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Biodegradation of UV light treated plastic waste using local bacterial isolates

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ABSTRACT

Environmental threats from the accumulation of plastic trash are getting worse. It is robust, lightweight, corrosion-resistant, affordable, and durable. Microorganisms play a significant role in protecting our environment by degrading plastic wastes that are harmful either naturally or by chemical modification. The current study aims to investigate the biodegradation of synthetic polyethylene through the utilization of a laboratory bioreactor. Various types of additives were introduced to the soil samples before subjecting them to a 30-day UV treatment. The degradation of polyethylene was shown through a reduction in weight following a 24-week incubation period with certain bacterial strains. Experimental findings have revealed that models subjected to UV radiation exhibit the highest degree of vulnerability and degradation. Approximately 52% of polyethylene (PE) films underwent degradation when exposed to soil enhanced with peat moss. In contrast, only 40% and 45% of PE films were destroyed when subjected to garden soil that was untreated and treated with UV radiation, respectively. In contrast, the addition of husk resulted in a 48% to 53% reduction in weight for PE films that were buried for the same duration of the experiment. The highest level of effectiveness was achieved by the disintegration of the plastic material that was introduced into the soil along with organic fertilizers, resulting in a value of 56.60%. The weight loss outcomes have been substantiated by the utilization of the Atomic Force Electron Microscope (AFM) images, which exhibited the highest magnitude in the experimental model using soil supplemented with fertilizers.

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INTRODUCTION

Biodegradable plastics are those that biodegrade through the process of biodegradation without producing noticeable, hazardous, or visible residues of carbon dioxide, water, inorganic chemicals, and biomass. Biodegradable bio-based plastics have been around for many years (Al-dahan *et al.,* 2022). However, due to their high price, they have never fully replaced traditional non-biodegradable plastics and are widely available. Polyethylene, PE which is obtained by polymerization of ethylene gas.

$$CH_2{=}CH_2 + R.\ \text{Ethen initiator} \longrightarrow CH_2\text{-}CH_2\text{-}R$$

$$CH_2{=}CH_2 + CH_2\text{-}CH_2\text{-}R \longrightarrow CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}R$$

The process continues to form polyethylene $[\text{-}CH_2\text{-}CH_2\text{-}]n$

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Classified into several different categories - based mostly on density and branching

E.g. LDPE (low density; 0.91-0.94 g/cm3), HDPE (high density; 0.95-0.97 g/cm^3 (Bonhomme *et al.,* 2003). Because of the same properties that make plastics so beneficial to humans, they also present a new environmental risk (Thompson *et al.,* 2009). In the globe, only 18% of plastic waste is recycled, while 24% is burned. The remaining 58% are buried in landfills or discharged into the environment, where they accumulate and linger for millennia. These polymers are now responsible for a significant portion (19%) of all municipal solid waste in the United States because of how quickly they are disposed of in landfills (Advancing Sustainable Materials Management:2014 Fact Sheet, 2016).). It is projected that more than 12,000 metric tons (Mt) of plastic waste will be present worldwide in landfills and the environment by 2050. According to the description above, degradation is any physical or chemical change in a substance brought on by an external element, such as light, heat, moisture, wind, chemical conditions, or biological activity.

Thus, biodegradation is considered a subcategory of degradation. As a result of the striking resemblance to the conditions in which plastics are used or disposed of, field tests that involve the routine burial of plastic samples in soil have frequently been carried out to ascertain how rapidly these materials break down. Active biodegradation can take place in different types of soil depending on the properties of the plastics that are being broken down. Because the bacteria that cause the deterioration are all different from one another and have different optimal conditions for flourishing in soil (Howard, 2002).In the scientific literature, there is a significant disagreement regarding the biodegradation of synthetic polymers between those who contend those who claim that the ability to break down synthetic polymers to extremely tiny chain lengths is necessary for microbiological attack and those who assert that synthetic polymers can also be digested at relatively large molecular weights. Experimental evidence suggests that the capacity to degrade synthetic polymers into shorter chain lengths plays a crucial role in facilitating microbiological degradation (Gu,2003). The degradation of polymers can be attributed to one or more processes, such as microbiological deterioration, wherein fungi and bacteria consume the substance. The degradation mechanisms of polymers may vary depending on the specific environmental conditions and intended use.

