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Research Article

Determination of the role of *Dermococcus nishinomiyaensis* and *Pseudomonas aeruginosa* in polyethylene (PE) and polyethylene terephthalate (PT) biodegradation

Ghada A. Mohammad*
Department of Biology, College of Sciences, University of Mosul, Mosul, Iraq
Amina G.O. Al-Ani
Department of Biology, College of Sciences, University of Mosul, Mosul, Iraq

*Corresponding author. E-mail: kadsbio32@uomosul.edu.iq

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Abstract
Plastic pollution is a worldwide menace to the ecosystem and human health, and only less than 10 percent of the plastic waste has been recycled and its continued existence causes climate change. The purpose of the study was to test the potential of biodegradation of *Dermococcus inshinomiyaensis* on two common types of plastics (Polyethylene Terephthalate (PET) and Polyethylene (PE)) when compared to the established degrader, *Pseudomonas aeruginosa*, at conditions that simulated the natural environment. The biodegradation was evaluated in terms of biofilm biomass determination, relative weight loss, scanning electron microscopy (SEM), and Fourier-transform infrared (FT-IR) spectroscopy. Biofilm quantification showed that *D. inshinomiyaensis* formed stronger biofilms on PET bottles (absorbance 0.16) than on PE bags (0.10–0.12), with activity comparable to that of *P. aeruginosa*. The analysis of weight loss showed that PE was severely degraded by *D. inshinomiyaensis* (12.2%), *P. aeruginosa* (40%), and PET was not severely degraded (0.7%). FT-IR spectra of PE indicated new absorption bands beyond 400 cm^{-1}, confirming structural defects induced by microbial activity. Distinct peaks between 1600–2653 cm^{-1}, including 1639 and 1737 cm^{-1} for *P. aeruginosa* and 1981–2325 cm^{-1} for *D. inshinomiyaensis*, suggested the formation of novel intermediates. SEM analysis also showed intense deformations in PE using *D. inshinomiyaensis* so that it generated a wide range of cracks, wrinkles, and grooves, which were much more effective compared to the action of *P. aeruginosa*.These results demonstrate the novelty of *D. inshinomiyaensis* as a potential biodegrader of PE, as it provided potential use in the management of plastic waste in a sustainable environment.

Keywords: Biodegradation, Fourier-transform infrared, Polyethylene, Sustainable development, Terephthalate

INTRODUCTION

Plastic materials are becoming more common in everyday life for the last several decades due to their low cost. The continual presence of non-biodegradable plastic waste in the environment results in a significant threat to all life forms. Therefore, there is a need to eradicate these wastes to protect and promote a healthy life and ecosystem. Plastic is a versatile material applicable to countless products widely used for domestic and industrial purposes because it has a set of useful properties: durability, strength, light weight, water and corrosion resistance, and low cost.

Plastic can also reach the oceans and exert lethal effects on aquatic organisms if ingested, and it can therefore be consumed by the human body through the food chain (Jung *et al*., 2020). Plastic waste burning contributes to air pollution and the transfer of these toxic substances into the human body, thereby threatening public health (ET).

One of the solutions used to get rid of plastic residues, the most important of which is biodegradation using microorganisms, which is cheap and environmentally friendly as it contributes to the fragmentation of organic compounds (Kong *et al*., 2022; Royer *et al*., 2018) due to the presence of internal and external enzymes in these microorganisms that can decompose the polymer into simple components (Zhang *et al*., 2020; Moharir and Kumar, 2019). Plastic garbage resists natural degradation, resulting in the accumulation of solid waste

that affects soil microorganism populations and activity (Lahive *et al.,* 2019).

Microorganisms have always played an indispensable role in nature, and because they can break down most organic and inorganic matter, they are used to prevent the accumulation of contaminants and to provide harmless treatment of various substances (Ahmed *et al*., 2018; Mahmoud and Mohammad, 2024).

Based on this idea and in view of the great importance of this topic, the study aimed to investigate the ability of *Dermococcus inshinomiyaensis* (*D*. *inshinomiyaensis*) bacterium (which are not commonly used in the field of environmental pollution removal application) to decompose common water's bottles which composed of Polyethylene Terephthalate (PET), or decompose black nylon bags consisting of polyethylene (PE) in an attempt to clean the natural environment from accumulations of this material and using *P. aeruginosa* as control factor considering it known in plastic degradation.

