Applied Microbiology: Theory & Technology
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Research Article

Performance of Bacteria *Lysinibacillus* and *Enterococcus Faecalis* to Degrade PA6 Composites

Oumayma Oulidi[1*], Ibtissam Elaaraj[1], Maria Jabri[2], Asmae Nakkabi[1,3], Aziz Bouymajane[4,5], Fouzia Rhazi Filali[5], Mohammed Fahim[1], Noureddine Elmoualij[1]

[1]Laboratory of Innovative Materials and Biotechnology of Natural Resources Faculty of Sciences, Moulay Ismail University, Meknes, Kingdom of Morocco
[2]Laboratory of Biology and Chemistry Applied to the Environment, URL-CNRST-N°13 Faculty of Sciences, Moulay Ismail University, Meknes, Kingdom of Morocco
[3]Department of Chemistry, Faculty of Sciences and Technologies, Moulay Ismail University, Errachidia, Kingdom of Morocco
[4]Department of Biology, Faculty of Sciences and Technologies, Moulay Ismail University, Errachidia, Kingdom of Morocco
[5]Laboratory of Chemistry-Biology Applied to the Environment, Microbiology, and Health Faculty of Sciences, Moulay Ismail University, Meknes, Kingdom of Morocco
Email: oumaymaoulidi@edu.umi.ac.ma

Received: 27 January 2024; **Revised:** 26 June 2024; **Accepted:** 12 August 2024

Abstract: The current research focuses on applying bio-reinforced composite materials, specifically emphasizing polyamide 6 (PA6), a widely used thermoplastic polymer known for its mechanical strength and versatility. To enhance the environmental sustainability of PA6, various natural reinforcements, including olive pomace powder (OPP), peanut shell powder (PSP), and plaster (PL), have been incorporated. This comprehensive study investigates the biodegradation of PA6 composites reinforced with these materials in the presence of specific bacteria such as *Lysinibacillus sp*. and *Enterococcus faecalis*. These bacteria were chosen for their known ability to degrade synthetic polymers. A range of analytical methods were employed to assess the biodegradation process thoroughly. Mass loss measurements provided quantitative data on the extent of polymer degradation. Scanning electron microscopy (SEM) was used to observe the surface morphology and structural changes in the composites, while infrared spectroscopy (IR) offered insights into the chemical modifications occurring during biodegradation. The results of this study reveal significant insights into the biodegradability of PA6 when reinforced with OPP, PSP, and PL. Notably, adding these bio-reinforcements enhanced the degradation rate of PA6, demonstrating their potential as effective agents for reducing the environmental impact of plastic waste. These findings are crucial in addressing the pressing challenges of ecological pollution caused by polymer waste, emphasizing the importance of developing sustainable materials. By providing a deeper understanding of the biodegradation mechanisms of PA6 composites, this research contributes to the advancement of environmentally friendly approaches in the design and utilization of plastic materials, paving the way for innovative solutions in waste management and pollution reduction.

Keywords: polyamide 6, composite, olive pomace, biodegradation, *Lysinibacillus*, *Enterococcus faecalis*

1. Introduction

Polyamide 6 (PA6) and its composites pose challenges in terms of biodegradation due to the polymer's inherent

DOI: https://doi.org/10.37256/amtt.5220244378

resistance to environmental breakdown, primarily attributed to robust interchain interactions, especially hydrogen bonding (Figure 1) [1]. PA6 is widely used in various industrial applications due to its exceptional physico-chemical, thermal, and mechanical properties. However, its resistance to microbial degradation poses significant ecological concerns.

Olive Pomace Powder (OPP), a byproduct generated in large quantities by olive oil refineries, has been successfully used as a reinforcement material in various polymers such as PVC [2], PP [3], and PS [4]. When incorporated into PA6, OPP enhances the thermal and chemical properties of the composite. Similarly, Peanut Shell Powder (PSP), a lignocellulosic material composed of cellulose, hemicellulose, and lignin, is of interest due to its low cost, availability, and potential to improve the biodegradability of polymer composites [5] groundnut shell powder (GSP). Plaster (PL), is widely used in construction for its thermal and acoustic insulation properties.

