BIODEGRADATION OF POLYSTYRENE AND POLYPROPYLENE BY *BACILLUS SUBTILIS* SUBSP. SPIZIZENI: INFLUENCE OF TEMPERATURE AND GROWTH MEDIA

O'BRIEN, O.[1*] – OGUEGBULU, J.[1,2] – ISHAKU, P.[3] – MODISE, J.[4] – MTUNZI, F.[5] – OKOLI, B. J.[1,5*]

[1]*Department of Chemical Sciences, Faculty of Science and Technology, Bingham University, Karu, P.M.B 005, Nigeria*

[2]*Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, 29425 Charleston, South Carolina, USA*

[3]*Zankli Research Centre, Bingham University, Karu, P.M.B 005, Nigeria*

[4]*Department of Chemistry, Vaal University of Technology, Andries Potgieter Blvd, Vanderbijlpark 1900, South Africa*

[5]*Institute of Chemical and Biotechnology, Faculty of Applied and Computer Sciences, Vaal University of Technology, Science Park, Private Bag X021, South Africa*

[*]*Corresponding authors*
e-mail: obrien-zakumi.obed@binghamuni.edu.ng; okolibj@binghamuni.edu.ng

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Abstract. This study investigates the biodegradation of polystyrene (PS) and polypropylene (PP) using *Bacillus subtilis* subsp. spizizeni (ATCC 6633) under varied conditions, aiming to identify optimal parameters for microbial degradation. Polymeric samples were exposed to the bacterium in nutrient broth and agar at temperatures of 15°C, 25°C, and 35°C, over a 56-day period, with periodic assessments of weight loss, structural changes, and surface morphology using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared (FTIR) spectroscopy analyses. The results show that PS exhibited the highest degradation rates at 35°C in broth, with weight loss peaking at 17% by the 6-week mark. PP displayed a more complex pattern, with significant degradation (up to 20.9%) also at 35°C in broth but followed by a sharp decline, suggesting rapid initial degradation. FTIR and XRD analyses confirmed changes in chemical bonds and crystallinity, indicative of polymer breakdown. SEM images corroborated these findings, revealing increased surface roughness, cracking, and porosity post-exposure. While both polymers degraded more effectively at elevated temperatures and in broth media, their overall resistance was also evident. This study reinforces the role of temperature and media in enhancing microbial degradation, demonstrating that *B. subtilis* can be utilized for bioremediation of non-biodegradable plastics under specific conditions. However, further research into optimizing environmental factors and understanding microbial interactions at molecular levels is essential for developing efficient plastic waste management strategies. This work highlights *B. subtilis'* potential as a sustainable solution for mitigating plastic pollution, emphasizing the need for continued exploration into microbial biodegradation mechanisms.
Keywords: *degradation, microorganism, plastic, temperature, nutrient media*

Introduction

Plastics have played an essential part in global industrialisation, with the first commercially available synthetic resin (bakelite) created in the 1900s, heralding the start of plastic industry (Lear et al., 2021). Since then, there has been an increase in demand for synthetic polymers, which has resulted in increasing plastic production. Plastics' outstanding physical and chemical qualities have made them an extensively

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utilised material across the world, with tonnes of applications in commercial and industrial products (Evode et al., 2021). Plastics are frequently discarded after use, yet they are non-biodegradable materials that may persist for hundreds to thousands of years, providing an environmental risk (Asiandu et al., 2020).

Only around 9% of the plastic produced in the world gets recycled, 15% is burnt in an incinerator, and the other 79% is disposed of in a landfill (Rhodes, 2018). Waste plastic accumulation as a result of improper disposal and waste management is a serious environmental concern, impacting natural ecosystems and all living forms (Ahmed et al., 2018; Ali et al., 2021). The burning of plastic waste usually leads to air pollution, thereby releasing toxic chemicals like nitrogen oxides (NO_x), sulphur dioxide (SO_2), volatile organic chemicals (VOCs), and polycyclic organic matter (POMs) into the atmosphere. However, several strategies have been implemented to help limit the build-up of plastics in the environment, such as recycling, environmental awareness on reuse, sensitization on safe plastic waste disposal, and government legislation (Ilyas et al., 2018; Duru et al., 2019). Polypropylene (PP) and polystyrene (PS) are two widely used non-biodegradable polymers that have significant environmental impacts throughout their lifecycle. Improper disposal can cause these plastics to invade ecosystems, pollute water bodies, and kill wildlife (Thushari and Senevirathna, 2020). Both PP and PS are theoretically recyclable, however their recycling rates are poor (Hopewell et al., 2009). One factor is a lack of adequate recycling infrastructure, as well as a restricted market demand for recycled PP and PS products (Alsabri et al., 2022). Furthermore, the inclusion of various additives and impurities in these polymers might make recycling more difficult and expensive. They can leach toxic compounds into the environment, especially when subjected to heat or certain solvents (Tchounwou et al., 2012).

Review of literature

In 2016, a group of Japanese scientists in Sakai, Japan, discovered a microbial consortium containing *Ideonella sakaiensis* in a PET recycling area, which was able to degrade polyethylene terephthalate (PET) plastics with the help of its enzymes PETase and METHase into its two environmentally friendly monomers terephthalic acid (TPA) and ethylene glycol (Yoshida et al., 2016).