MATERIALS AND METHODS

Bacterial isolates

Two bacterial isolates, *D. inshinomiyaensis* and *Pseudomonas* (*P.*) *aeruginosa* (pre-diagnosed by the 16S rRNA gene), were obtained from the University of Mosul / College of Science / Department of Biology.

Plastic materials:

Because the plastic decomposes very slowly and incompletely, thus it is considered an environmental problem, thus wastes of a common sort were utilized which were:

Plastic water buttles: manufactured industrially from Polyethylene Terephthalate (PET).

Black polyethylene bags (Commonly known as black nylon bags in our society**)**, manufactured industrially from Polyethylene (PE).

Two types above were cut into pieces of equal area with dimensions of 2x2 cm^2 and then weighed each piece separately.

Biodegradation process:

A natural environmental mimics method was used by soil to investigate the decomposition of (PET) buttles and (PE) bags pieces, bakers with a capacity of 250 cm^3 were prepared and put a quantity of soil in them, weighted pieces of (PET) buttles and (PE) bags were placed in the middle, then covered by another layer of soil, then all of them were sterilized by autoclave at 121°C and pressure 1 atmosphere for 20 minutes.

After removing the bakers from the autoclave and cooling them, the soil was impregnated with (10) ml of each of the *D. inshinomiyaensis* and *P. aeruginosa* separately at a concentration of (1.5×10^8) colony-forming unit/ml in natural environmental conditions, left at room temperature for 30 days. After that, they were re-impregnated with bacteria again after 30 days.

After that, they were re-impregnated with 10 ml of sterile physiological solution for moisturization and left for 15 days; finally, they were re-impregnated for the fourth time with 10 ml of sterile physiological saline solution and left for 15 days. Eventually the necessary tests were performed. The experiment was done for each bacterium with three replicates.

Biodegradation confirmation measurements

Estimation of biofilm mass on plastic surfaces

The total protein content of bacterial biofilm biomass was measured as described by Gupta and Devi (2020). After 90 days of applying the bacteria with (PET) buttles and black nylon bags (PE), they were gently washed with water to remove any soil residue or any weakly adhered bacterial cells. The biofilms formed from the pieces of black nylon bags were then removed for measurement by adding 1 ml of 0.85% saline solution with shaking for 4 minutes. Normal saline was used as a blank to spectrophotometrically determine the protein concentration at 595 nm.

Relative weight loss

Weight loss was determined according to the following equation:

Percentage of weight loss = Initial weight –final weight /Initial weight x 100

For initial weight, each piece was recorded separately before placed in the soil.

For final weight, any biofilm formed on the piece was first removed (i.e., removal of adherent bacterial cells), so the pieces were washed with 2% sodium dodecyl sulfate for 4 hours and vortexed, and finally rinsed with deionized water. Pieces were dried on filter paper at 40 °C for about 12 hours (Han *et al*., 2020).

Fourier-transform Infrared spectrum Analysis

The effects of bacterial treatment on the structure and functional groups of PET and PE membranes were analyzed using Fourier-transform infrared (FTIR) spectra (Alpphaii, Bruker-germany) in the frequency range 4000-400 cm^{-1}. They were used with an accuracy of 1 cm^{1-}.

Scanning electron microscopy (SEM)

Pieces of (PE) not treated with any bacteria and another pieces treated with *D. inshinomiyaensis* and *P. aeruginosa* bacteria were subjected after 90 days to SEM analysis after washing them with 2% aqueous SDS and distilled water (V/V) repeatedly with a slight mixing for a few minutes in addition to washing with 70% ethanol to remove cells to obtain a surface free of bacterial biofilm to watch the changes in the outer surface found (Das

and Kumar, 2015).

The samples were then fixed in position on the sample holder using carbon, coated with gold for 40 seconds, and observed under a high-resolution SEM (TESCAN MIRA3/FRAN).