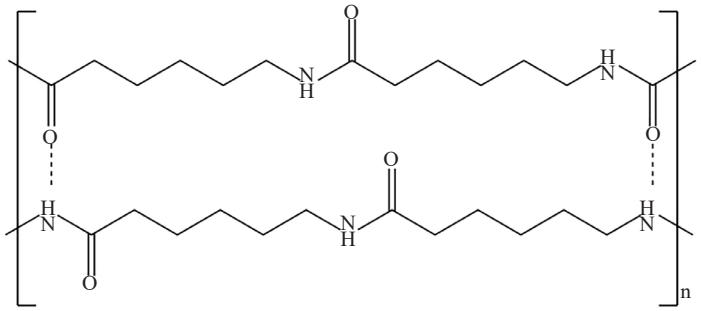

Figure 1. Hydrogen bonding between molecular chains of PA6

This study aligns with the growing interest in natural fibers as reinforcing agents in polymer composites, driven by their low cost, availability, and reported effects on biodegradability [6]. Investigating the ability of selected bacterial strains to degrade PA6 reinforced with PSP, an unexplored area, will contribute valuable insights into the biodegradation potential of such composites.

The chemical composition of olive pomace and peanut shells, comprising cellulose, hemicelluloses, and lignin [7], suggests a substantial content of organic matter crucial for bacterial life. Drawing from scientific insights on the potential of fibrous or fibrillated cellulosic reinforcements to enhance biodegradation, we aim to study the degradation of PA6 reinforced with olive pomace and peanut shells. Previous research has highlighted the ability of certain bacteria, including *Lysinibacillus*, to degrade PA6, making them viable candidates for this study. Building on our previous work demonstrating the ability of these bacterial strains to degrade unmodified PA6, the current study delves into their effectiveness in degrading PA6 reinforced with olive pomace and peanut shells.

Thus, according to the literature, the biodegradation of composites has been considered. The biodegradation of rPP/PSP by *Aspergillus niger* was studied by Usman M. et al. [5] groundnut shell powder (GSP; they revealed that the addition of treated or untreated PSP enhances the biodegradability of recycled PP. Similarly, Rogovina et al investigated the biodegradability of PLA/starch composites [6], as well as PA11/chitosan composites [8]. No study has been conducted on the biodegradation of the PA6/PSP composite until the writing of this article. Therefore, the present work aims to investigate the ability of two bacterial strains; *Lysinibacillus sp*. (MH717277.1) and *Enterococcus faecalis* (KR137541.1), to degrade the PA6 composite reinforced with PSP. These bacteria were chosen based on their successful degradation of untreated PA6 [9].

Studies have demonstrated the capability of certain bacteria, such as *Flavobacterium sp*. [10] and *Pseudomonas sp*. (NK87) [11], to degrade the oligomers of PA6, but they are unable to degrade the polymers of PA6. Conversely, other research has revealed that *Alcaligenes faecalis* degrades PA6 [12] along with its monomer (ε-caprolactam) [13]. Additionally, separate studies have confirmed the degradation of ε-caprolactam by *Lysinibacillus* [14]. This bacterial strain is also known for its ability to degrade polyethylene [15].

Similarly, plaster, widely used in construction for its thermal and acoustic insulation properties, prompted our exploration into its potential as a reinforcement material for PA6. Encouraging results from incorporating plaster into

the PA6 matrix led us to investigate its impact on the polymer's thermal and physico-chemical properties. Considering the ubiquity of PA6 in various industrial applications due to its exceptional physico-chemical, thermal, and mechanical properties, its resistance to microbial degradation poses an ecological concern [16-17]. Thus, our study aims to comprehend the consequences of incorporating plaster into PA6, specifically focusing on the ability of selected bacterial strains to degrade PA6 reinforced with plaster.

In summary, our research addresses the ecological concerns associated with the limited biodegradability of PA6 by exploring the use of olive pomace, plaster, and peanut shell powder as reinforcement materials. We aim to deepen our understanding of the microbial degradation of these reinforced PA6 composites, paving the way for environmentally sustainable alternatives in various industrial applications.

