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Investigating the Biodegradation Potential of Extreme Thermophilic Bacteria for Plastic Waste in Harsh Environmental Conditions

Dunia Al Farraj[1*]

[1*]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. E-mail: dfarraj@ksu.edu.sa

Abstract

There has been a high rate of plastic waste accumulation, especially of synthetic polymers like polyethylene, high-density polyethylene, and polyethylene terephthalate, which is a great environmental challenge. Waste management methods that are traditional have been proven inefficient and unsustainable. This paper examines the biodegradability of the extreme thermophilic bacteria found in high-temperature industrial air, and it aims to investigate the capacity of the bacteria in degrading PE, HDPE, and PET under high temperatures. The main aim of the research is to isolate and test extreme thermophilic bacterial strains for their ability to biodegrade common plastic polymers at temperatures higher than 60°C. Their enzyme activity and their degradation rates, particularly in harsh industrial and natural conditions, will also be studied in the research. Bacterial strains were further anthocultured and incubated with plastic samples in thermophilic conditions (60°C, 70°C, and 75°C) in industrial pipeline systems. To quantify the modifications in the chemical, plastic degradation was quantified as to weight loss, biofilm production, and Fourier Transform Infrared spectroscopy, which indicate that strain TPB02 and TPB05 exhibited maximal degradation of PE and HDPE at 2.3% - 4.0%. PET was unsuitable for degradation, with a weight loss of 0.5 % to 18%. The aggressive biofilm development was linked with an increased degree of the degradation capacity, particularly of PE and HDPE. The current paper puts an emphasis on the potential of extreme thermophilic bacteria, TPB02 and TPB05, as promising agents in the plastic waste bioremediation process, and more so at high temperatures. Their future research ought to be aimed at maximizing the environment of these strains and the enzymatic processes during plastic degradation.

Keywords:

Biodegradation, biofilm formation, extreme thermophiles, FTIR analysis, polyethylene, thermophilic bacteria, waste management.

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Corresponding Author: Dunia Al Farraj, E-mail: dfarraj@ksu.edu.sa

Introduction

The problem of plastic waste has become one of the most pressing problems of the contemporary world, with the widespread usage of synthetic materials in the form of polymers like polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET). These substances are extremely robust and unable to degrade naturally, and hence they become long-lived in the terrestrial and aquatic ecosystems. Traditional approaches to plastic waste, such as landfilling, incineration, and mechanical recycling, tend to be energy-demanding, harmful to the environment, or economically unfeasible, especially in severe environmental conditions (Kotova et al., 2021).

Attention to the biological degradation of plastics as a promising, environmentally friendly alternative has increased over the last couple of years. Biodegradation of microbes is based on the process of enzymes that can break polymer chains into small assimilable molecules. Despite their low potential for plastic degradation, mesophilic and thermophilic microorganisms can perform poorly since they possess very low temperature-working ranges and enzyme stability (Finore et al., 2023). Extreme thermophilic bacteria, which survive at temperatures over 70 °C in hot springs, geothermal vents, and industrial reactors, have highly stable enzymes, which exhibit increased catalytic activity and resistance to severe physicochemical factors (Pham et al., 2024; Gallo et al., 2024; Abdullah, 2025). These peculiarities make extreme thermophiles one of the possible candidates in the biodegradation of plastics in the high temperature and chemically hostile environment, where other microorganisms cannot survive (Baker et al., 2020).

The main aim of this research is to examine the potential of plastic biodegradation by extreme thermophilic bacteria in harsh environmental conditions. Specifically, the study will seek to (i) isolate and screen the extreme thermophilic bacterial strains that have the capacity to interact and degrade common plastic polymers, (ii) determine the degradation rates of the strains at high temperatures, and (iii) determine the mechanism behind the thermophile-mediated degradation of plastics.

Although there is an increasing concern about microbial plastic biodegradation, current studies are mainly in the form of mesophilic or moderately thermophilic microorganisms deployed under controlled conditions within the laboratory. A major gap in the literature is a deficiency of systematic studies investigating extreme thermophilic bacteria as the degraders of plastics in particular conditions simulating an industrial or natural extreme environment. In addition, the enzymatic activities, thermal properties, and degradation of the plastic-degrading enzymes of thermophilic nature are not well known (Ray & Sankhyan, 2022; Bosco & Aleem, 2023). This disadvantage restricts the scaling of the microbial biodegradation processes to those that are rooted in high-temperature scale waste treatments (Goswami et al., 2024; Ruggero et al., 2023).

The hypothesis of the current study is that extreme thermophilic bacteria have thermostable enzymatic machinery capable of triggering and catalyzing the process of synthetic plastic polymers biodegradation in severe environmental conditions. High temperatures will enhance the mobility of the polymer chains and enzyme and substrate interactions, and decomposition under high temperatures will work well compared to mesophilic systems (Bhattacharya & Gupta, 2025).

The key contributions of this research are as follows:

Recognition and characterization of ultra-thermophilic bacterial isolates having prospective plastic-degrading features.

Plastic biodegradation efficiency under extreme environmental and industrial conditions (high temperature).

The data regarding the enzymatic activities and thermal stability of the enzymes obtained from thermophiles in plastic degradation.

Basic proof of thermophile-based biodegradation systems as an alternative to the sustainable management of plastic waste in harsh environments.

Linking microbiology and environmental biotechnology, which offers a blueprint for the future construction of high-temperature bioprocesses that can be used to treat plastic waste.