In the context of the natural environment, it would be very useful to conduct experiments aimed at evaluating the biodegradation potential of plastic waste when exposed to certain physical agents (Greene, 2007). In the literature, the term degradation may include depolymerization, chemical modification, alteration of physical properties, overall mass loss by any mechanisms, or complete mineralization to CO_2 and H_2O (Lambert & Wagner, 2016). This study was conducted to investigate the biodegradation of polyethylene (PE) plastic bags under artificial soil conditions.

MATERIAL AND METHODS

Preparation of Polyethylene samples

The Polyethylene bags collected from the sampling site (local markets) were cut into beads of equal sizes (12 x 10 x 1 mm) and used for degradation studies.

Pre-treatment of the samples

Pre-weighed strips of the sample were subjected to UV Irradiation in the UV chamber (365nm exposure) for as long as four weeks to achieve an average molecular weight (MW) decrease, (Figure 1).

Aerobic composting bioreactors

The 1-kg working volume of the aerobic reactor was housed in a plastic box that measured

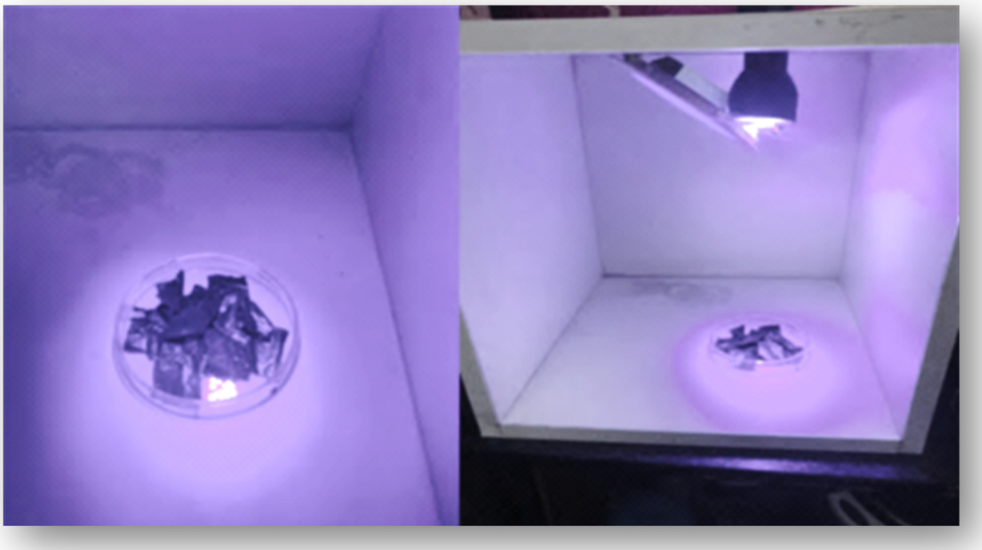

Fig. 1. Exposures of plastic strips in the chamber of UV light source.

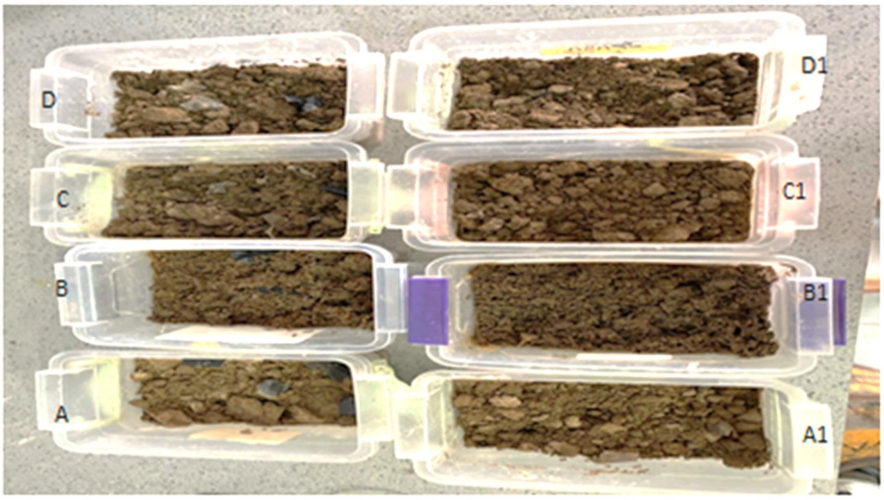

Fig. 2. Aerobic lab composting bioreactor of UV exposure PE; (A, A1) soil without additives before and after degradation;(B, B1) soil with the addition of rise husk before and after degradation;(C, C1) soil with the addition of compost before and after degradation; (D, D1) soil with the addition of organic fertilizer before and after degradation

20 cm in length, 15 cm in width, and 10 cm in height. (Figure 2). The process of adding plastic pieces (10 pieces) to several previously prepared reactors by preparing them to be of equal dimensions and sizes to conduct the fermentation experiment as follows:

A - The first basin contains soil in addition to the plastic pieces

B- Soil with rice husks added.