2. Materials and methods

2.1 *Synthesis of the composites PA6/PSP, PA6/OPP and PA6/PL*

The detailed synthesis procedures for the PA6/PSP, PA6/OPP, and PA6/PL composites are outlined in the referenced article [18-19]. The synthesis involved anionic polymerization of ε-caprolactam (ε-CL) using NaH as an initiator and N-acetylcaprolactam as an activator, both sourced from Sigma-Aldrich. The process was carried out in two oil baths at different temperatures: 80 °C (B1) and 130 °C (B2). Initially, ε-caprolactam was melted in two test tubes (T1 and T2) at 80 °C for 10 minutes. Subsequently, 0.15g of NaH was added to T1, resulting in hydrogen release. A 10% weight of reinforcement (PSP, OPP, or PL) was then added to T1, while T2 received 0.320 mL of N-acetylcaprolactam. After heating T2 at 80 °C for 10 minutes and then at 130 °C for another 10 minutes, its content was poured into T1 and homogenized. The mixture was agitated at 100 rpm to ensure proper dispersion of the reinforcement, followed by stirring at 140 °C for 10 minutes to achieve homogenization. The grain diameter of PSP and OPP are 396 µm and 228 µm, respectively. while the PL had a diameter of less than 160 µm.

For clarity and consistency, Table 1 provides the details of sample preparation along with the acronyms used throughout this manuscript. The composite material is then subjected to crushing and pressing, at room temperature, using an ICL hydraulic laboratory press (International Crystal Laboratories) with a force of 20 tonnes. The resulting pellets take the form of discs, measuring 13 mm in diameter and approximately 1 mm in thickness, as measured by a HOLEX caliper. Before inoculation into the test medium, the PA6/PSP, PA6/OPP, and PA6/PL pellets underwent sterilization through autoclaving at 120 °C for 10 minutes. It's noteworthy that there were no observed alterations in the shape of the pellets following the sterilization procedure.

Table 1. Sample preparation details and acronyms

	Peanut Shell (PSP)	Olive Pomace (OPP)	Plaster (PL)
Polyamide 6 (PA6)	PA6/PSP	PA6/OPP	PA6/PL

2.2 *Source and maintenance of bacterial strains*

The source of the bacterial strains degraded plastic materials was gathered in April at the Meknes public landfill, which is situated in the Kingdom of Morocco at (33° 53′ 42″ north, 5° 33′ 17″ west) (average temperature: 20 °C, humidity: 78%). After being removed in sterile bags, these plastics were delivered to the Microbiology Laboratory of the Meknes Faculty of Sciences in the Moroccan Kingdom. These plastic materials were used to gather bacterial strains, which were then isolated and distinguished using a selective culture medium. These separated bacterial cultures were then kept in a freezer at a temperature of -20 °C. Before being applied to the breakdown process, bacteria are inoculated in a solid medium on nutrient agar and incubated for 24 hours at 37 °C.

2.3 *Experimental setup and cultivation conditions for bacterial degradation of PA6 composites*

The compounds employed in this investigation, along with the quantities of minimal medium (MM) [11], are detailed in the provided table (Table 2). The basic requirements culture medium and special requirements culture medium were prepared using 1 L and 300 mL of distilled water, respectively. Subsequently, a mixture of these two media was created to form the MM medium utilized in this study. In a nutshell, 25 ml of minimal medium was dispensed into test tubes, and a bacterial aliquot was introduced into each test tube using a loop. Homogeneity of the medium was achieved through Vortex agitation. Subsequently, the particles of PA6, PA6/PSP, PA6/OPP, and PA6/PL composites were meticulously submerged into each tube and then incubated at 37 °C for 48 days in aerobic conditions.

Table 2. Composition of the minimum medium in basic and specific needs of bacteria

Basic needs	Quantity	Specific needs	Quantity
KH_2PO_4	1,000 mg	$MnSO_4$	145.1 mg
NaCl	30 mg	H_3BO_3	188.6 mg
$MgSO_4$, $7H_2O$	140 mg	$CuSO_4$, $5H_2O$	86.4 mg
$FeSO_4$, $7H_2O$	30 mg	$ZnSO_4$, $5H_2O$	103.4 mg
$CaCO_3$	10 mg	$Co(NO_3)_2$, $6H_2O$	108.8 mg
H_2O	1 L	H_2O	300 ml

2.4 *Characterization techniques*

2.4.1 *Optical density (OD)*

The kinetics of bacterial growth is monitored by measuring the turbidity (optical density) of the bacterial culture using a V-1100D type spectrophotometer from the SELECTA brand, at a wavelength of 600 ± 0.5 nm [12, 20].

