*., 2017).

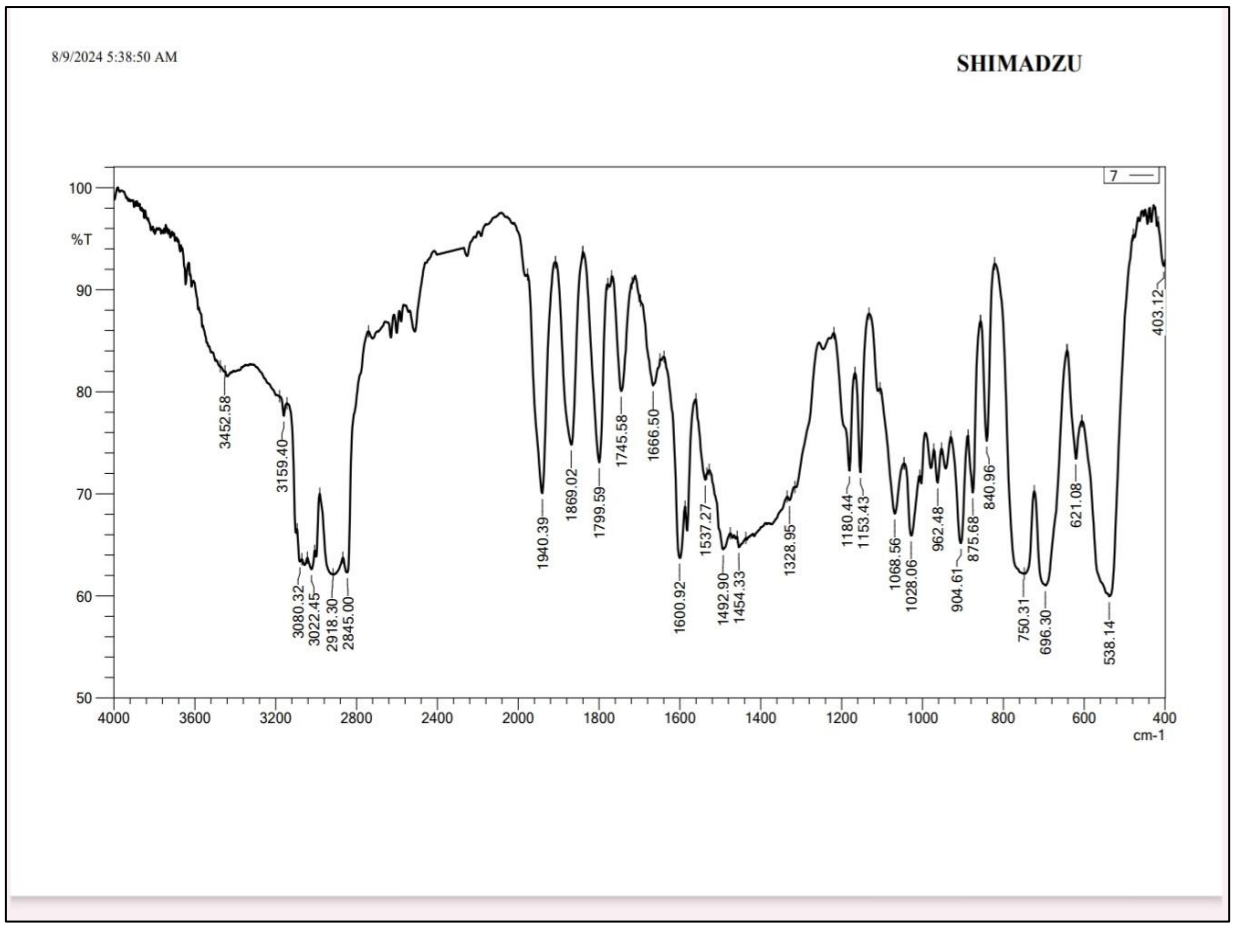
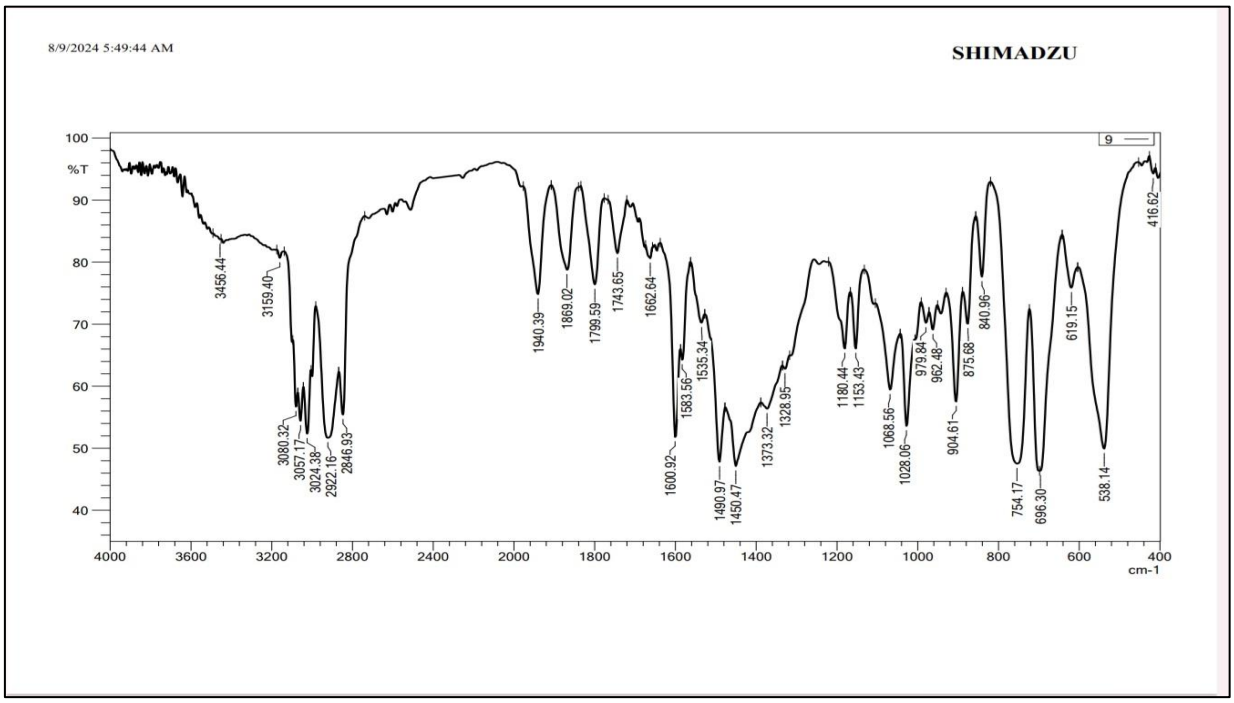