The Introduction defines the global problem of plastic waste and presents extreme thermophilic bacteria as one of the possible solutions to the biodegradation in severe conditions. The research on the microbial degradation of plastics focuses the Literature Review on the thermophilic and extremophilic bacteria. In the Materials and Methods, the isolation of bacteria, the preparation of plastic substrates, and the experiment of biodegradation are explained. Results provided show the biodegradation efficiency and biofilm formation of the strains, in addition to FTIR analysis, which shows better performance of TPB02 and TPB05 strains. These findings are discussed in the Discussion, and the conclusion highlights the future research direction and use (Możejko-Ciesielska et al., 2023).

Literature Survey

Environmental tenacity of plastic waste is an imperative global issue in the world because synthetic polymers are inherently resistant to environmental degradation processes. The traditional methods of waste management, like landfill, burning, and mechanical recycling, tend to be inefficient, polluting, and unsustainable economically, especially when the environmental conditions are extreme. Consequently, biological degradation has become a possible and viable option, which is based on the metabolism of microorganisms and enzymatic action to convert polymeric bodies into less toxic materials (Kotova et al., 2021; Cai et al., 2023; Nazeer et al., 2024). Nevertheless, the effectiveness of biodegradation is highly conditioned by the environment, polymers, and microbial diversity, which indicates the necessity to investigate systems other than mesophilic microorganisms.

An immense amount of evidence has shown that multiple microbial species can break down plastics both by colonization of the surface, by formation of biofilms, and by enzyme depolymerization. Esterases, lipases, cutinases, hydrolases, oxygenases, and so on are enzymes that help to break down the backbones of polymers and initiate their degradation (Lv et al., 2024; Gupta et al., 2025). Recent advances in metagenomics and plastisphere research have also shown metabolite microbial assemblies of plastic surfaces of complex metabolism and functional redundancy of plastic-degrading microbiomes (Saleem et al., 2023; Herrera et al., 2023). The studies that are available, although with these advances, are mainly based on ambient or moderately high temperatures and do not apply as well to extreme environmental or industrial conditions.

Extremophilic microorganisms, capable of surviving under high temperature, extreme pH, salinity, or radiation, have gained increasing attention for environmental biotechnology. The active presence of enzymes and stability of the enzymes have led Atanasova et al., (2021) and Pham et al., (2024) to discover the idea of extremophiles as an unutilized resource for the biodegradation of plastics. Microorganisms that grow in unfavorable environments, including deserts, geothermal, and compost products, were also more adapted to the conditions that cause the inactivity of mesophilic organisms during bioremediation of plastics and microplastics under the conditions where the microorganisms are capable of survival (Ribeiro et al., 2024).

Bacteria with extreme and thermophilic growth at temperatures over 60°C -70°C have enzymes enabling the degradation of polymers. High temperatures enhance chain mobility, lower crystallinity, and enhance the enzyme-substrate interactions, resulting in faster rates of degradation (Bhattacharya & Gupta, 2022; Gallo et al., 2023). Thermophilic bacteria's degradation of polyethylene and microplastics has experimentally proved their ability, and the effectiveness of degradation depends on particle size and incubation temperature (Özdemir et al., 2022; Akarsu et al., 2022). There is further evidence via high-temperature stages that special microbial communities are found that produce enhanced degradation of biodegradable plastics and PET microplastics (Ruggero et al., 2023; Cazaudehore et al., 2023; Li et al., 2024).

The ability of extreme thermophiles to adapt is also shown by the studies in extreme conditions of nature. The study by Hakakzadeh et al., (2025) showed the degradation of high-density polyethylene under the influence of desert bacteria found in the Lut desert, which indicated the possibility of biodegrading plastic under adverse thermal environments. Although the interest is increasing, the existing literature is still disjointed and does not feature much mechanistic integration and systematic analysis under sustained severe conditions (Urbanek et al., 2021; Bher et al., 2021). Lack of knowledge in the extreme thermophilic degradation mechanisms and lack of comparison with mesophilic systems do not allow application in the real world. To overcome these gaps, the current research aims to assess the biodegradation capabilities of extreme thermophilic bacteria under adverse environmental factors to promote sustainable and high-temperature-driven plastic waste management plans.