2.4.2 *Determination of the mass loss of PA6/PSP, PA6/OPP and PA6/PL*

The PA6, PA6/PSP, PA6/OPP, and PA6/PL pellets are recovered from the incubation medium by conventional filtration. The biofilms colonizing the plastic particles were removed by washing with water and then the pellets were dried in a hot air oven at 50 °C for 6 hours. The percentage mass loss of PA6 was determined using the equation 1:

$$\text{Weight loss } (\%) = \left(\frac{w0 - w}{w0} \right) \times 100$$

With w0: initial mass, w: residual mass in grams.

Weighing is carried out using a NAHITA-BLUE series 5133 balance with a precision of 220 g/0.001 g and repeatability ± 0.0002 g.

2.4.3 *Scanning electron microscopy (SEM)*

The samples' morphology and elemental distribution are assessed using a JEOL JSM-IT 500 HR scanning electron microscope equipped with an energy-dispersive X-ray spectrometer (EDX). The instrument operates at an acceleration voltage of 3 kV, capturing images in secondary electron mode. To secure the pellets, each one is individually affixed to the sample holder using double-sided carbon adhesive. This analysis is conducted at the Technology Transfer Innovation Center of Moulay Ismail University (CITT-UMI) in Meknes, Kingdom of Morocco.

3. Results and discussions

3.1 *Macroscopic examination: Unveiling the appearance of pellets*

As one of the key properties of biodegradable composites, the ability to degrade under environmental influence has been investigated. Pellets were exposed to two specific bacterial strains, *Lysinibacillus (LB)* and *Enterococcus faecalis (EF)*, to assess their impact on the PA6 composites. Figure 2 illustrates the outcomes of PA6 composites degradation. It can be seen that the pellets lost their initial shape once introduced into the culture medium.

The formation of a biofilm, appearing as a thick dark-gray crust, is observed, and primarily caused by the production of colored spores, notably in black, when PA6/OPP is exposed to *Enterococcus faecalis* bacteria. This confirms the material's biodegradability and similar observations were made by Sivan et al. on polyethylene incubated with the same bacteria [21]. Microorganisms irreversibly attach to the surface, forming biofilms, as shown in Figure 2 for both *Lysinibacillus* and *Enterococcus faecalis*.

Microbial biofilm formation leads to surface deterioration due to the action of enzymes secreted by *Enterococcus faecalis*, especially in the case of PA6/PL as shown in Figure 3. A similar behavior was observed for the other strains *Lysinibacillus* although their levels of degradation were less pronounced.

Figure 2 also illustrates the changes in PA6/ PSP pellets in contact with these bacteria. A distinct color change is observed, with the pellets taking on a darker hue compared to the control. Regarding shape and texture, the pellets lost their initial form, showing signs of deterioration, especially those incubated with *Lysinibacillus sp*., which may be attributed to the action of enzymes secreted by bacteria adapted to the new culture medium. Conversely, the shape of the pellets in contact with *Enterococcus faecalis* remained virtually unchanged, with the observation of a biofilm adhering to the surface, a feature also noted in other studies (6) [22] which is one of the most utilized thermoplastics, is highly durable and is considered to be non-biodegradable. Hence, polystyrene waste accumulates in the environment posing an increasing ecological threat. In a previous study we have isolated a biofilm-producing strain C208 [21].