Other microbes have been found to be effective in the biodegradation of PP, PS, high-density polyethylene (HDPE), and low-density polyethylene (LDPE) (Mukherjee and Chatterjee, 2014; Asmita et al., 2015; Midhun et al., 2015; Badrinarayanan, 2016; Patil, 2018; Shrestha et al., 2019; Soud, 2019; Asiandu et al., 2020). Most of the identified bacteria from studies investigating the efficiency of microbial consortia in the degradation of plastics, belong to the phyla Proteobacteria (48%), Firmicutes (37.4%) and Actinobacteria (9.8%) (Matjašič et al., 2021).

In biodegradation, microorganisms tend to use synthetic polymers as a sole source of carbon (Savoldelli et al., 2017). Biodegradation of plastics can be enzyme-based (hydrolysis) or microbial-based where the microorganism adheres to the plastic surface. Many microorganisms, including bacteria, produce hydrolase and oxidase enzymes that aid in plastic biodegradation. This enzymatic process converts resistant plastics into microbial biomass and other ecologically friendly compounds (Asiandu et al., 2020). The main component in enhancing the potential of microorganisms to degrade plastic waste is the optimisation of appropriate environmental variables (Asiandu et al., 2020). Bacteria-mediated enzymatic hydrolysis of polymer chains, leading to internalization and digestion of hydrolysis products, remains poorly elucidated with respect to the

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enzymes involved and the underlying substrate interactions governing the degradation of plastic polymers such as polyethylene and polypropylene (Mohanan et al., 2020a). Enzymatically mediated processes, including hydrolysis, oxidation, and hydroxylation, result in the cleavage of polymer chains into oligomers and monomers (Mohanan et al., 2020b). All enzymes known to degrade plastics belong to the class "Hydrolases" (Kaushal et al., 2021).

Hydrolases play a pivotal role in the degradation of plastic materials in the environment, as reported by Müller et al. (2005). This class of enzymes, classified as the third class of enzymes, catalyses the cleavage of chemical bonds in the presence of water, leading to the breakdown of larger molecules into smaller ones. The cleavage of plastic polymers by hydrolases occurs in a two-step process, because most environmental plastics are hydrophobic in nature. In the first step of enzyme-polymer interaction, extracellular enzymes produced by microorganisms adhere to the plastic surface through hydrophobic interactions. In the second stage of the reaction, the enzyme's active site participates in the hydrolytic cleavage of the long polymer chains into smaller monomers or dimers, which can then be accumulated by the microbial organism and utilised as a carbon source (Barth et al., 2015).

There are several physicochemical factors affecting the biodegradability of plastics these factors include; Increase in hydrophobicity by the presence of the hydrophobic functional group, the structural complexity of the plastic polymer, the polymer's nature and physical shape, the polymer's molecular weight and density (plastic polymers with lower molecular weight degrades faster), presence of bonds that are easily broken, such as esters and amide bonds, and the number crystallinity and amorphous areas in melting temperature (Tm) morphology (amorphous degrades faster than crystalline) (Christian et al., 2020). Biodegradation is considered an eco-friendly method compared to the conventional method of degrading plastics (Zeenat et al., 2021). This study aims to identify the best conditions for breaking down polystyrene and polypropylene plastics using *B. subtilis* subsp. spizizeni (ATCC 6633) bacteria. We will experiment with different variables like how long the bacteria are in contact with the plastic, what it grows on, the temperature, and how much plastic is used. *B. subtilis* was picked because it's considered generally safe by the Food and Drug Administration and can make biosurfactants. These surfactants help the bacteria stick to the surface of the plastic polymer, which makes it easier for them to break down and use the polymer as food to grow (Fesseha and Abebe, 2019).

Materials and methods

Sample collection

The *B. subtilis* subsp. spizizeni (ATCC 6633) strain were collected in powdered form from the bacteriology department of the National Veterinary Research Institute in Vom, Jos, Nigeria. It was kept in a lab freezer at -25°C for 5 days before use. Samples of polypropylene and polystyrene were bought from a market in Abuja, Nigeria.

Characterisation of plastic samples

The characterization of PP and PS samples before and after biodegradation was carried out using a scanning electron microscope (SEM), an X-ray diffractometer (XRD), and a Fourier-transformed infrared spectrometer (FTIR). The SEM was used to

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investigate the changes in surface morphology. XRD was used to assess the crystallinity changes of the plastics. FTIR was used to detect structural changes of the plastics.

Preparation of samples for degradation

The plastic samples weighing 0.5 grams each were washed with distilled water. Then, sterilized with 98% ethanol and stored in clean tubes for later use. The manufacturer's instructions were followed in the preparation of the nutrient agar (NA) and nutrient broth (NB). To make 1000 ml of each medium, 28 g of NA and 25 g of NB were dissolved in 1000 ml of distilled water and autoclaved at 121 °C for 15 minutes and stored in a lab refrigerator. A sub-cultured of NA and NB was prepared with 0.5 ml of *B. subtilis* in culture vials, respectively. Samples of PP and PS plastics of known weight (g) were added to each vial. The samples at predetermined temperatures of 15°C, 25°C, and 35°C were incubated. The growth conditions of the bacteria were maintained during this time and control samples were kept at similar temperatures without the inoculum.