Figure 4b: FTIR spectra of polyethylene (PE) by bacterial *Pseudomonas*



Figuer 4c: FTIR spectra of polyethylene (PE) by bacterial *Klebsiella*

The FTIR spectrum of the cut polyethylene sample treated with Escherichia coli bacteria showed signs of oxidation and surface degradation. The broad band at 3439.08 cm^{-1} is O-H stretching, whereas in the area around 3024.38 cm^{-1}, 2924.09 cm^{-1}, and 2848.86 cm^{-1} (marked N–H and C–H stretching), polymer

molecules interact differently with bacterial proteins than do their chain segments. Another peak at 1741.72 cm^{-1} indicates carbonyl formation to cause oxidation, while 1600.92 cm^{-1} and 1654.92 cm^{-1} represent C=C stretching out of unsaturated byproducts. There are minor structural changes in the 1543.05 cm^{-1}, 1450.47 cm^{-1}, and 1371.39 cm^{-1} regions of bending vibration. C-o stretching may occur at 1153.43 cm^{-1} and 1086.56 cm^{-1}, showing oxidation to the chain. 906.54 cm^{-1}, 754.17 cm^{-1}, and 698.23 cm^{-1} are bands that reveal some of the structure remains after deformation. It can be concluded from the overall picture that the FTIR spectrum of the sample may be deemed to show surface-level oxidative degradation with retention of core structure (Mohammed *et al*., 2019).