C- Soil to which peat moss is added

D- Soil to which animal organic fertilizer is added

(All additives were (25%). Soil moisture levels were consistently measured to be between 65 and 50 percent throughout the duration of the study.

Screening for Polyethylene Degradation:

Strips recovered after the composting period of up to 12 weeks. Using sterile forceps, each film was taken out and put into a beaker of sterile water, where it was swirled for 60 minutes at room temperature. After being aseptically transferred, the films were kept in a 70% (vol/vol) ethanol solution for 30 minutes. Then, each film was put into a fresh, sterile petri dish (Gerhardt et al., 1981). After allowing the dishes containing the films to acclimate to room temperature and dry overnight at 45 to 50°C, they were weighed to an accuracy of +0.1 mg, allowing the weight of the film to be calculated. Utilizing the formula, the percentage of residual film weight will be determined;

$$Weight\ loss\% = \frac{Initial\ film\ weight - Final\ film\ weight}{original\ weight\ of\ the\ sample}\ x\ 100$$

Inoculum

In a prior investigation, bacteria from the heat-resistance section of composted food wastes were identified as belonging to the thermophilic Bacillus genus was cultured in a 250 ml culture dish with 150 ml of the following media (weighted in grams per liter): Five grams of peptone, one gram of glucose, five grams of sodium chloride, and three grams of meat extract were combined in a pH 6.8 rotary shaker and shaken for three days at 200 rpm and 55 °C to create an inoculum that was then added to the soil.

Screening of microorganisms

Following the addition of 99 ml of sterile, distilled water to one gram of soil, the sample was serially diluted. The nutrient agar was inoculated with the diluted samples. plates of Sabouraud Dextrose Agar (SDA) were incubated at 32° C for 24 hours. Isolates of bacteria, fungi, and yeast were documented.

AFM Analysis of PE Films

Variations in the substance were documented through photography, microscopy (AFM), and visual inspection (Rydz *et al.*, 2015).

RESULTS AND DISCUSSION

UV Pretreatment

Films that have been subjected to ultraviolet radiation (Figure 2) end up becoming brittle and lose their elastic properties. They are anticipated to continue to shrink in length until they reach the “bioactive” stage, which is something that cannot be predicted with absolute certainty at this time. It is envisaged that the size of these molecules would decrease to the point where they can be taken up by bacteria. Even the least reactive polyolefin, PE, deteriorates with time. The absence of UV-visible chromophores and the use of C-C single bonds in the construction of PE’s backbone chains make it resistant to hydrolysis and photo-oxidative destruction. Chromophores can be formed by fortuitous impurities or structural flaws (Grassie & Scott, 1988), that appear in PE during production or later during weathering. A minor number of unsaturated (C═C) bonds (usually vinyl groups in HDPE and vinylidenes in LDPE) may also be present in PE’s main chain or chain ends. Extremely unstable hydroperoxides form at these sites when O_3, NOx, or other tropospheric radicals oxidize them; these are then changed to UV-absorbing carbonyl groups, which are more stable. (Rabek, 1995). Due to the higher frequency of reactive branch points in LDPE, the low-density polymer is known to undergo photo-oxidation at a faster rate than HDPE. (Craig *et al.,* 2005). At temperatures below 100 °C, the thermal oxidative breakdown of PE is negligible when exposed to no sunlight.

Table 1. Weight loss of plastic film after biodegradation (After 12 weeks).