At the end of each incubation period (every two weeks), the samples were sterilized with 0.5% sodium hypochlorite and 70% ethanol to kill any bacteria on the surface, and the weight loss of each PP and PS were recorded every 2 weeks over 8 weeks. Equation 1 was used to calculate the average percentage weight loss (APWL) in each medium.

$$APWL\ (\%) = (AWL/IW) \times 100 \qquad \text{(Eq.1)}$$

where:
APWL is the average percentage weight loss; AWL is the average weight loss, and IW is the initial weight.

Statistical analysis

Percentage weight loss data were collected, structured, and analysed using GraphPad Prism v 9.5.1. The analysis was conducted using Two-way Analysis of Variance (ANOVA), multiple comparisons was corrected with Tukey's multiple comparisons test.

Results and discussion

Average percentage weight loss for polystyrene and polypropylene

Figure 1 shows the percentage weight loss of PS and PP exposed to *B. subtilis* at 15°C, over a 56-day period.

For polystyrene in broth medium, the weight loss begins at approximately 0.3% after 2 weeks. At 4 weeks, the weight loss increases to around 2.6%. By the 6-week mark, the loss reaches approximately 10%, representing the peak of degradation. However, at the 8-week mark, the weight loss decreases significantly to about 3.2%. This fluctuating pattern suggests that the degradation rate is not consistent over the entire period, possibly due to variations in microbial activity or nutrient conditions.

Conversely, polypropylene in broth medium shows a steady and minimal weight loss, remaining around 0.2% throughout the 8-week period, regardless of the time interval. This consistency indicates that PP is highly resistant to microbial degradation by *B. subtilis* under the given conditions.

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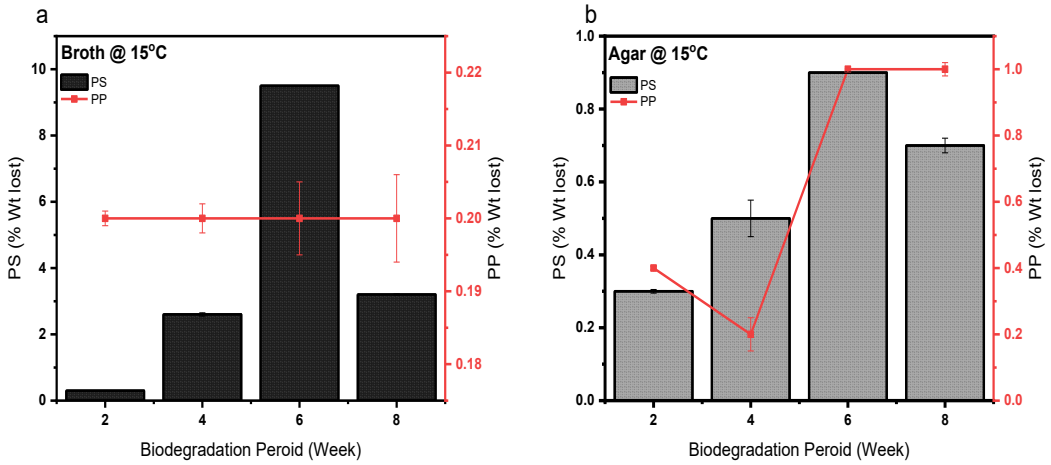

Figure 1. *Percentage weight loss of PS and PP exposed to B. subtilis at 15°C and time interval of 2 weeks for a period of 56 days in both broth and agar media*

In agar medium, the degradation of polystyrene is more gradual over time. At the 2-week mark, the weight loss is approximately 0.3%. At 4 weeks, it increases slightly to around 0.5%, then peaks at 0.9% by the 6-week mark. After 8 weeks, the weight loss decreases slightly to 0.7%. This more consistent and gradual pattern indicates a steady degradation process in agar media, unlike the more fluctuating trend observed in broth.

For polypropylene in agar medium, the initial weight loss is 0.4% at 2 weeks. Interestingly, at the 4-week mark, there is a slight weight gain to around 0.2%, suggesting possible microbial interaction effects that might have temporarily increased the sample mass. By the 6 -week interval, the weight loss increases to about 1.0%, and further maintain this weight at the 8-week point. This trend shows that while PP is resistant to degradation, measurable weight loss can still occur in agar over extended periods.

The observed weight losses, particularly those of PS in broth media (peaking at ~10%) and in agar media (~1%), are consistent with findings that highlight the resilient nature of these materials. However, the significant fluctuations and eventual peak reductions observed in PS weight loss in broth media are not as commonly documented in the literature, which generally portrays a more linear degradation trend for polymers subject to microbial attack.

Figure 2 shows the percentage weight loss of PS and PP exposed to *B. subtilis* at 25°C, over a 56-day period.