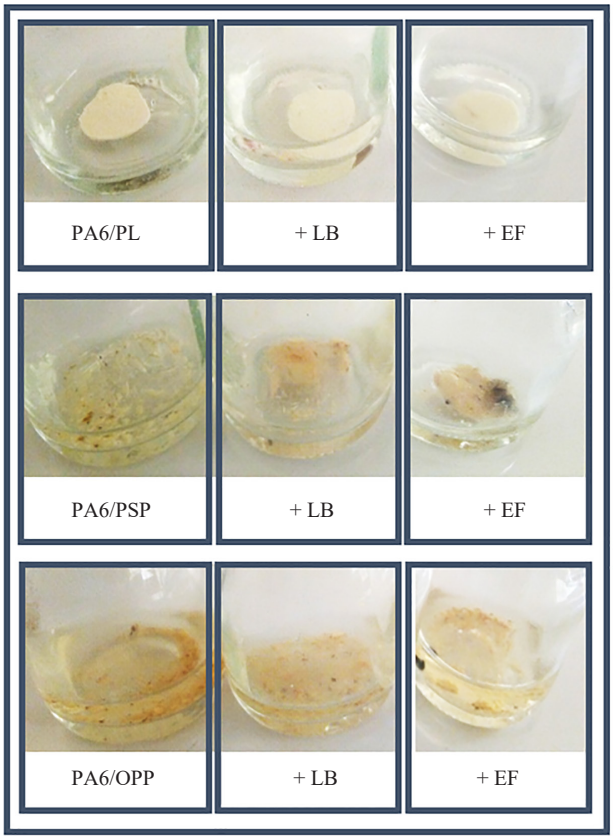

Figure 2. The composite pellets alone and in contact with: *Lysinibacillus sp. (LB)* and *Enterococcus faecalis (EF)* after incubation

Figure 3. Image taken after incubation showing the formation of the biofilm on the surface of the pellet PA6/PL placed in contact with *Enterococcus faecalis*

3.2 *Determination of the weight loss of the composites incubated with the two bacterial isolates*

A quantitative assessment of bacterial activity was conducted by measuring the weight loss of PA6/PSP pellets after incubation with *Lysinibacillus sp*. and *Enterococcus faecalis*.

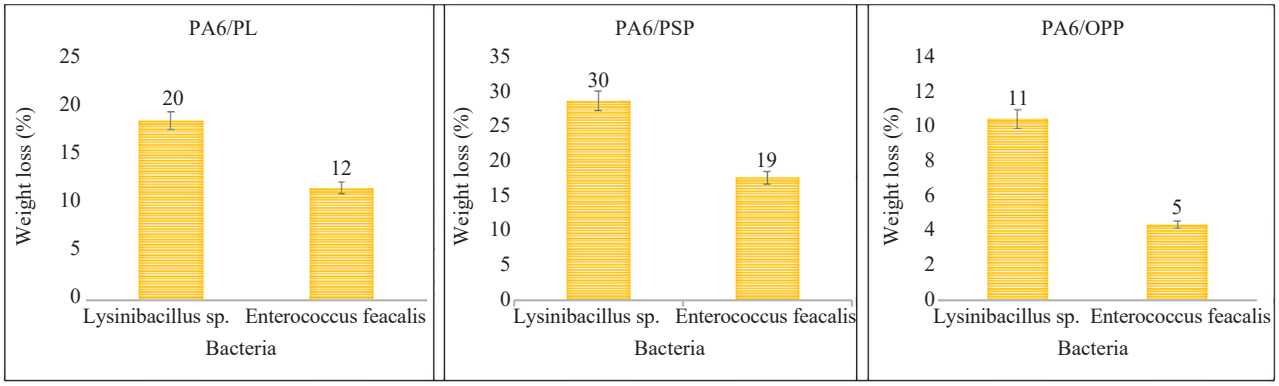

Figure 4. Weight loss of PA6 composite pellets after incubation for 48 days with the bacteria *Lysinibacillus sp*. and *Enterococcus faecalis*

The outcomes, illustrated in Figure 4, reveal substantial weight reductions of 30%, 20%, and 11% for PA6/PSP, PA6/PL, and PA6/OPP, respectively, following 48 days of incubation with *Lysinibacillus sp*. Remarkably, *Lysinibacillus sp*. exhibited the most pronounced degradation, underscoring its impressive ability to degrade these composites. This underscores the efficacy of the bacterial isolates in secreting specific enzymes that target the 10% by weight reinforcement particles, resulting in their degradation. In contrast, *Enterococcus faecalis* led to weight losses of 19%, 12%, and 5% for PA6/PSP, PA6/PL, and PA6/OPP, respectively, after the same incubation period. Although slightly lower than the other isolate, these results remain notably significant. The distinct behavior of these two bacteria in the context of PA6 composites can be elucidated by their capability to adhere to pellets through the hydrophilic-hydrophobic interface of the composite. Notably, the hydrophilic nature of PSP and OPP has further facilitated the integration of bacterial strains into the composite matrix.