Materials and Methods

Study Design and Experimental Overview

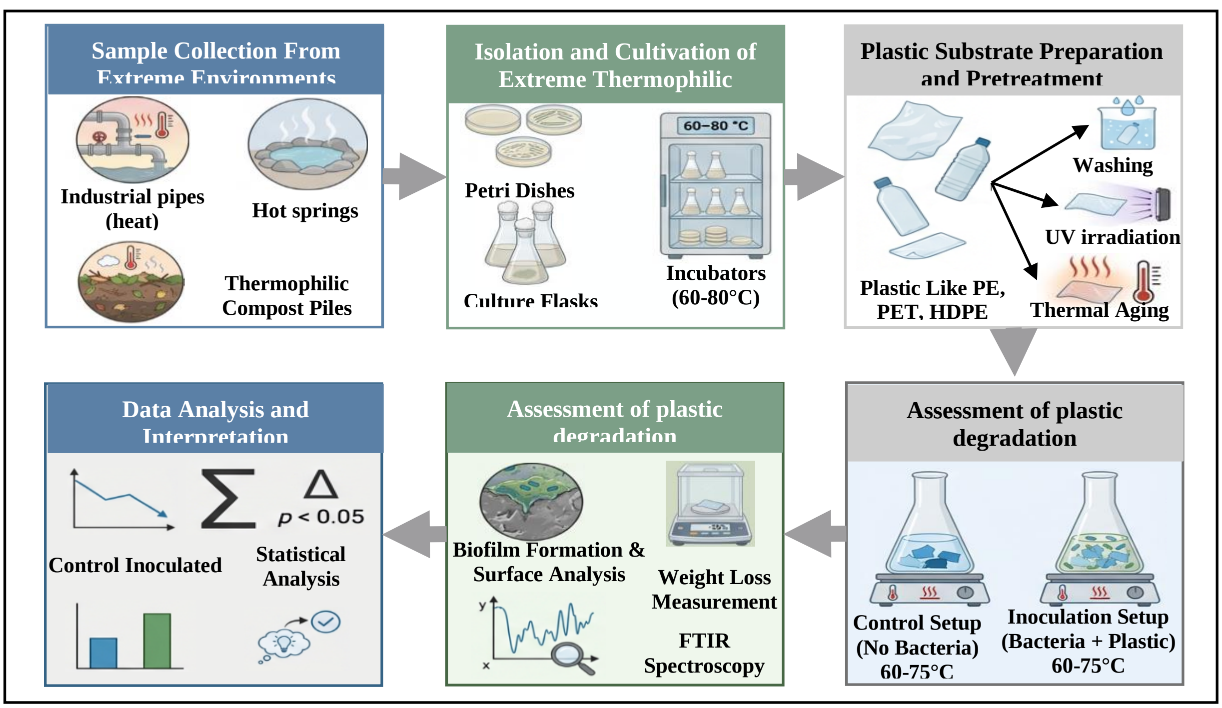

Figure 1. Plastic biodegradation experimental workflow examining the extreme thermophilic bacteria under severe environmental conditions

This paper is based on a controlled laboratory-based experimental method to evaluate the potential of extreme thermophilic bacteria to biodegrade plastic waste in severe conditions of high temperatures and extreme environmental conditions. The experimental study will combine microbial isolation, polymer degradation experiments, and the physicochemical characterization of plastic waste to test the applicability of microbial degradation of plastics in extreme conditions (Ruggero et al., 2023). Figure 1 shows a summary of the process of the experiment that will be used in this study.

Sampling and Source of Extreme Thermophilic Bacteria

The extreme thermophilic bacteria were sourced from the environmental samples taken in naturally occurring hot conditions, which are geothermal hot springs, composting, and industrial effluent sites (Akarsu et al., 2022). It is also known that these habitats carry microbial communities that are adapted to high temperatures, and hence, the best way to isolate high-temperature-adapted thermophilic bacteria, which can be used to degrade plastic polymers. Sample collection was done aseptically, and the samples were transported to the laboratory under temperature-controlled conditions to ensure that the microbes remained alive during transportation.

Isolation and Cultivation of Thermophilic Bacterial Strains

Extreme thermophilic bacteria were cultured by centrifuging environmental samples in selective enrichment media that are specific to thermophilic bacteria (e.g., tryptic soy agar or R2A agar). The incubation temperature was set between 60°C and 80°C to encourage the growth of thermophilic bacteria while inhibiting mesophilic species. After the initial incubation, serial sub-culturing was performed to obtain pure isolates. The purity of bacterial isolates was confirmed by microscopic examination and Gram staining. Molecular identification of isolates was carried out using 16S rRNA gene sequencing to accurately identify and classify the bacterial strains (Hakakzadeh et al., 2025; Pham et al., 2024).

Preparation and Characterization of Plastic Substrates

Three types of plastic waste materials, namely polyethylene (PE), high-density polyethylene (HDPE), and polyethylene terephthalate (PET), were taken as the biodegradation substrates. Plastic samples were cut into standard pieces (1 cm x 1 cm) and were subjected to some method of surface treatment, such as washing in distilled water, 2-hour UV irradiation, and 24-hour thermal treatment at 70°C. These pretreatment procedures were meant and developed to remove surface contaminants, enhance the adhesion of microbes, and mimic the exposure to the environment. The physicochemical characteristics of the plastics, such as surface roughness (atomic force microscopy), molecular weight distribution (gel permeation chromatography), and surface functional groups (Fourier transform infrared spectroscopy, FTIR), were described before the biodegradation experiment (Goswami et al., 2024; Özdemir et al., 2022).

Biodegradation Experiments Under Thermophilic Conditions

The isolates in which experiments of biodegradation were conducted were the thermophilic bacterial strains incubated in mineral salt media (pH 7.0) under aerobic conditions. Thermophiles were used to incubate plastic samples at 60°C to 75°C for incubation times of between 1 and 6 weeks. In the absence of bacterial inoculation, control experiments were performed to take into consideration the abiotic degradation. The degradation of plastics was monitored by periodic sampling, and the influence of temperature and bacterial strain on degradation efficiency was evaluated (Li et al., 2024; Ruggero et al., 2023).

Assessment of Plastic Degradation

Plastic degradation was evaluated using the following analytical techniques:

Weight Loss Analysis: The Weights of the plastic samples were taken before and after incubating. The degree of degradation was determined by computing the percentage of weight lost.

Fourier Transform Infrared Spectroscopy (FTIR): The alterations in the functional groups on the plastic surfaces were determined with the help of the FTIR analysis, as per which the presence of the changes in the degradation of the microbes (Cazaudehore et al., 2023; Saleem et al., 2023).

Biofilm Formation and Microbial Adhesion Studies

Thermophilic bacteria were analyzed with the help of crystal violet stain under a microscope (light and fluorescence microscopy) to determine their relative ability to bind to plastic surfaces and develop biofilms. The formation of biofilm was determined by measuring the absorbance at 570nm. The correlation between the formation of biofilm and degradation efficiency was determined to gain insight into the contributions made by microbial colonization in plastic degradation (Bhattacharya & Gupta, 2022; Parray & Li, 2025).