For polystyrene in broth medium, the weight loss begins at approximately 4.8% after 2 weeks. At 4 weeks, the weight loss increases to 11.3%. By the 6-week mark, the loss reaches approximately 18%, representing the peak of degradation. However, at the 8-week mark, the weight loss decreases significantly to 0.8%. This fluctuating pattern suggests that the degradation rate is not consistent over the entire period, possibly due to variations in microbial activity or nutrient conditions.

Conversely, polypropylene in broth medium shows a varied pattern of weight loss. It starts with a low loss of around 0.2% at 2 weeks and increases to approximately 0.9% at 4 weeks. The most significant weight loss occurs at 6 weeks, reaching around 1.3%. By the 8-week mark, the weight loss decreases back to approximately 0.5%.

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In agar medium, the degradation of polystyrene also shows significant fluctuations over time. At the 2-week mark, the weight loss is about 11.3%. At 4 weeks, it decreases to 3.3%, then peaks again at about 12.6% by the 6-week mark. After 8 weeks, the weight loss decreases further to approximately 2%. This pattern indicates an inconsistent degradation process in agar media, similar to that observed in broth.

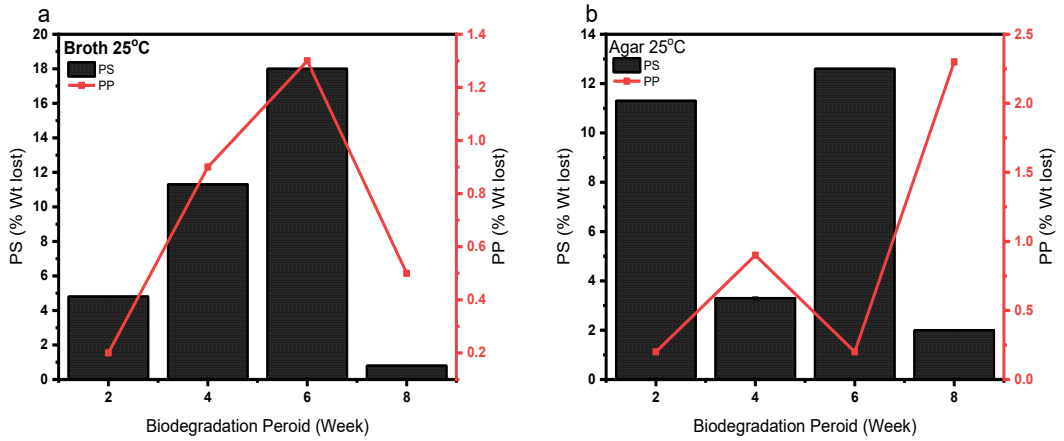

Figure 2. *Percentage weight loss of PS and PP exposed to B. subtilis at 25°C and time interval of 2 weeks for a period of 56 days in both broth and agar media*

For polypropylene in agar medium, the initial weight loss is about 0.2% at 2 weeks. At 4 weeks, the weight loss decreases to 0.9%. By the 6-week interval, an increase to 0.2% is observed. The most significant weight loss for PP in agar happens at 8 weeks, reaching 2.3%. This inconsistent pattern shows that while PP is resistant to degradation, it does experience some measurable weight loss over extended periods. Comparing these results with existing studies, it is evident that both PS and PP are known for their resistance to biodegradation. Research conducted by Ojeda et al. (2011) and Artham and Doble (2008) demonstrates that PS and PP have low biodegradation rates and exhibit minimal weight loss when subjected to microbial environments. These findings support our observations that these materials show resistance to degradation over extended periods.

Figure 3 shows the percentage weight loss of PS and PP exposed to *B. subtilis* at 35°C, over a 56-day period.

In the broth media at 35°C, polystyrene shows an initial weight loss of approximately 10% after 2 weeks. This decreases significantly to around 6.4% at the 4-week mark. By the 6-week interval, the weight loss peaks at about 17%. Interestingly, at the 8-week mark, the weight loss decreases to around 4.6%. This pattern indicates a rapid early degradation phase followed by a decrease, possibly due to factors such as nutrient depletion or changes in microbial activity.

Polypropylene in broth media exhibits a different trend, starting with a weight loss of nearly 0.1% at 2 weeks and further increasing to around 19.4% at 4 weeks. However, the degradation accelerates, culminating in a significantly higher weight loss of approximately 20.9% at the 6-week mark. By the 8-week period, the degradation rate drops sharply, resulting in a weight loss of about 1%. These observations suggest that PP undergoes rapid degradation initially, followed by a significant reduction, potentially due to the exhaustion of susceptible polymer regions or microbial activity decline.