Samples	Percentage of lost substance without UV treatment (%)	Percentage of lost substance after UV treatment (%)
Garden soil	40	45
Garden soil+ rise husk	53	48
Garden soil+ peatmos	55	52
Garden soil+ organic fertilizer	56	60

Composting process in bioreactor

The rate of loss was recorded as (56,60%) for untreated and treated samples respectively, indicating that the best treatment to enhance the degradation process is using organic fertilizer, after the requisite tests were conducted to measure the efficiency of the decomposition of plastic pieces. The Patmos and rise fiber contributed to 52% and 53%, respectively, of the substance loss. The experiment additionally validated that the degradation rate was lowest in the unaltered soil, with a decomposition percentage of forty percent for the exposed strips and forty-five percent for the non-exposed strips, respectively. The microbe plays a crucial part in the breakdown of plastic. Different types of microorganisms can degrade different classes of plastic. The microbial biodegradation process has been recognized for its enhanced efficiency and is now being explored. Table (1) displays the list of additive samples together with their plastic degradation rates. The ability of microorganisms to remove pollutants from contaminated locations is one of the potential treatment approaches (8). Bioremediation, an alternate technique, is favored because of its efficiency, reduced hazard, monetary worth, and environmental safety (Mousa *et al.,* 2019). This study showed how Bacillus sp. can enhance the decomposition of plastic in soil by collaborating with consortia of microorganisms and their enzymes.

Polyethylene degrades in two ways: by light and through microbes. Essential abiotic precursors are obtained before the inclusion of selected thermophilic bacteria via abiotic oxidation (exposure to UV radiation) or heat treatment (Bonhomme *et al*., 2003). In the early stages of degeneration, there may be little to no mass loss. Instead, because of the adhesion of microbes and/or the uptake of oxygen, the mass may rise at brief exposure times (Azimi *et al*., 2014). During degradation, surface pits and fissures can also become clogged with clinging biomass and other debris (Yabannavar & Bartha, 1994). Therefore, to get relevant results, very extensive experimental times are typically required (Kumaravel *et al.,*2010). This method should be used in conjunction with some of the other analytical techniques, as mass loss measurements by themselves can be difficult to understand and extrapolate. Furthermore, weight loss, tensile strength loss, current elongation variations, and polymer molar mass changes are utilized to determine biodegradability (Erlandsson *et al.,* 1997; Sowmya *et al.,* 2014).

Involvement of microorganisms in the degradation of plastics

The degradation mechanisms of a polymer, as well as any elements that speed up the process, influence how and how quickly it degrades. Polymer breakdown can be influenced by a variety of environmental factors, including oxygen, temperature, sunlight, water, stress, living organisms, and pollutants (Gu, 2003). Therefore, from a soil microbiology perspective, evaluating the biodegradability of plastics and the microbes responsible for it in different soil conditions is necessary before any broad conclusions can be drawn regarding the biodegradability of plastics. Figure 3 shows the possibility of the presence of many microorganisms in soil contaminated with plastic, whether they are bacteria, yeasts, or fungi.

In addition to bacteria, are likely to have played a significant role in the biodegradation of degradable plastics, which can be broken down under favorable conditions via physical,

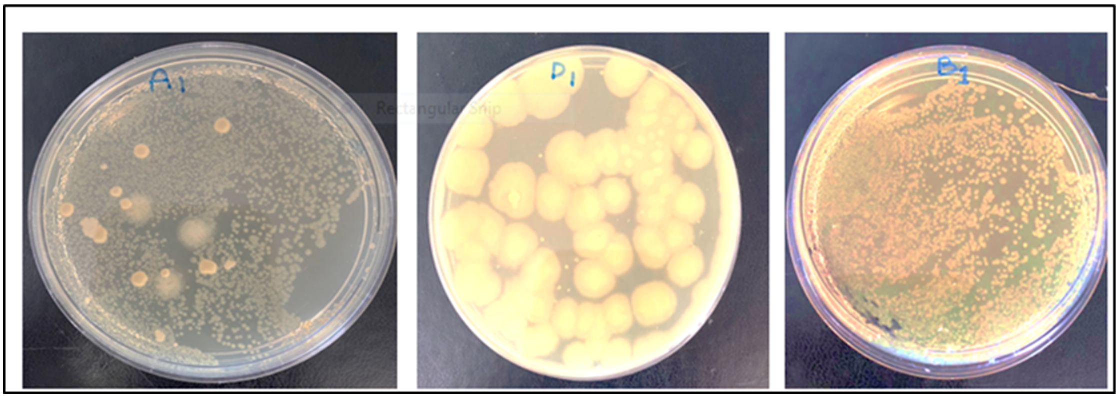

Fig. 3. Fungal, yeast and bacterial species isolated from bioreactor indicating microbial degradation of plastic.