RESULTS AND DISCUSSION

Biodegradation is defined as the process of removing pollutants and polymers mediated by living organisms such as bacteria and fungi by using it as a food source, biodegradation provides advantages including low cost and strong effectiveness, and it does not release toxic gases and harmful compounds into the environment, making the biodegradation process environmentally friendly (Webb *et al*., 2012). In the present research, a new bacterium, D. inshinomiyaensis, which had not been previously used in this field, was used to determine whether it benefits in degrading the two types of plastic: PET and PE, and to compare its degradative activity with that of *P. aeruginosa*, well known for its degradative activity. So the results were as follow:

Measurement of bacterial biofilm biomass

Quantification of biomass adhered to the surface is an efficient method for determining bacterial colonization of PET or PE and biofilm development. Growth kinetics and biofilm formation of *D. inshinomiyaensis* and *P. aeruginosa* cells on both plastic surfaces were estimated by measuring the total protein content of adhering biomass. Here we have two important results: the first, the study proved that the *D. inshinomiyaensis* has the same ability as *P. aeruginosa* for biofilm formation on plastic surfaces, the second that the biofilm formed on the bottle (where the absorbance was 0.16) more than on the black bag (where the absorbance was 0.1, and 0.12 respectively), which indicates the possibility of removing PET by them from the environment. This is due to the use of plastic as a carbon source in their's metabolism.

The majority of bacterial surfaces are hydrophilic; however, bacteria cannot adhere to PE surfaces because PE is hydrophobic. It is widely recognized that for PE to be broken down by microbes, bacteria must adhere to the surface and then form biofilms, where they thrive in low-nutrient conditions and use solid substrates (Bardají *et al*., 2019). Research indicates that the attachment of microbes and the formation of biofilms may convert the hydrophobic to hydrophilic characteristics of plastic film surfaces (Chen *et al*., 2020). Han *et al*. (2020) reported that every PE surface became more hydrophilic when bacteria were present. Implying that our isolated bacterial strain's adhesion may enhance the hydrophilic process, making it easier for microorganisms to break down chemical bonds in the process of biodegradation that follows:

Relative weight loss

The weights of PET and PE were recorded before the experiment i.e. before being placed with bacteria, also the weights were recorded after 90 days of the end of their treatment, and the results were a massive decrease in relative weight of PE by *P. aeruginosa* (40 %) as compared with PET (0.70 %). This may be due to high binding affinity of *P. aeruginosa* enzymes to PE, or to the fact that some PE-degrading enzymes are more highly expressed than others (Kim *et al*., 2020).

As well as the comparison with *D. inshinomiyaensis*, which showed a decrease in the relative weight of PE to 12.2% and 0.7% only for PET, these results are considered a decent value. The inverse relationship between the results of the amount of biomass measured and the loss of relative weight of both the black bag and the bottle samples is attributed to the fact that both bacteria were easier to use the black bag as a nutrition source in the absence of a carbon source, while requiring a larger biomass on the water bottle because it is difficult to consume it.

It was noteworthy that no relative weight loss was observed in the control group, indicating the absence of mechanical abrasion or abiotic hydrolysis in the test groups. Thus, the relative reduction in weight within the experimental groups can be attributed to bacterial activity.

As PE and PET were present in the same soil layer during the experiment, PE may have been consumed first by bacteria as a carbon source, while PET may have required more time to be consumed. Whereas PET is a more complex compound consisting of ethylene glycol polymers and terephthalic acid (Tari and Kannan, 2020). A slight reduction in weight is due to the fact that plastic particles must undergo biochemical reactions to break long, water-soluble polymer molecules so microorganisms can transport them into their cells through the cell's outer membrane (Mohammad and Taha 2021). In order for the polymer to be absorbed and broken down by the cell microorganisms, it must first undergo a process known as depolymerization, in which it fragments into smaller monomers inside the cells, so it can be converted to simpler compounds (Mir *et al*., 2017). The biodegradation of polymers involved two active enzymes: intracellular depolymerases and extracellular enzymes. The biological degradation of polymers is initiated by extracellular and intracellular enzymes that catalyze depolymerization (Raziyafathima *et al*., 2016).

PE, composed of linear or slightly branched carbon-carbon bonds, is readily biodegradable, while PET contains rigid aromatic rings and ester bonds that increase crystallinity and chemical stability, thus limiting microbial access and degradative activity. As a result, PET exhibits significantly slower biodegradation rates compared to PE.