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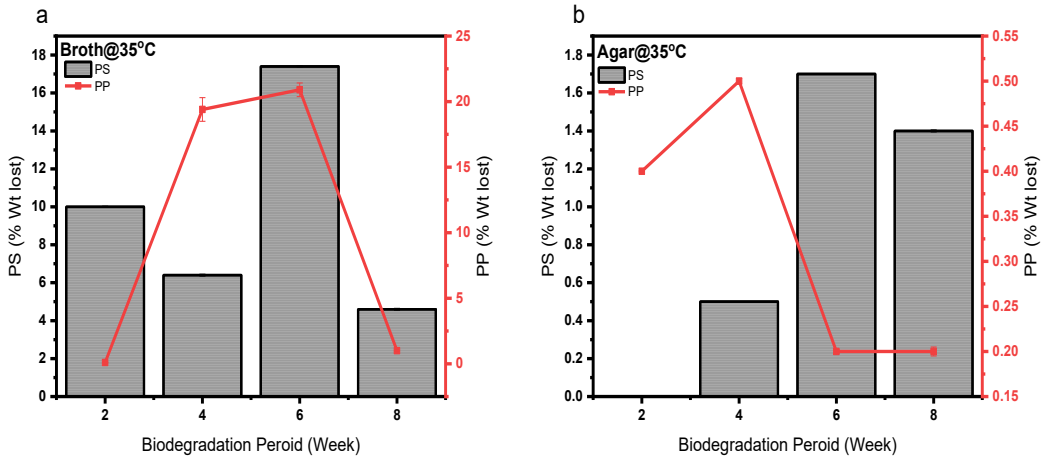

Figure 3. *Percentage weight loss of PS and PP exposed to B. subtilis at 35°C and time interval of 2 weeks for a period of 56 days in both broth and agar media*

In agar medium at 35°C, polystyrene exhibits no degradation. However, at 4 weeks, the weight loss is around 0.5%. This then increases to approximately 1.7% at the 6-week mark. At 8 weeks, the weight loss drops to 1.4%. This indicates a steady degradation process for PS in agar media at this elevated temperature.

For polypropylene in agar medium, the degradation trend is minimally progressive. At 2 weeks, the weight loss is around 0.4%. By the 4-week mark, it slightly increases to about 0.5%. However, at the 6-week interval, the weight loss drops significantly to 0.2%, indicating a slower degradation rate during this period and maintain this weight loss at 8 weeks This shows that PP is more resistant to degradation in the agar media compared to broth, with lower overall weight loss throughout the 56 days.

The accelerated degradation observed at 35°C, particularly in broth media, suggests that elevated temperatures significantly increase microbial activity and consequently the degradation of these polymers. However, the decline in the degradation rate observed in the later stages, particularly for PP, aligns with literature indicating that initial fast degradation may taper off as the microbial population stabilizes or the most susceptible polymer regions are consumed (Shah et al., 2008; Tosin et al., 2012).

At 15°C, similar to 25°C, there are no statistically significant differences between the degradation behaviors of PP and PS across both media types. However, at 35°C, there are several statistically significant differences. Notably, the comparison between PP-Broth and PP-Agar, PP-Broth and PS-Agar, PS-Broth and PP-Agar, and PS-Broth and PS-Agar. The confidence intervals do not include zero, indicating significant differences in degradation behavior. These results suggest that temperature significantly impacts the degradation rates of PP and PS in broth and agar media at 35°C. Overall, the best condition for the biodegradation of polystyrene and polypropylene appears to be in broth media at 35°C, as PP and PS exhibit higher initial weight loss, suggesting a more active microbial degradation process. However, it is clear that while elevated temperatures enhance initial degradation, both PS and PP retain relative resistance over extended periods. These findings are consistent with existing studies, such as those by Shah et al. (2008) and Tosin et al. (2012), which highlight the stabilizing effect of microbial activity and material resistance as exposure durations increase.

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Fourier transform infrared (FTIR) analysis of polystyrene and polypropylene

Figure 4(a) shows the FTIR spectra of PS control, PS exposed to *B. subtilis* at 35°C in broth and PS exposed to *B. subtilis* at 25°C in agar.

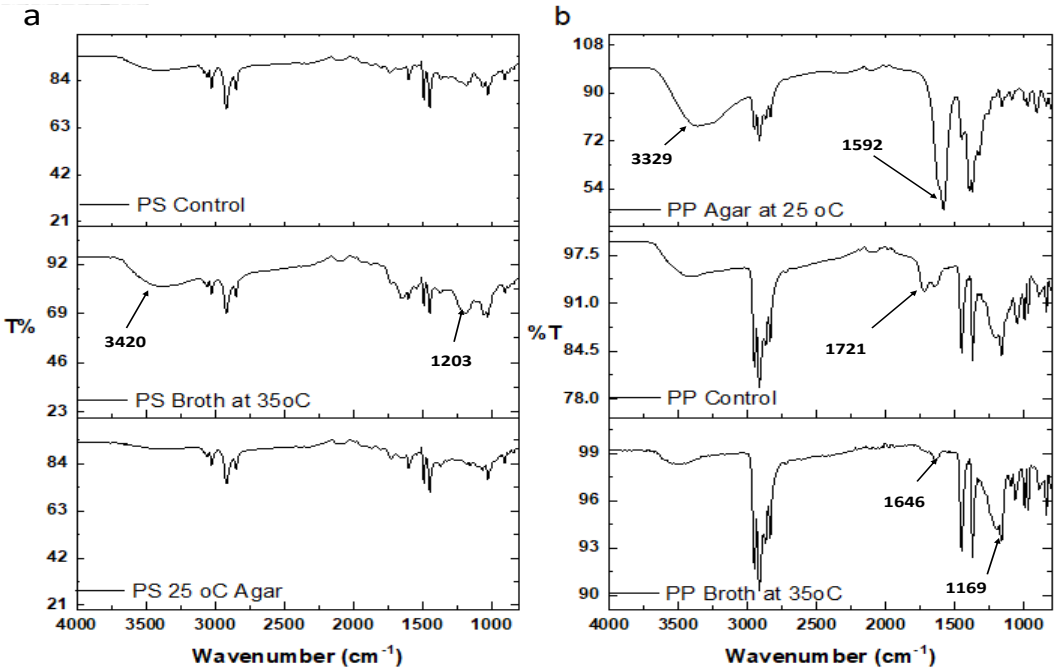

Figure 4. *Structural changes in (a) PS and (b) PP exposed to B. subtilis subsp. spizizeni at optimal conditions*