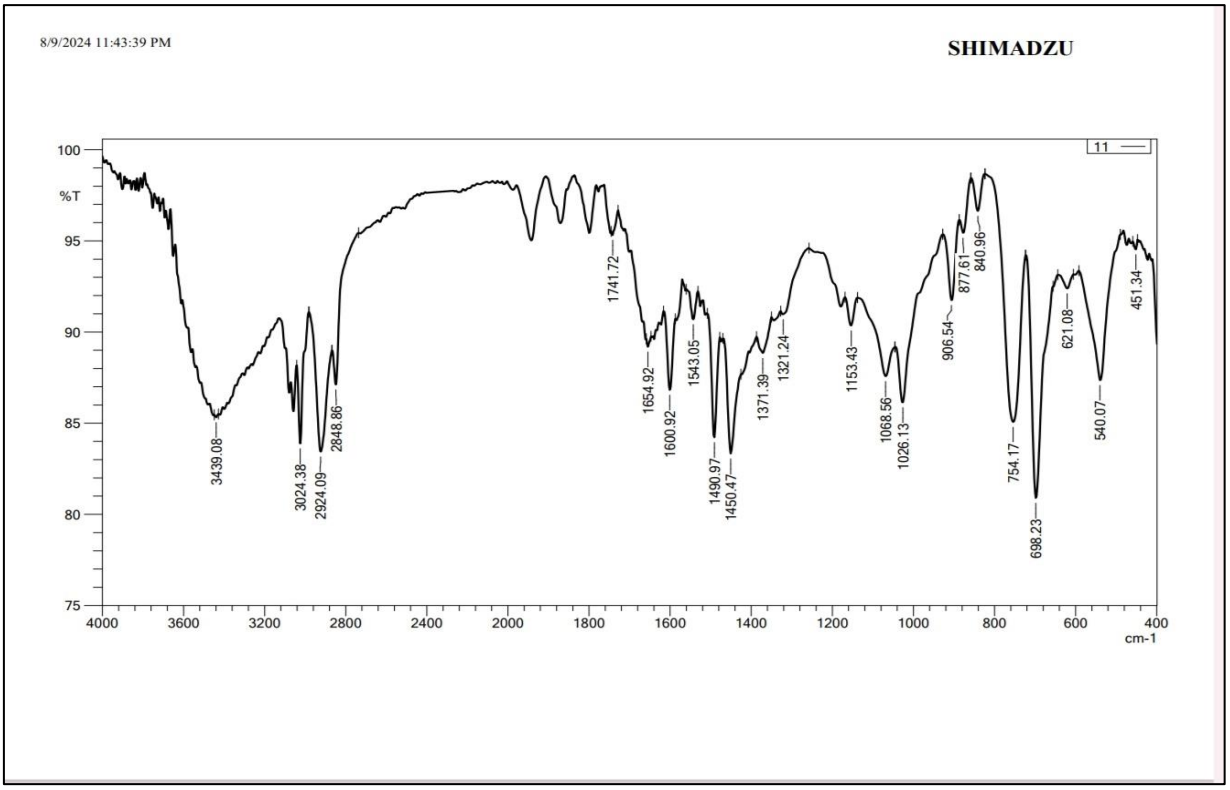
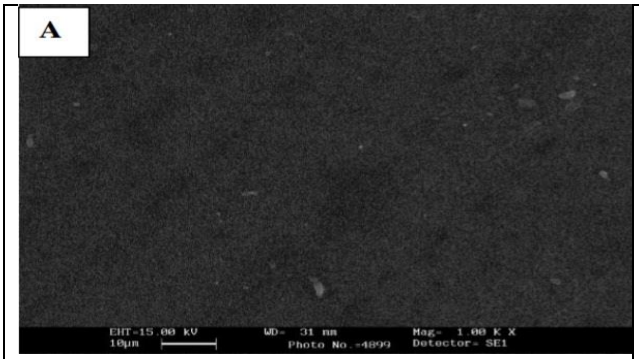


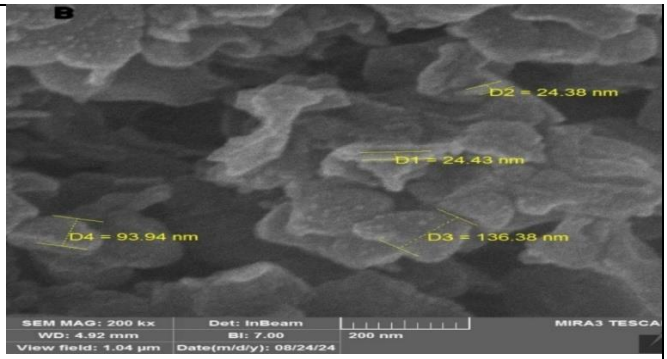
Figure 4d: FTIR spectra of polyethylene (PE) by bacterial *Escherichia coli*