Infrared spectrum analysis

For the substance PE, comparing with control (Fig. 1), all the replicates of *D. inshinomiyaensis* showed different changes from what *P. aeruginosa* caused as shown in Fig. 3 and Fig. 2, respectively as a selective model for each, Fig. 1 demonstrate the spectra of control (without treatment with bacteria) many peaks reflecting the chemical structure of PE. the characteristic absorption peaks were assigned at 718 cm^{-1} indicates for (C–H bend-mono), and 1,461 and 1,472 cm^{-1} which for (C=C stretch), as well as 2,663 cm^{-1} (CHO stretch), lately 2,847 and 2,914 cm^{-1} (both due to C–H stretch). Significant variations, as well as similar peaks to the control, were observed for both bacterial isolates. For the PE tested with both bacteria, a New band has been observed at 400 cm^{-1} (fingerprint of the material), indicating a defect in the material's identity, which consequently supports the activity of the bacterial isolates. The absorption peaks from after 1600 cm^{-1} to about 2653 cm^{-1} appeared in wobble state especially for *P. aeruginosa* (Fig. 2) more than *D. inshinomiyaensis* (Fig. 3) indicating the formation of new intermediates products through the CHO stretch region in particular 1639 cm-1 and 1737cm^{-1} in *D. inshinomiyaensis* , and 1981, 2049m 2150,2163, 2187 and finally 2325 cm-1 in *P. aeruginosa* . In addition, there are other new peaks in *P. aeruginosa* at 787 cm^{-1} and 1037 cm^{-1}, and in *D. inshinomiyaensis*. There are peaks at 1038, 1082, 1639, and 1737 cm^{-1}. in the treated sample than the control. Changes in the peak values of functional groups supporting the conformational change on PE surfaces. At the beginning of the degradation process, the main chain is cleaved into oligomers or monomers by an enzyme secreted by bacteria (Dwicania *et al.,* 2019).As for the PET substance, no new functional groups appeared in the FT-IR analysis of the bottle for

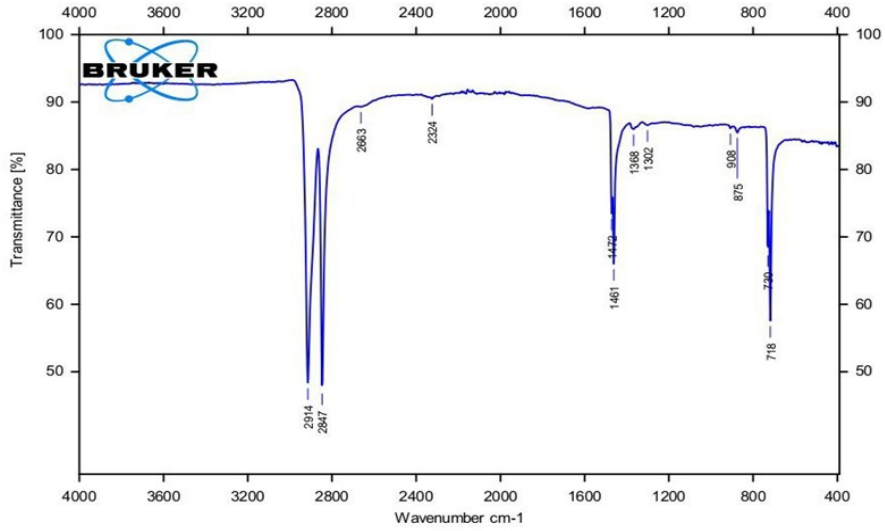

Fig. 1. *Fourier-transform infrared (FT-IR) spectra of polyethylene (PE) without treatment (control)*