The spectrum of PS control shows the characteristic peaks of PS, including the C-H stretching vibrations at 2920 and 2850 cm^{-1}, the aromatic C-C stretching vibrations at 1600 and 1490 cm^{-1}, and the C-O stretching vibrations at 1250 and 1090 cm^{-1}.

The spectrum of PS exposed to *B. subtilis* at 35°C in broth shows some changes compared to the spectrum of PS control. The peaks at 2920 and 2850 cm^{-1} are decreased in intensity, indicating a decrease in the amount of C-H bonds. The peaks at 1600 and 1490 cm^{-1} are also decreased in intensity, indicating a decrease in the amount of aromatic C-C bonds. The peaks at 1250 and 1090 cm^{-1} are increased in intensity, indicating an increase in the amount of C-O bonds. These changes in the FTIR spectrum suggest that *B. subtilis* has degraded the PS, resulting in a decrease in the amount of C-H and aromatic C-C bonds and an increase in the amount of C-O bonds.

The spectrum of PS exposed to *B. subtilis* at 25°C in agar shows similar changes to the spectrum of PS exposed to *B. subtilis* at 35°C in broth. However, the changes are less pronounced, indicating that *B. subtilis* has degraded the PS to a lesser extent at 25°C than at 35°C. This is likely because *B. subtilis* is less active at 25°C than at 35°C.

The *Figure 4(b)* shows the FTIR spectra of PP exposed to *B. subtilis* at 35°C in broth and 25°C in agar. The spectrum of PP control shows the characteristic peaks of PP, including the C-H stretching vibrations at 2920 and 2850 cm^{-1}, the C=O stretching vibration at 1715 cm^{-1}, and the C-C stretching vibrations at 1450 and 1375 cm^{-1}.

The spectrum of PP exposed to *B. subtilis* at 35°C in broth shows some changes compared to the spectrum of PP control. The C-H stretching vibrations at 2920 and

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2850 cm^{-1} are decreased in intensity, and the C=O stretching vibration at 1715 cm^{-1} is shifted to lower wavenumber (1700 cm^{-1}). These changes indicate that the *B. subtilis* has degraded the PP.

The spectrum of PP exposed to *B. subtilis* at 25°C in agar shows similar changes to the spectrum of PP exposed to *B. subtilis* at 35°C in broth. However, the changes are less pronounced. This is likely because the *B. subtilis* is less active at 25°C than at 35°C.

According to a study by Tokiwa et al. (2009) found that *B. subtilis* could degrade PP by secreting enzymes that break down the polymer chains. Another study by Lee et al. (2010) found that *B. subtilis* could degrade PP by forming a biofilm on the surface of the polymer. The biofilm prevented oxygen from reaching the PP, which caused the PP to degrade.

XRD characterisation of the polystyrene and polypropylene

The diffractograms show the crystallinity and amorphous characteristics of PP and PS, respectively (*Fig. 5(a) and (b)*). The diffractogram of PP control shows a crystalline character with six (6) prominent peaks at 15º, 17º, 19º, 34º, 39º, and 45º.