2_8 Bacterial growth scanning by SEM

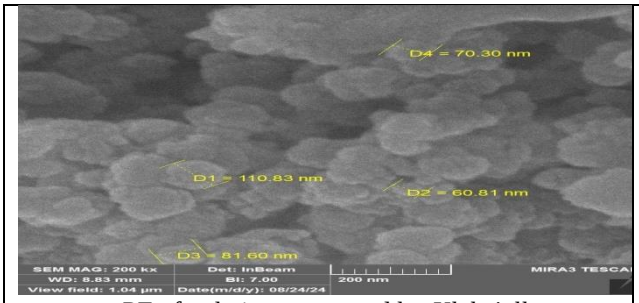
All types of isolated bacteria showed the formation of biofilms on the surface of the plastic samples (PE) but in different percentages of formation and this showed by SEM. The result of SEM showedThe extent of biodegradation was examined by detecting changes in surface morphology through scanning electron microscopy analysis. Several changes in the physical appearance of the Pseudomonas-treated PE film were observed after 90 days of incubation. However, the untreated PE sample showed a smooth surface (Fig 5). The microbially treated sample confirms the surface deformation by 2showing various cracks and irregularities formed as a result of microbial activity The change displayed in the surface (Shraddha Awasthi *et al*., 2017), Cai *et al*., 2023, Crystal Thew *et al*., 2023, Gilani *et al*., 2023)



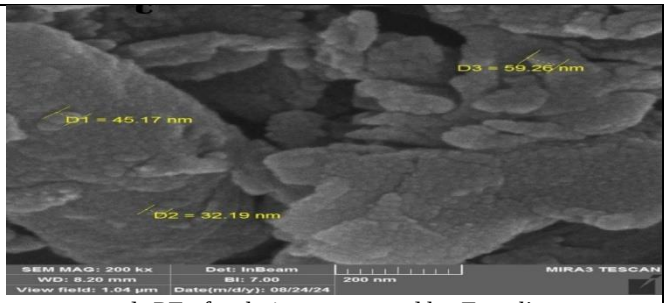
a-Polyethylene (PE) before consumed



b- PE after consumed by *Pseudomonas*



c- PE after being consumed by *Klebsiella*

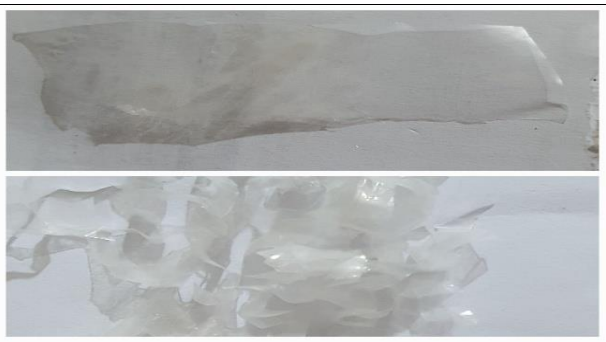


d- PE after being consumed by E. *coli*

Figure 5: Scanning electron microscopy shows polyethylene before (a) and after consumption by isolated bacteria (b, c and d) by *Pseudomonas, Klebsiella, and Escherichia coli*

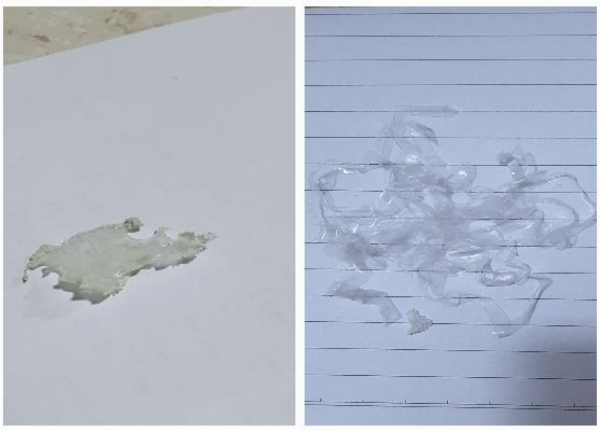
2_10 Physiological changes of bacteria isolated from plastic

We noticed a difference in the plastic Before and after treatment with the isolated bacteria. There is a difference in the shape, size and thickness of the plastic and this ensure the degradation of polyethylene by bacteria (Abbas and Abdulhay, 2024) (Figure 6).



a- Polyethylene (PE) before consume

b- PE after consumed by *Pseudomonas*



c- PE after being consumed by *Klebsiella*

d- PE after being consumed by *E. coli*

Figure 6: Physiological changes show polyethylene before (a) and after consumption by isolated bacteria (b, c, and d) by Pseudomonas, Klebsiella, and Escherichia coli

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