Fig. 4. Morphology of plastic film. Control1strips before exposure –control2: strips after exposure-A:(A, A1) in soil without additives after degradation; (B, B1) soil with addition of rise husk after degradation; (C, C1) soil with addition of peatmos after degradation; (D, D1) soil with addition of organic fertilizers rafter degradation

chemical, and most crucially biological processes and satisfy the requirements of the standard testing techniques; Plastics that break down in the presence of naturally occurring microorganisms are known as biodegradable. Plastics that decompose biologically during use are called compostable plastics (Stevens, 2002; Stevens & Goldstein, 2002; Greene, 2007).

Degradable plastics as commercially labeled may not be a biodegradable reality as the disintegration may not be due to the actions of microorganisms (Stevens and Goldstein, 2002), but it is caused by UV radiation, heat, or hydrolysis (Darby, 2012). Compostable plastics are one type of biodegradable (Kyrikou and Briassoulis, 2007); the biodegradation of plastics may be so slow that cannot be compostable (Albertsson *et al.,* 1994; Stevens and Goldstein, 2002). At the end of the experiment, the color of the white polypropylene granules changed to yellow, indicating bacteria colonization.

Microscopic Investigation of Polymer Film and Composites

Surface changes in plastic sample

Numerous analytical procedures and methods are capable of quantifying the extent of polythene degradation. The vast majority of tests permit the assessment of polymers that have experienced discernible alterations. Surface roughness, hole or crack formation, de-fragmentation, color changes, and biofilm formation on the surface are all signs of degradation. Visual alterations, meanwhile, have the potential to function as a first indication of a microbial incursion. These modifications offer metabolic evidence to support the existence of a biodegradation route. Morphological changes may be observed on the surface of both the extra UV-treated and non-treated plastic samples, as depicted in (Figure 4). The objects had visible cracks and underwent a transition from a smooth to a rough texture.

Atomic force microscopy, scanning electron microscopy, and transmission optical microscopy are all state-of-the-art methods that can shed light on the degradation mechanism (Gu, 2003). In surface science labs, atomic force microscopy is used to produce images with an atomic resolution of 10-10 m, or one-tenth of a nanometer. To analyze the surface properties of a polymer sample, this sort of microscopy can be successfully used in the field of polymers. There were bright and dark areas. The bright areas show a 180 phase difference from the soft phase, which was represented by the dark areas (6). The films examined in this study exhibited increased surface roughness during the degradation process, with variations in roughness observed at the nanometer scale, (Figures 5, 6, 7, 8).

In the AFM height image, the low portions are depicted in dark colors, while the high regions are depicted in bright ones. After 90 days in a composting experiment, the pore depths of the stiff film surfaces were consistently 45 to 50 millimeters. In their unworn state, polymers' surfaces are typically quite flat and smooth; nevertheless, our measurements of the biggest pores after degradation revealed that their average depth was 45 m, their length was 2.14 m, and their breadth was 1.7 m. As time passes, the surface becomes rougher. It is common knowledge that erosion and the dissolution of degradation products increase roughness by creating cracks and pores. The findings demonstrated that the polyethylene had been broken down effectively. Both extracellular and intracellular depolymerizing enzymes are involved in the active destruction