Data Analysis and Statistical Evaluation

The experiments were conducted three times, and the results were in the form of mean ± standard deviation. The statistical procedures were used to identify significant differences between treatments and control groups using statistical measures such as one-way analysis of variance (ANOVA) and post-hoc test (Tukey). A p-value of < 0.05 showed high significance, and the correlation of the relationship between the degree of biodegradation and the factors such as bacterial strain, temperature, biofilm formation, and enzyme activity was demonstrated (Li et al., 2024; Ruggero et al., 2023).

Environmental and Biosafety Considerations

Institutional biosafety and environmental guidelines were observed for all the experimental procedures. During experimentation, microbial cultures as well as plastic substrates were sterilized to avoid contamination. The disposed waste products (disposed plastic samples and bacterial cultures) were also disposed of according to their applicable environmental laws, and the environmental impact was minimized in such a way (Goswami et al., 2024; Bhattacharya & Gupta, 2022).

Results

Isolation and Growth Characteristics of Extreme Thermophilic Bacteria

Surveys of the environment of high-temperature industrial pipeline systems produced six thermophilic strains of bacteria that were able to grow under severe thermal conditions. All the isolates exhibited steady growth at a wide temperature span (55-80 °C) with an optimum growth between 65°C and 70°C, which is typical of extreme thermophiles. Microscopic evidence showed that their morphologies were mostly Gram-positive and rod-shaped, with the only exception being a strain, TPB04, which had a Gram-negative morphology and a short-rod morphology (Table 1).

Table 1. Characteristics of extreme thermophilic bacterial strains isolated from industrial pipes

Strain ID	Sampling Source	Gram Reaction	Morphology	Growth Range (°C)	Optimum Temp (°C)
TPB01	Industrial hot-water pipe	Gram-positive	Rod-shaped	55–75	60
TPB02	Industrial steam pipe	Gram-positive	Rod-shaped	60–85	70
TPB03	Industrial effluent pipe	Gram-positive	Rod-shaped	55–70	50
TPB04	Industrial thermal pipeline	Gram-negative	Short rods	60–75	75
TPB05	High-temperature discharge pipe	Gram-positive	Rod-shaped	60–80	65
TPB06	Industrial cooling-return pipe	Gram-positive	Rod-shaped	55–65	60

The table is a summary of the sample source, Gram reaction, cell morphology, temperature range of tolerance, and optimum growth temperature of six thermophilic bacterial isolates (TPB01-TPB06) that had been obtained in an industrial high-temperature pipeline. All of the isolates were able to grow continuously at higher temperatures (≥55 °C), and the best growth was at 65-70°C, indicating great adaptation to thermophilic industrial conditions.

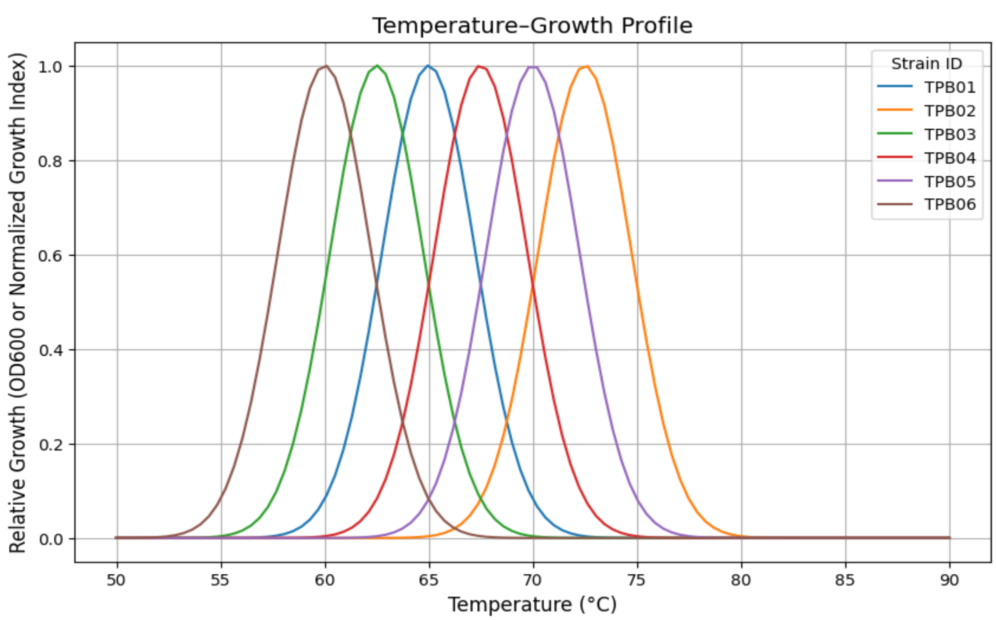

Figure 2. Growth characteristics and morphology of extreme thermophilic bacteria isolated from industrial pipelines

Figure 2 depicts normalized relative growth (OD_{600} or growth index) of six thermophilic bacterial strains (TPB01 -TPB06) under a temperature gradient (50-90°C). All the isolates demonstrate unique growth curves with respect to temperature, with maximum growth at room temperature of 65°C to 75°C. TPB02 and TPB05 can be cultivated with high temperatures (approximately 70 -75°C), but TPB01, TPB03, and TPB06 have optima around 60 - 65°C, meaning that there is variation in thermotolerance between strains. On the whole, the profiles support the Discussion of the isolates as extreme thermophiles that can be used in high-temperature industrial settings.