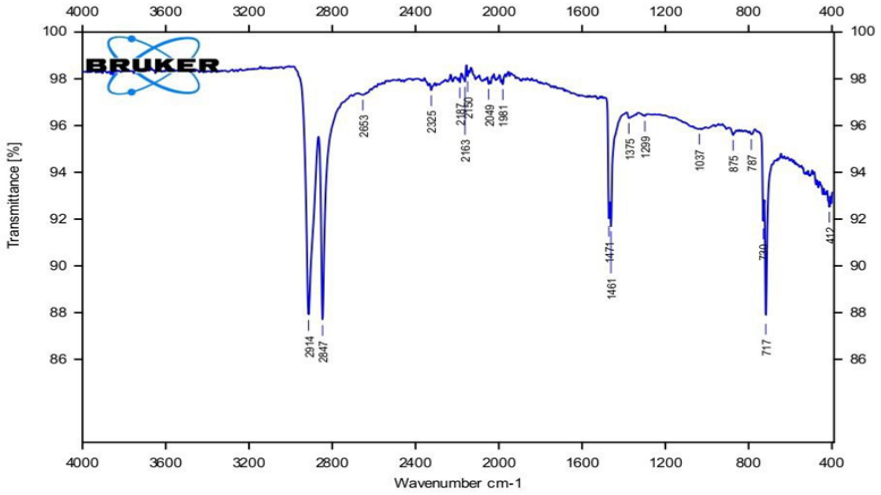

Fig. 2. *Fourier-transform infrared (FT-IR) spectra of polyethylene (PE) treated with Pseudomonas aeruginosa*

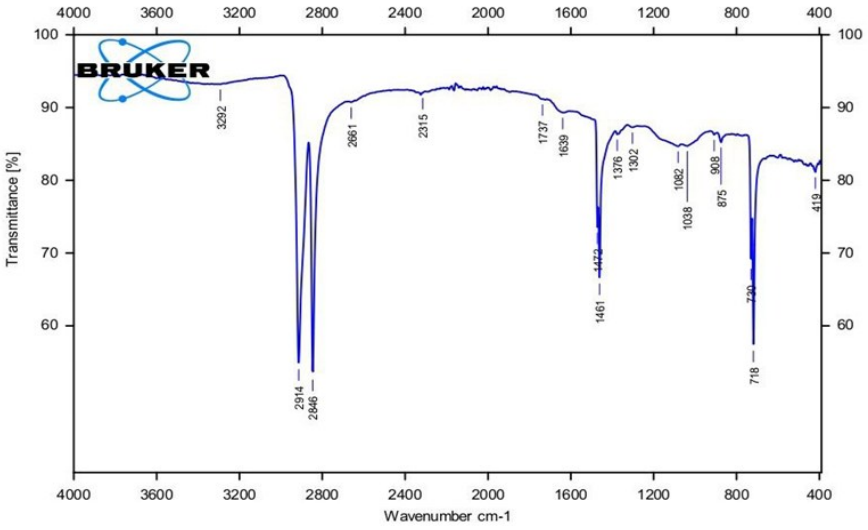

Fig. 3. *Fourier-transform infrared (FT-IR) spectra of polyethylene (PE) treated with Dermococcus inshinomiyaensis*

both bacteria under study. This may be due to the absence of detectable cleavage of ester bonds constituting its structure, or to a degradation level below the spectroscopic detection limit.

Scanning electron microscopy (SEM) results

From comparing images obtained by SEM with a magnification of 15 000,times of *D. inshinomiyaensis* with both the control samples Fig. 4 A (which appeared with a smooth surface free of cracks) and the *P. aeruginosa* sample Fig. 4 B (which showed significant changes and cracks on the surface of the PE segment), the results showed deformations on its surface, where *D. inshinomiyaensis* succeeded in achieving impressive results in terms of the appearance of large cracks and clear cracks, as well as the shape of wrinkles and uneven grooves. On the surface of PE, as shown in Fig. 4C, this indicates the tremendous ability of this bacterial species in this field to consume this substance as a carbon source, potentially enabling it to live and survive after adhering to it.

According to Bolo *et al*., (2015), SEM analysis revealed that *Kocuria kristinae*, *Dermacoccus nishinomiyaensis*, *Pseudomonas stutzeri*, and *Acinetobacter haemolyticus* could all degrade plastic. The emergence of cracks and crevices, which may be an indication of microbial activity, is a sign of biodegradation. Scratches and particle buildup on plastic film surfaces are signs of the presence of molecules such metabolites and organic acids, which are byproducts of bacterial metabolism. These studies support the hypothesis that microorganisms use this plastic as the source of carbon to be metabolized.