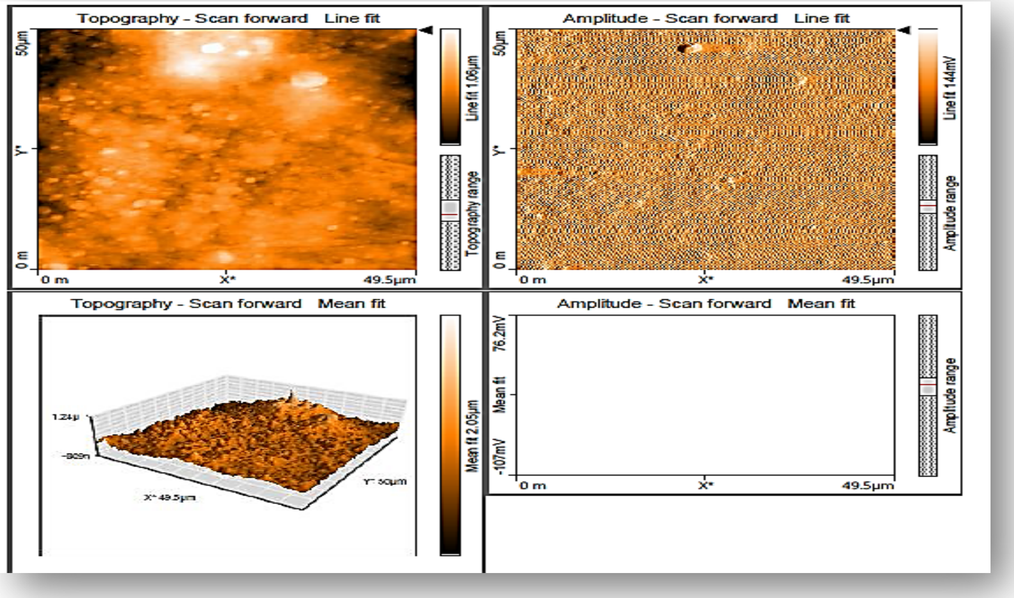

Fig. 5. AFM images of the surface of PE strips before degradation.

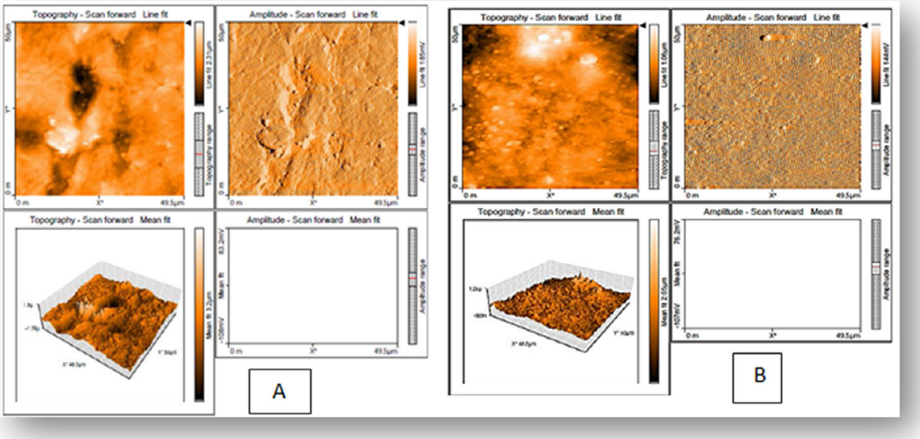

Fig. 6. AFM images of the surface after degradation in soil composting reactor of UV treated;(A) And non- treated (B) PE for 90 days of erosion.

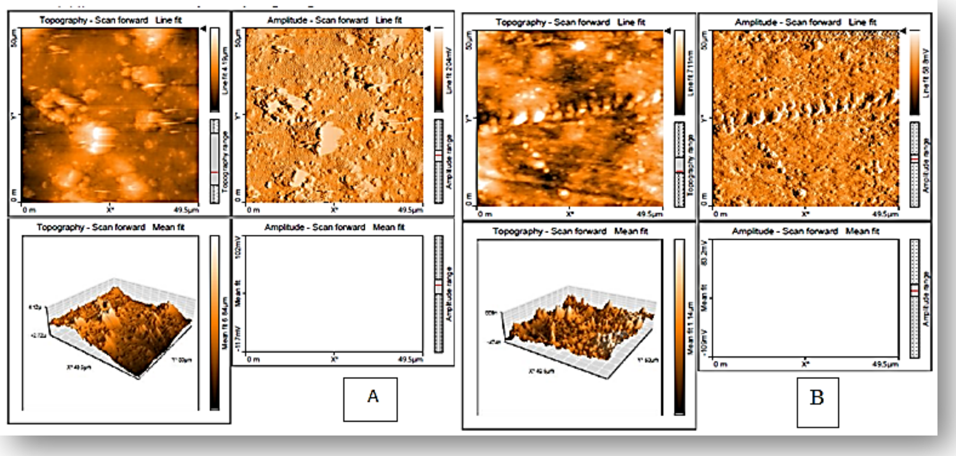

Fig. 7. AFM images of the surface after degradation in rise husk supplemented soil composting reactor of UV treated;(A) and non-treated; (B) PE for 90 days of erosion.