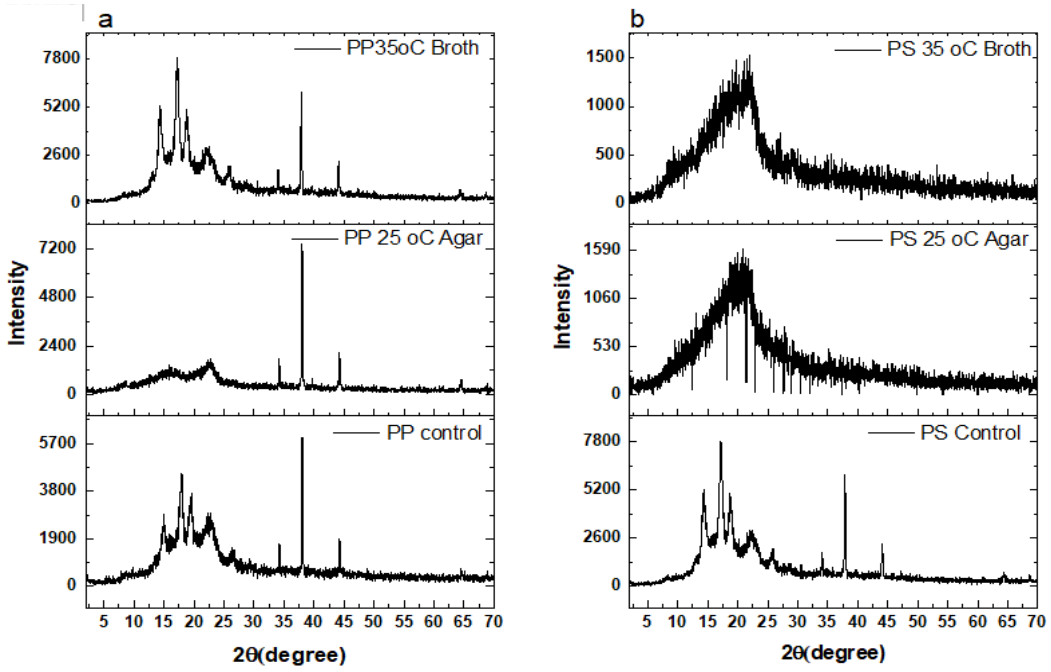

Figure 5. *Crystallinity and amorphous characteristics of (a) PP and (b) PS exposed to B. subtilis at optimal conditions*

The XRD patterns of PP exposed to *B. subtilis* at optimal conditions at 35°C in broth and 25°C in agar are shown in *Figure 5(a)*. The PP control sample showed a sharp peak at 2θ = 21.5°, which is characteristic of the α-crystallinity of PP. This peak became more intense and broader in the PP 35°C broth sample, indicating that the *B. subtilis* exposure at 35°C in broth caused an increase in the α-crystallinity of PP. The PP 25°C Agar sample showed a decrease in α-crystallinity, but the peak less pronounced as that of the PP at 35°C broth sample. *B. subtilis* exposure induces changes in the nucleation and growth of PP crystals. At 35°C in broth, the bacteria facilitate the formation of a greater number of nucleating agents, leading to more homogeneous nucleation and

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increased α-crystallinity. However, at 25°C on agar, the interactions between the bacteria and PP are limited, resulting in fewer nucleating agents and a lower degree of α-crystallinity (Khanna et al., 2010).

The XRD characterisation of PS exposed to *B. subtilis* at optimal conditions at 35°C in broth and 25°C in agar is shown in *Figure 5(b)*. The XRD pattern of PS control shows two broad peaks at around 2θ = 15° and 25°, which are attributed to the amorphous nature of the PS. The pattern of PS exposed to the bacteria at 35°C in broth shows a decrease in the intensity of the broad peaks, indicating a decrease in the crystallinity of the PS. This decrease in crystallinity is likely due to the enzymatic degradation of the PS by the *B. subtilis*. The pattern of PS exposed to the bacteria at 25°C in agar shows a slight increase in the intensity of the broad peaks, indicating an increase in the crystallinity of the PS. This increase in crystallinity is likely because the PS is able to crystallize more slowly at 25°C than at 35°C.

These results are consistent with the study by Bai et al. (2009) found that *B. subtilis* was able to degrade PS at a faster rate at 35°C than at 25°C. Another study by Kimata-Kino et al. (2011) found that *B. subtilis* was able to degrade PS more effectively in broth than in agar. These studies suggest that the biodegradation of PS by *B. subtilis* is a complex process that is influenced by a number of factors, including the temperature and the environment in which the biodegradation is taking place.

Surface morphology of polystyrene and polypropylene

SEM was used to examine changes in the morphological structure of PP and PS at optimal conditions, and the micrographs are shown in *Fig. 6*. Each SEM image was captured at three distinct magnifications (X 10,000) and at 20 KV, with particle sizes of 20 μm. The micrographs of PP and PS at X 10000 at 35°C in broth and 25°C in agar show significant differences in their morphology.