These findings are consistent with previous research where PA6/PSP, PA6/OPP, and PA6/PL composites were incubated with the bacteria *Alcaligenes faecalis*. It was found that the addition of PSP improved the biodegradation of PA6 (38% weight loss), unlike OPP, which delayed it (19% weight loss) due to its richness in polyphenols known for their inhibitory activity. Plaster's incorporation caused a similar degradation to that of raw PA6. Overall, the previous study demonstrated the potential of the *Alcaligenes faecalis* strain (KM222194.1) to effectively biodegrade PA6 and its composites. These results are in harmony with those found with the *Alcaligenes faecalis* strain in our recent article [23].

3.3 *Exploring optical density: Insights into bacterial growth and degradation dynamics*

The degradation of the PA6/OPP composite under the action of two bacterial strains: *Lysinibacillus sp. (LB)* and *Enterococcus faecalis (EF)* was estimated. The curves (Figure 5) highlight the optical density measurements taken during the entire incubation period.

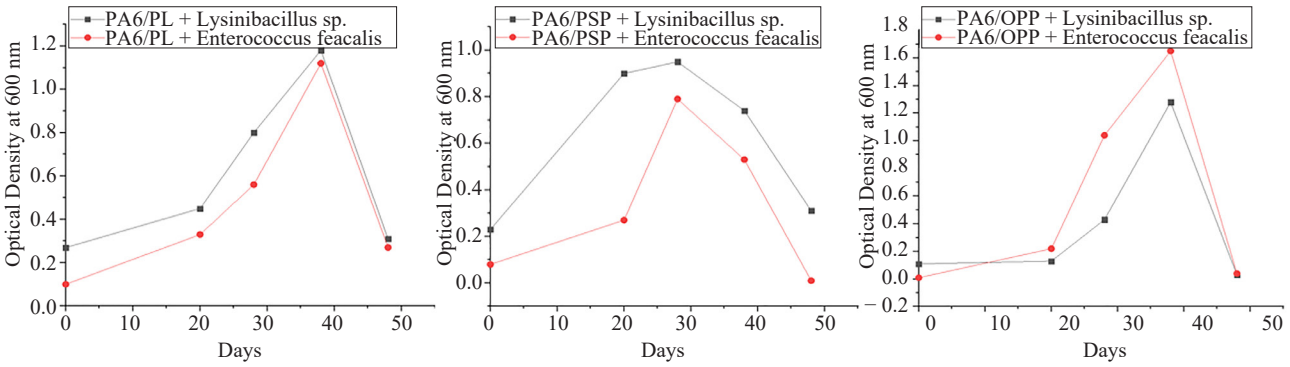

Figure 5. Evolution of the optical density of PA6 reinforced by (a): PL, (b): PSP, and (c): OPP, during the incubation period with *Lysinibacillus sp.* and *Enterococcus faecalis*

According to Figure 5, it can be observed that the adaptation phase to new culture conditions varied in duration for each case, as bacterial strains synthesize enzymes adapted to the new culture environment. This phase is shorter for PA6/PSP than for the PA6/PL composite, which, in turn, is shorter than that of PA6/OPP. The adaptation phase was not recorded for PA6/OPP. The duration of this period depends on the nature of the host environment, the physiological state of the inoculated cells, and possibly the size of the inoculum [24]. Several authors have observed that the thermal history of an inoculum had a significant influence on the duration of the latency phase [25] canned dog food, and raw ground beef (untreated and irradiation-sterilized [26].

Also evident from the same (Figure 4) is that OPP alone is not sufficient for the survival of the two strains, despite the richness in elementary organic components essential for bacterial life.

The growth trajectory of the two bacterial strains follows the same growth cycle for PA6 in our previous study [9]. The addition of OPP to the PA6 matrix seems to have favored bacterial growth. Therefore, it can be concluded that the bacterial strains *Lysinibacillus sp.* and *Enterococcus faecalis* have succeeded in growing in an environment devoid of carbon and nitrogen sources, except those present in the PA6 composites.

Two well-known factors that tend to inhibit the biodegradation of cellulose-based materials are increased levels of lignin and increased levels of crystallinity. An inhibitory effect of lignin on the degradation of biopolymers has been reported by Anstey et al. [27].