According to Lee *et al*., (2020), the *P. aeruginosa* strain had an extraordinary capacity to degrade polystyrene (PS) and polyvinyl sulfide (PPS). Polypropylene (PP), as well as polyethylene (PE). It was found that the biodegradation of PP was the most gradual and the PE

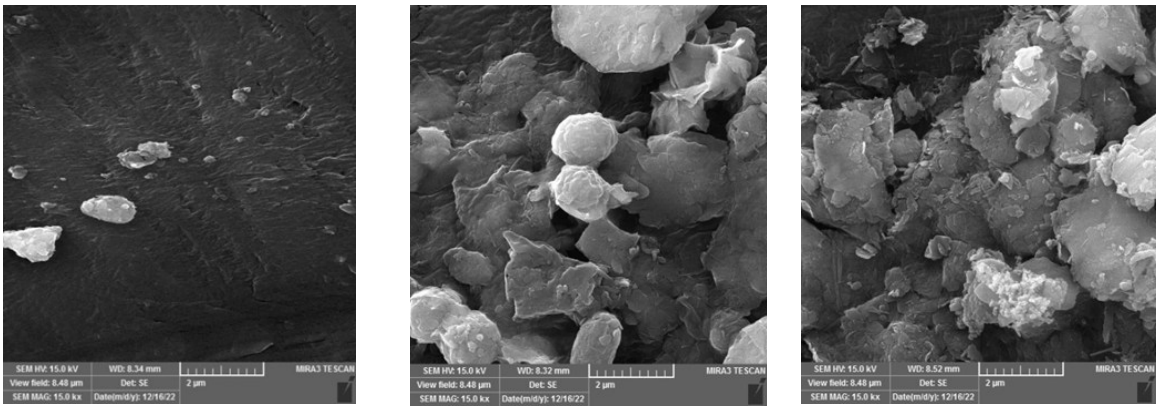

Fig. 4. *Scanning electron microscopy (SEM) pictures of PE, A) before treatment as Control, B) PE treated by Pseudomonas aeruginosa treatment, and, C) PE treated by Dermococcus inshinomiyaensis. with magnification 15.0 kx*

was the quickest. Furthermore, the rates at which *P. aeruginosa* grew were not always in line with the rates of plastic biodegradation, indicating that the characteristics and composition of the intermediate molecules produced during this process could affect bacterial growth rates and potentially provide useful cellular precursors and energy. *Pseudomonas* species account for 21% of other bacterial genera, according to Lv *et al*. (2014), and are among the bacterial genera linked to the breakdown of plastic, although some species are pathogenic (Mohammad *et al*., 2024).

Physical and chemical degradation methods for plastic waste are now considered adequate due to the environmental pollution they cause. Therefore, an alternative remediation technique is needed. Recently, biodegradation of plastic by bacteria and fungi had increased attention due to their sustainability, efficiency, environmental friendly, and low-cost characteristics.

Future research ought to include the use of more sophisticated methods of analysis including gas chromatography-mass spectrometry (GC-MS) to identify certain byproducts of the degradation. The direct evidence of the presence of metabolic activity could be the identification of compounds such as alkanes or fatty acids and elucidate the enzymatic processes that may have taken place. Urbanek *et al*. (2018) highlighted that these molecular-based studies are invaluable in the determination of superficial fragmentation or actual biodegradation.

Another strong method of mineralization confirmation is through respirmetric assays and especially carbon dioxide evolution. The direct conversion of microbial activity into plastic carbon conversion to natural biogeochemical cycles can be achieved by quantifying the release of carbon dioxide. Ru *et al*. (2020) established that these techniques offer solid proof of microbial mineralization other than just a weight-loss measurement.

The combination of these biochemical and analytical methods would make the scientific input of the biodegradation research much more robust. It was observed that the combination of physical, chemical and metabolic evidence provides more detailed insight into plastic degradation by microbes (Shah *et al*., 2008). This integrated approach increases the environmental relevance and makes laboratory results reliable in terms of environmental transfer.

In the natural ecosystem, interactions with microbes can have synergistic effects with bacteria, fungi and other organisms to either degrade or change competitive behaviour. This kind of complexity indicates that, biodegradation is not an individual action of a particular species but a collective activity of microbes, which provides more stable ecological results. Thus, although it is of great importance to find one bacterium that can degrade plastic in the laboratory, its work in nature is subordinated to larger microbial communities.

Conclusion

Both bacteria *P. aeruginosa* and *D. inshinomiyaensis* were found to be able to consume PE more than PET, this finding motivate to use them to rid environment of black polyethylene bags and interfere in the sustainable development field. The recommendation for future research is to investigate additional parameters, such as mineralization and polymerization, to further strengthen the understanding of plastic biodegradation further.

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Disclosure and conflict of interest

The authors declare that they have no conflicts of interest

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