Weight Loss Analysis of Plastic Substrates

After incubation with thermophilic bacterial cultures, plastic substrates were biodegraded moderately but significantly. Six weeks later, the weight loss in polyethylene (PE) samples was recorded as between 1.4% and 3.8% depending on bacterial strain and incubation temperature (Table 2). The degradation profile of high-density polyethylene (HDPE) also exhibited very similar but slightly lower values of weight loss, ranging between 1.2% and 3.4% (Table 3).

In contrast, polyethylene terephthalate (PET) exhibited relatively low degradation rates, a weight reduction of 0.5% -1.5%, which implies an increased resistance to microbial attack at thermophilic conditions (Table 4). The mass loss in the control samples incubated in superficial conditions and not inoculated with bacteria showed minimal loss (less than 0.3 %) of mass at all temperatures and plastic types, which can be deemed as a confirmation that the degradation was mainly caused by the activity of the micro-organisms. In all plastic substrates, degradation rates were always higher at 70°C than at 60°C and 75°C; though these differences were not significant among all strains. TPB02 and TPB05 were the most degradable of the isolates with the highest efficiency to degrade PE and HDPE (Table 2 and Table 3).

Biofilm formation assays further supported these findings, revealing stronger surface colonization on PE and HDPE relative to PET. Strains TPB02 and TPB05 showed the highest biofilm biomass across all plastic types, suggesting a positive association between biofilm formation and plastic degradation potential under thermophilic conditions (Table 5).

Table 2. Percentage weight loss of plastic substrates after 6 weeks in polyethylene

Polyethylene (PE)	**60 °C (%)**	**70 °C (%)**	**75 °C (%)**
TPB01	1.8 ± 0.3	2.7 ± 0.4	2.3 ± 0.3
TPB02	2.3 ± 0.4	3.9 ± 0.5	3.5 ± 0.5
TPB03	1.7 ± 0.3	2.4 ± 0.3	2.0 ± 0.3
TPB04	2.0 ± 0.3	3.1 ± 0.4	2.7 ± 0.4
TPB05	2.4 ± 0.4	4.0 ± 0.6	3.8 ± 0.5
TPB06	1.6 ± 0.3	2.5 ± 0.3	2.2 ± 0.3
Control	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1

The table shows the mean percentage weight loss (± SD) of polyethylene films incubated with thermophilic bacterial strains TPB01-TPB06 at 60°C, 70°C, and 75°C for a period of six weeks. Control experiments were conducted in the absence of bacterial inoculation in order to consider the abiotic degradation.

Table 3. Percentage weight loss of plastic substrates after 6 weeks in high-density polyethylene

High-density polyethylene (HDPE)	**60 °C (%)**	**70 °C (%)**	**75 °C (%)**
TPB01	1.4 ± 0.3	2.5 ± 0.4	2.2 ± 0.4
TPB02	2.0 ± 0.3	3.5 ± 0.5	3.0 ± 0.4
TPB03	1.3 ± 0.3	2.2 ± 0.3	1.9 ± 0.3
TPB04	1.7 ± 0.3	2.8 ± 0.4	2.3 ± 0.3
TPB05	2.2 ± 0.4	3.6 ± 0.6	3.2 ± 0.5
TPB06	1.5 ± 0.3	2.4 ± 0.4	2.0 ± 0.3
Control	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

The weight loss (means ± SD) of HDPE substrates was calculated as a percent change after 6 weeks of incubation with each bacterial strain at three high temperatures (60°C, 70°C, and 75°C). The results indicate moderate yet constant bio-degradation in comparison with the abiotic controls.

Table 4. Percentage weight loss of plastic substrates after 6 weeks in polyethylene terephthalate

Polyethylene terephthalate	60 °C (%)	70 °C (%)	75 °C (%)
TPB01	0.7 ± 0.1	1.2 ± 0.2	1.1 ± 0.2
TPB02	1.0 ± 0.2	1.6 ± 0.3	1.4 ± 0.2
TPB03	0.6 ± 0.1	1.0 ± 0.2	0.9 ± 0.2
TPB04	0.8 ± 0.1	1.3 ± 0.2	1.1 ± 0.2
TPB05	1.1 ± 0.2	1.8 ± 0.3	1.5 ± 0.3
TPB06	0.7 ± 0.1	1.1 ± 0.2	1.0 ± 0.2
Control	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

The table also presents the efficiency of PET degradation in terms of mean percentage weight loss (± SD) after a long period of incubation with thermophilic bacterial strains at high temperatures. The rate of degradation of all strains at all temperatures of PET was relatively low compared to PE and HDPE.