For PA6/PL, it can be shown that the two strains, *Lysinibacillus sp.* and *Enterococcus faecalis* were able to adapt to a new growth environment composed only of the PA6/PL composite. It is discernible, from the same figure, that these strains exhibited a different behavior compared to other raw. This can be explained by the immobility or very slight mobility of these strains within the macromolecule, indicated by a very low growth rate, marked in the graph by the short duration of the growth phase. This phase reflects bacterial multiplicity, and the maximum growth rate depends on the characteristics of the culture medium [28].

The rapid transition to the decline phase, characterized by a slowdown and cessation of growth, is due to the depletion of nutrients or possibly a change in the properties of the culture medium that makes it unsuitable for microbial growth [24]. There is a consensus that an increase in the crystallinity of the polymer matrix will essentially slow down or stop the progression of biodegradation [29-31].

In summary, our study explores the biodegradation potential of PA6 composites reinforced with olive pomace (PA6/OPP), plaster (PA6/PL), and peanut shell powder (PA6/PSP) using bacterial strains *Lysinibacillus sp.* and *Enterococcus faecalis*. The investigation reveals varying adaptation phases for each composite, with PA6/PSP exhibiting the

shortest, followed by PA6/PL, while PA6/OPP shows no recorded adaptation phase. The bacterial strains demonstrated remarkable growth in environments devoid of carbon and nitrogen sources, owing to the inherent properties of the composites. These findings contribute valuable insights into the intricate interplay of adaptation dynamics, bacterial growth, and composite degradation, offering potential applications in eco-friendly material development and waste management strategies.

3.4 *Microstructural analysis: Scanning electron microscopy characterization of PA6 composites (SEM)*

SEM images were produced for PA6 pellets undergoing incubation, both in the absence and presence of bacteria, specifically *Lysinibacillus sp*. and *Enterococcus faecalis*. Figures 6 to 8 illustrate the SEM images depicting the degradation of PA6/OPP. Subsequently, Figures 8 to 10 display the SEM images illustrating the degradation of PA6/PL, and finally, Figures 12 to 14 showcase the SEM images capturing the degradation of PA6/PSP.