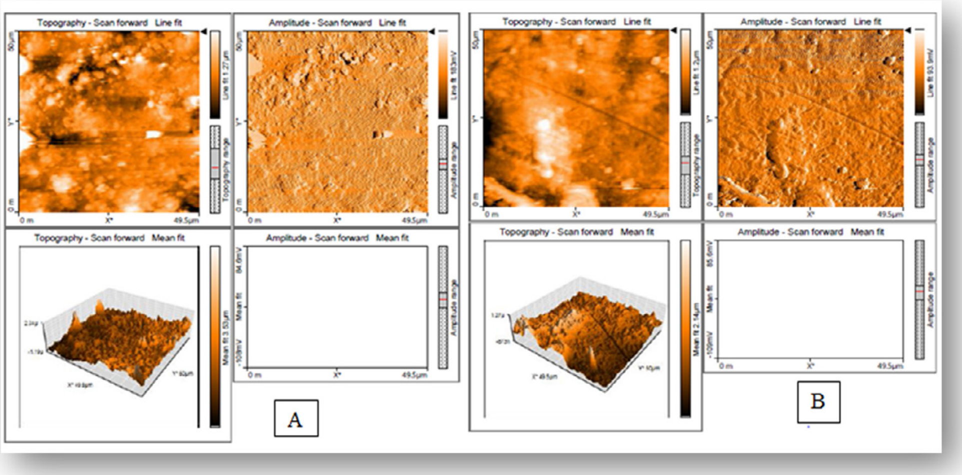

Fig. 8. AFM images of the surface after degradation in compost-supplemented soil composting reactor of UV-treated (A), and non-treated (B) PE for 90 days of erosion.

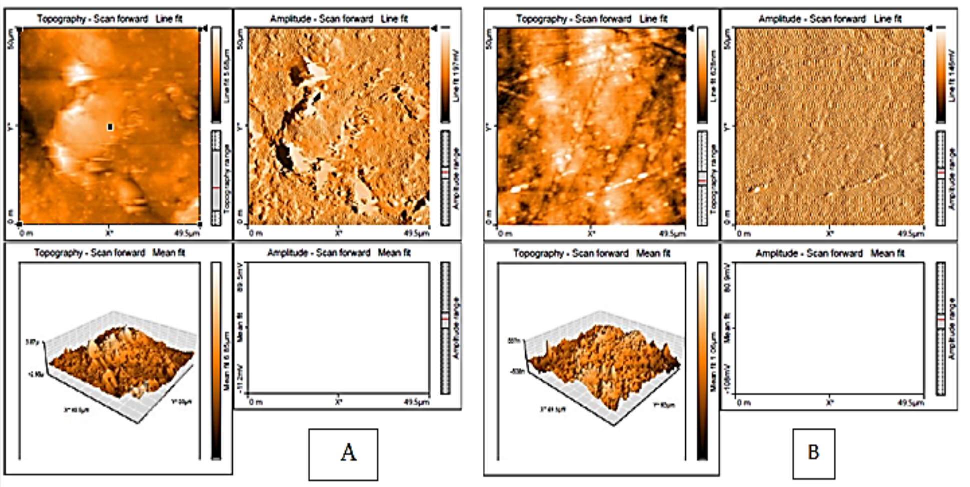

Fig. 9. AFM images of the surface after degradation in organic fertilizer supplemented soil composting reactor of UV treated (A), and non-treated; (B) PE for 90 days of erosion.

of polymers in living organisms. In the process of degradation, microbial exoenzymes break down long chains of polymers into oligomers, dimers, and monomers. These molecules can serve as carbon and energy sources because of their tiny size, which makes them soluble in water and allows them to pass through semi-permeable bacterial outer membranes (Gross and Kalra, 2002; Gu, 2003; Mohan and Srivastava, 2010). All the examined films grow rougher on the surface as they degrade; however, the variation in surface roughness was on the nanometer scale. Dark and light colors in the AFM height image indicate low and high areas.

CONCLUSIONS

Conventional plastics can biodegrade in the soil for decades, although both abiotic and biological mechanisms are implicated in the observed biodegradation This study examined the biodegradation of synthetic polyethylene in lab-bioreactors with different soil additions before and after 30 days of UV irradiation. Weight loss after 24 weeks of incubation with selected bacterial isolates showed polyethylene degradation. UV-exposed models degraded the most easily in experiments. PE films decomposed 52% and 55% in soil containing Peat moss, but only 40% and 45% in garden soil untreated and UV-treated. During the same experiment, PE films buried in husk-supplemented soil lost 48% and 53% weight. Plastic breakdown in organic fertilizer-supplied soil was most effective, reaching 56-60%. Atomic Force Electron Microscope (AFM) images show that weight loss peaked in the model with fertilized soil.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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