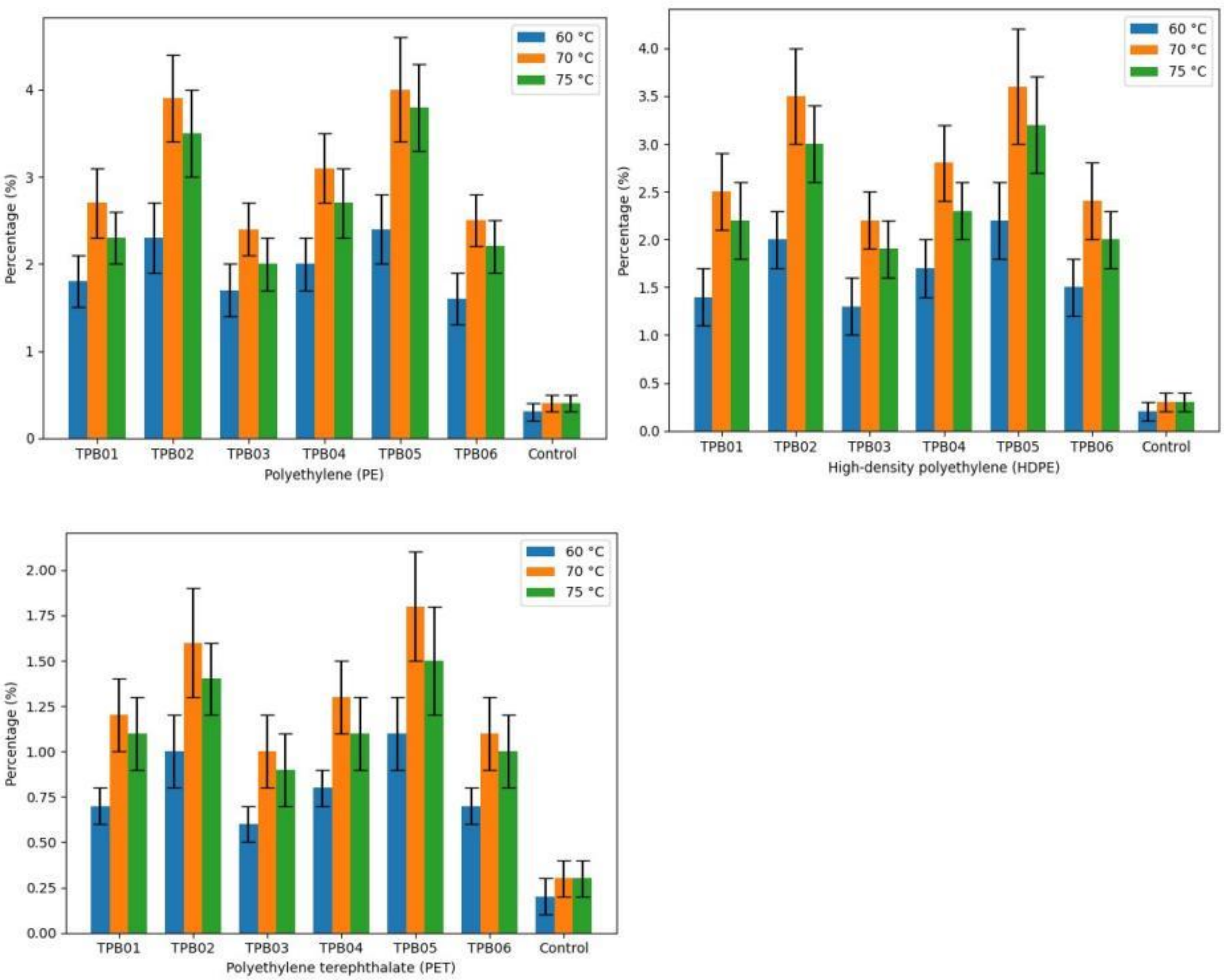

Figure 3. Weight loss of plastic substrates under thermophilic conditions

Figure 3 presents three bar graphs of the biodegradation of Polyethylene (PE), High-Density Polyethylene (HDPE), and Polyethylene Terephthalate (PET) in the presence of six thermophilic bacterial strains (TPB01-TPB06) at three temperatures (60°C, 70°C, and 75°C). The graphs show the percentage weight loss of each plastic substrate after incubating it for six weeks. PE and HDPE are more degraded than PET and TPB02, and TPB05 strains exhibit the highest degree of biodegradation, especially at 70°C.

Table 5. Biofilm Formation on Plastic Surfaces (OD_{570})

Strain	**PE**	**HDPE**	**PET**
TPB01	0.42 ± 0.04	0.38 ± 0.03	0.24 ± 0.03
TPB02	0.61 ± 0.05	0.55 ± 0.04	0.36 ± 0.04
TPB03	0.39 ± 0.04	0.35 ± 0.03	0.22 ± 0.03
TPB04	0.48 ± 0.05	0.44 ± 0.04	0.30 ± 0.03
TPB05	0.62 ± 0.05	0.57 ± 0.05	0.38 ± 0.04
TPB06	0.41 ± 0.04	0.37 ± 0.03	0.25 ± 0.03

The thickness of biofilm on PE, HDPE, and PET surfaces inoculated with thermophilic bacterial strains expressed as optical density at 570 nm (mean ± SD). Greater biofilm growth on PE and HDPE indicates that they have a higher colonization of the surface than PET.

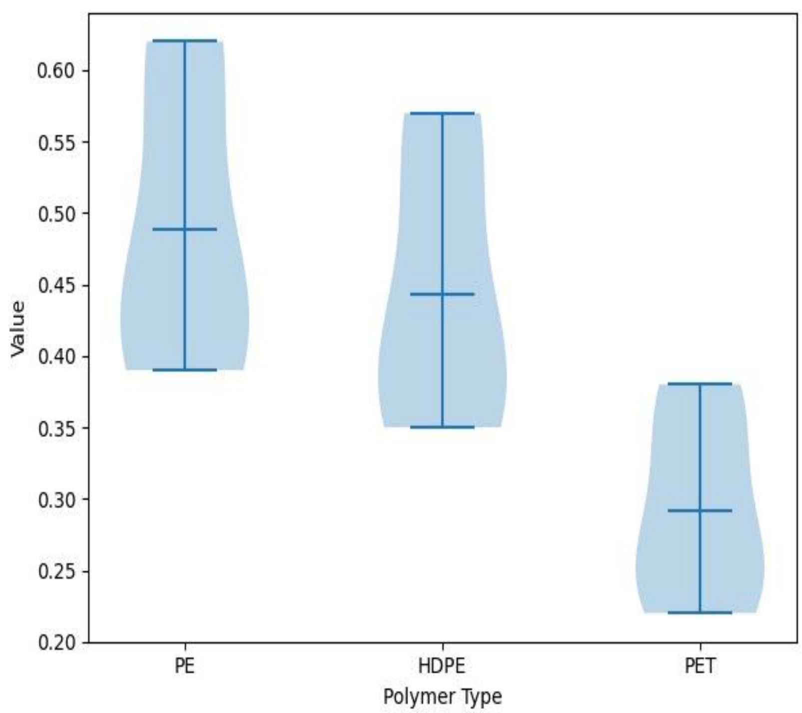

Figure 4. Biofilm formation of thermophilic bacterial isolates on different plastic surfaces