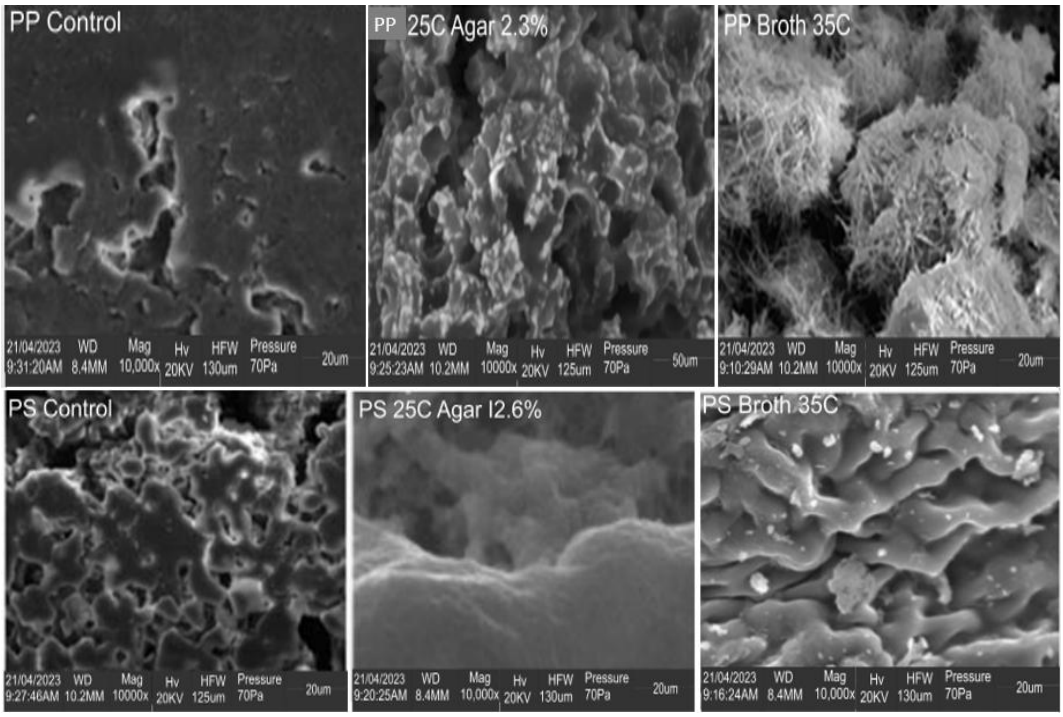

Figure 6. *Micrographs of PP and PS at optimal conditions at X 10000*

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The micrograph of PP control shows a smooth and featureless surface, this is because PP is a semi-crystalline polymer, and the crystals are too small to be seen at this magnification. The micrograph of PP 25°C Agar 2.3% shows a rough and porous surface. This is because the agar has absorbed water and swelled, which has caused the PP to crack and craze. The micrograph of PP Broth 35°C shows a similar surface to the PP 25°C Agar 2.3%, but the cracks and crazes are more severe. This is because the broth is a more aggressive environment than the agar.

The micrograph of PS control shows a rough and porous surface, similar to PP. The micrograph of PS 25°C agar shows a smooth and featureless surface, similar to PP 25°C agar. The micrograph of PS Broth 35°C shows a very rough and porous surface, with large cracks and crazes. This is because PS is an amorphous polymer, and the molecules are not as tightly packed as they are in a semi-crystalline polymer. This makes PS more susceptible to attack by water and other chemicals.

Conclusion

In conclusion, our study has highlighted the variable biodegradation outcomes of PS and PP when exposed to *B. subtilis* under different conditions. We have shown that temperature plays a significant role in the microbial degradation of these polymers, with 35°C providing the most conducive environment for enhanced microbial activity and subsequent degradation, particularly in nutrient broth. For PS, the most substantial degradation was observed at 35°C in broth, with a significant weight loss up to 17% by the end of the 56-day period. This finding aligns with the FTIR results, which showed notable changes in the chemical bonds indicative of degradation. XRD analysis further confirmed these findings, highlighting alterations in crystallinity, particularly at elevated temperatures where microbial interactions were most pronounced.

For PP, although generally more resistant to microbial degradation, the 35°C broth conditions again demonstrated the highest levels of weight loss (up to approximately 20.9%). This suggests that higher temperatures substantially enhance the enzymatic activity and overall biodegradation process, making PP more susceptible to microbial attack under these conditions. This result was corroborated by FTIR and XRD analyses, which showed significant structural changes in the polymer matrix, indicative of effective biodegradation.

The SEM analyses provided visual evidence of the physical surface changes in both PS and PP as a result of microbial degradation. While the control samples retained smooth surfaces, the treated samples exhibited increased roughness, cracking, and porosity, consistent with microbial and enzymatic activity acting on the polymer surfaces.

Overall, our findings underscore the potential of *B. subtilis* as a viable microbial agent for the biodegradation of non-biodegradable plastics, specifically PS and PP, under optimized temperatures. While biodegradation rates are influenced by several factors, including temperature and media type, this study provides crucial insights for future bioremediation strategies aimed at mitigating plastic pollution. The focus on optimizing environmental conditions to enhance microbial degradation efficiency is key to developing sustainable solutions for plastic waste management. Further research should explore the genetic and enzymatic mechanisms governing these interactions to fully harness the biodegradation potential of *B. subtilis* and similar microorganisms.

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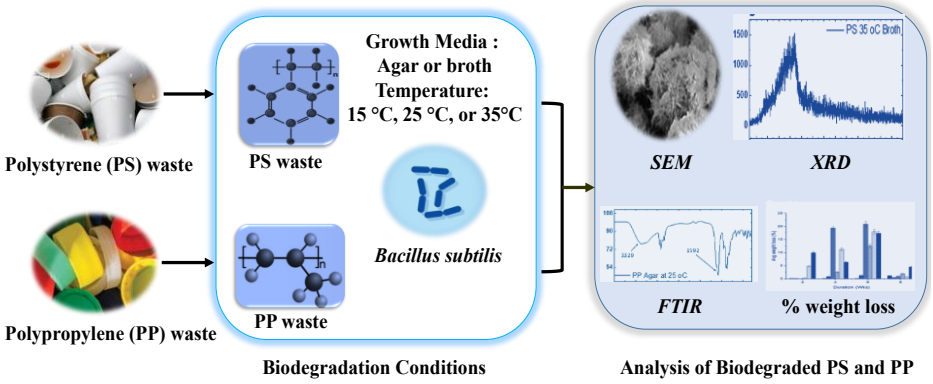
Appendix

Graphical abstract



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