The violin plot presented in Figure 4 shows the degradation values of three kinds of plastic substrates, consisting of Polyethylene (PE), High-Density Polyethylene (HDPE), and Polyethylene Terephthalate (PET). The plot of degradation values of each violin plot displays the distribution of degradation values of each type of polymer, and the width of each plot is the number of data points at different degradation levels. Horizontal lines, which are the median values of each polymer, are also used in the plot. Based on the plot, PE and HDPE demonstrate rather higher values of degradation than PET, meaning that these polymers have dissimilar aptitudes to degradation by bacteria.

Strain Performance and Biodegradation Potential

Out of the six thermophilic strains used, TPB02 and TPB05 were the only two strains that showed the maximum degradation potential of Polyethylene (PE) and High-Density Polyethylene (HDPE). These strains exhibited the highest weight loss at the temperatures tested (60°C, 70°C, and 75°C), and TPB02 was the best at 70°C, whereas TPB05 had the highest degradation rates at all the temperatures.

For PE: TPB02 was the most degraded with a weight loss of 2.3%, 3.9% and 3.5% at 60°C, 70°C, and 75°C, respectively. TPP05 gave other similar results of 2.4%, 4.0%, and 3.8% at the same temperatures.

For HDPE: TPB02 recorded the best degradation with the values of 2.0%, 3.5%, and 3.0% as the weight loss, and TPB05 also had 2.2%, 3.6%, and 3.2%.

These data show that TPB02 and TPB05 are the most potent strains in the biodegradation of PE and HDPE, and that TPB02 is slightly superior at the elevated temperatures. They also show a positive relationship between biofilm density and biodegradation efficiency (their increased biofilm formation on PE and HDPE surfaces).

FTIR Analysis of Plastic Degradation

FTIR spectral analysis showed that there were specific chemical changes on the surfaces of plastic substrates after exposure to the bacterial cultures that were thermophilic. Biodegraded plastics exhibited consistent variations in characteristic absorption bands relative to untreated and abiotic control samples, with the mediation of surface variations by biology.

In the case of polyethylene (PE) and high-density polyethylene (HDPE), a rise of carbonyl (C=O) stretching vibrations (~1715 cm^{-1}) and hydroxyl (-OH)-stretching bands (~3300 cm^{-1}) was noticed, which indicated that the polymer chains were oxidatively modified during thermophilic incubation. HDPE also exhibited a slight decrease in C H bending vibrations (~1470 cm^{-1}), which is evidence of early-stage chain scission reactions (Table 6).

On the contrary, polyethylene terephthalate (PET) samples showed only a weak spectral variation, such as a slight reduction of the intensity of the ester carbonyl peaks (~1730 cm^{-1}) and a slight change in C-O stretching (~1100 cm^{-1}), which indicates a low hydrolytic activity under the conditions of the test (Table 6). These FTIR results, in general, show that the surface-level oxidative and hydrolytic alterations of thermophilic bacterial activity occur instead of more basic polymer backbone degradation, which is consistent with early-stage thermophilic biodegradation.

Table 6. FTIR Functional group changes after biodegradation

Plastic	Functional Group	Wavenumber (cm^{-1})	Change Observed	Interpretation
PE	C=O stretch	~1715	Slight increase	Surface oxidation
PE	–OH stretch	~3300	Moderate increase	Oxidative modification
HDPE	C–H bending	~1470	Minor reduction	Chain scission
PET	Ester C=O	~1730	Slight decrease	Limited hydrolysis
PET	C–O stretch	~1100	Minor shift	Early ester bond cleavage

The table gives key functional groups of change as can be observed in high-density polyethylene (HDPE), polyethylene (PE), and polyethylene terephthalate (PET) due to thermophilic biodegradation. Alteration of typical absorption bands, such as carbonyl, hydroxyl, and esthetic bands, depicts oxidative and restricted hydrolytic alterations of polymer surfaces as compared to untreated and abiotic control samples.

Biofilm Formation on Plastic Surfaces

Crystal violet staining further indicated that all thermophilic isolates developed biofilms on plastic substrates with different densities of the biofilm. vPE and HDPE surfaces were selective to grow moderate biofilm with a value of optical density (OD570) of 0.35-0.62. PET surfaces showed lower biofilm formation (OD570: 0.20–0.38). A positive correlation was observed between biofilm density and plastic weight loss, particularly for PE substrates. Strains with a greater biofilm formation were more likely to give a higher degradation value, indicating that biofilm formation and adhesion to microbes facilitate the thermophilic biodegradation of plastics.

Influence of Temperature on Biodegradation Efficiency

The incubation at 70°C led to a slightly higher weight loss and biofilm formation than at 60°C, and the performance decreased to some extent at 75°C in some of the isolates, which may be because of the thermal stress. These results suggest that the thermophilic biodegradation process has an optimum temperature range within which it performs, rather than increasing directly with the temperature.

Statistical Analysis

One-way analysis of variance (ANOVA) demonstrated that plastic weight loss in inoculated samples was significantly higher than in abiotic controls across all plastic types and incubation temperatures ($p < 0.05$). Differences among bacterial strains and temperature conditions were observable but comparatively moderate. The post-hoc test by Tukey found that strains TPB02 and TPB05 caused a much higher degradation of polyethylene (PE) and high-density polyethylene (HDPE) in comparison with other strains, especially at 70 °C ($p < 0.05$). Conversely, polyethylene terephthalate (PET) degradation did not reveal any significant differences between pairs within the group.

The biofilm formation was also significantly different among different bacterial strains and plastic surfaces (ANOVA, $p < 0.05$), with the TPB02 and TPB05 strains having the largest biofilm biomass on PE and HDPE. Regression analysis showed weak to moderate positive correlations between biofilm density and plastic weight loss (R^2 = 0.45 -0.58), which shows that biofilm formation is a factor that can determine the biodegradation efficiency, but not the only factor that determines thermophilic plastic degradation. This relationship was measured by the strength of the coefficient of determination (R^2), which was determined as given in Equation (1):

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (1)$$

Where yi represents the values of the observed plastic weight loss, $\hat{y}_i$ represents the values that are predicted by the regression model, $\bar{y}$ depicts the mean of the observed values, and n is the number of observations.

Discussion

This paper examined the biodegradation capabilities of thermophilic bacterial isolates found in high-temperature industrial pipeline systems with the aim of examining their capacity to degrade Polyethylene (PE), High-Density Polyethylene (HDPE), and Polyethylene Terephthalate (PET). The findings affirm that TPB02 and TPB05 are the most apt ones that can be employed to biodegrade PE and HDPE under a thermophilic environment. The isolates were found to have a stable growth within a wide temperature range of (55-80°C), but optimally at (65°C-70°C). This is characteristic of extreme thermophiles that are able to survive in high temperatures. The strains were Gram-positive and rod-shaped, with a majority of the strains being Gram-negative: short-rod morphologies indicating strain-specific morphological diversity. TPB02 and TPB05 were found to grow with high temperature (around 70°C to 75°C), hence they are particularly fit for thermophilic biodegradation.

The results of biodegradation were that PE and HDPE were more prone to attack by microorganisms than PET, and more weight loss was recorded in the former two plastics. The findings were that PE and HDPE depicted significant weight loss (1.4% -4.0%), whereas PET was less degraded in terms of its weight (0.5% - 1.8%). The present results are in agreement with the earlier reports that found that PET does not degrade easily by microbes based on its chemical composition. PE and HDPE are more vulnerable to oxidative and hydrolytic degradation, and hence, they biodegrade better in thermophilic temperatures, particularly at 70°C. The biofilm formation studies revealed that TPB02 and TPB05 strains produced the highest biofilm biomass on PE and HDPE surfaces and, therefore, demonstrated that biofilm formation and degradation potential had a positive correlation. Formation of biofilm leads to improved adhesion and degradation of hydrophobic surfaces such as plastics, and for strains that formed stronger biofilms, they degraded them faster. This brings into light the significance of biofilm formation in the process of biodegradation.

The analysis of the FTIR also supported the findings of biodegradation, i.e., when subjected to oxidative and hydrolytic stress, significant changes in the surface occurred in PE and HDPE due to the activity of the bacteria. The general absorptions of the carbonyl (C=O) and hydroxyl (–OH) stretching were also increasing, and that is a sign of the oxidation of the polymer chains. PET, on the other hand, had a low spectral response, implying that it was not sensitive to the change of microbes. The biodegradation process also employed temperature, with 70°C being the optimum temperature to degrade plastics since more weight was lost and more biofilm was formed at 70°C. However, the activity of some strains decreased at 75°C, which means that the thermophilic bacteria are adapted to high temperatures, but high temperatures can cause thermal stress and slow down the activity of microbes. In general, this paper indicates that TPB02 and TPB05 are very efficient strains that can be used in the biodegradation of PE and HDPE and could be used in the bioremediation of plastic waste under thermophilic conditions.

Conclusion

The paper illustrates that thermophilic bacteria strains obtained after high-temperature industrial pipeline systems can be used to biodegrade Polyethylene (PE), High-Density Polyethylene (HDPE), and Polyethylene Terephthalate (PET). Out of the six isolates tested, there was high efficiency of biodegradation of TPB02 and TPB05, especially of PE and HDPE, at temperatures of 60°C, 70°C, and 75°C. ANOVA- one-way showed that all inoculated treatments had a significant plastic weight loss compared to all abiotic controls ($p < 0.05$), which indicated the biological source of degradation. Post-hoc analysis also found that TPB02 and TPB05 had a larger effect on PE and HDPE degradation, particularly at 70°C ($p < 0.05$), but no statistically significant degradation in PET. The control of biofilm formation varied greatly among strains and plastic types ($p < 0.05$), and biofilm biomass was greater on the surfaces of PE and HDPE. The regression analysis indicated a positive moderate relationship between biofilm density and plastic weight loss (R2 = 0.45 -0.58), which means that the surface colonization positively affects biodegradation, but does not entirely control it. These quantitative results were corroborated by FTIR analysis, showing oxidative and hydrolytic surface changes in PE and HDPE, and PET, showing little chemical change. Optimization in temperature conditions revealed the highest biodegradation at 70°C, with decreased efficiency at 75°C, possibly as a result of thermal stress. Future studies need to find out the most important enzymes and metabolic routes of thermophilic plastic degradation, how to enhance the susceptibility of PET to degradation by pretreatment or microbial consortia, and the long-term degradation rates. Enhancing these statistically optimized processes in bioreactors and in industry is a promising future of sustainable plastic waste management in extreme environmental conditions.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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