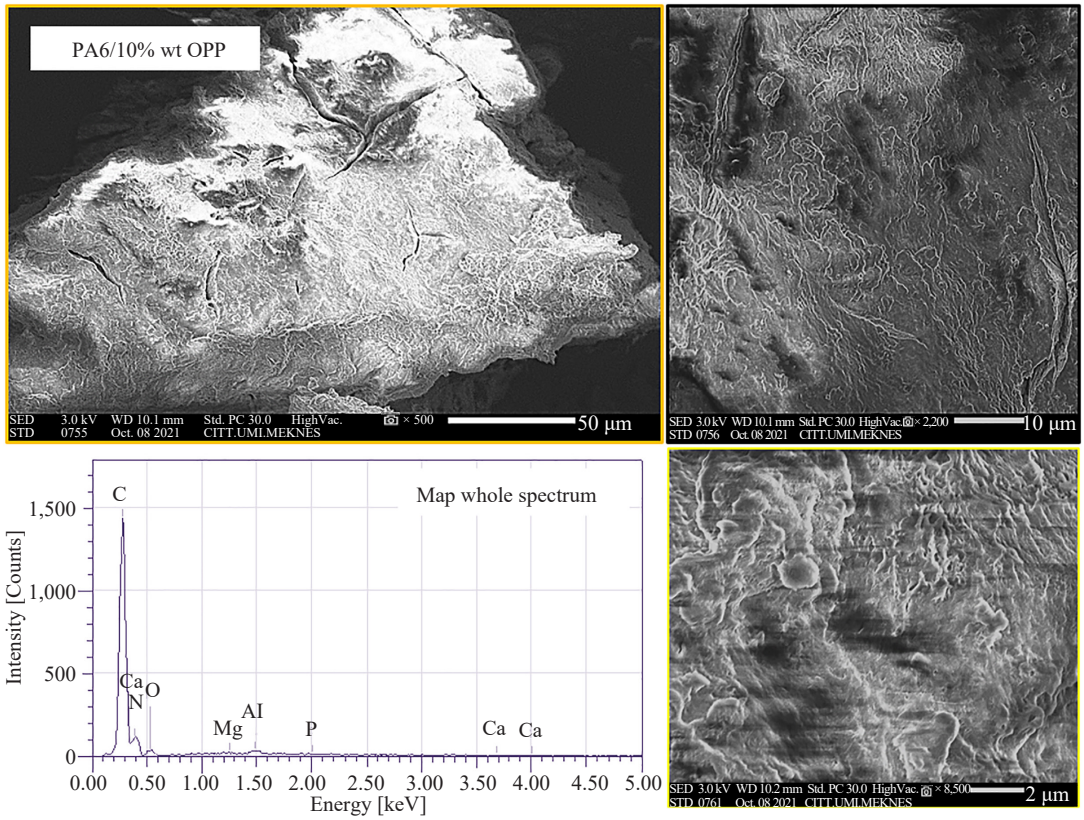

Figure 6. SEM image of the surface of a PA6/OPP pellet without contact with bacteria

From the image above (Figure 6), it can be seen that the surface of the pellet was affected slightly under the influence of the incubation medium, it became rough, with the appearance of cracks showing thus the superposition of the crystalline layers. In Figure 6, voids and pores are observed throughout the surface, with a crack (yellow arrows) showing the well-ordered distribution of macromolecular chains.

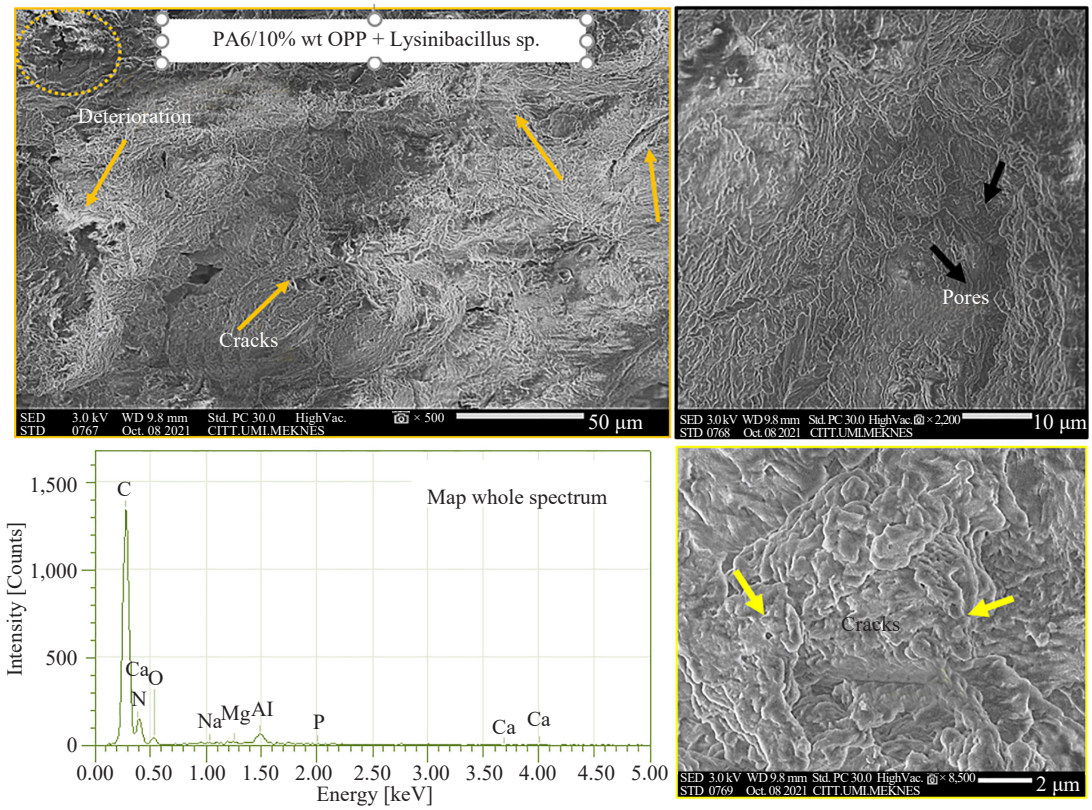

Figure 7. SEM-EDX of the surface of a PA6/OPP contact with *Lysinibacillus sp*.

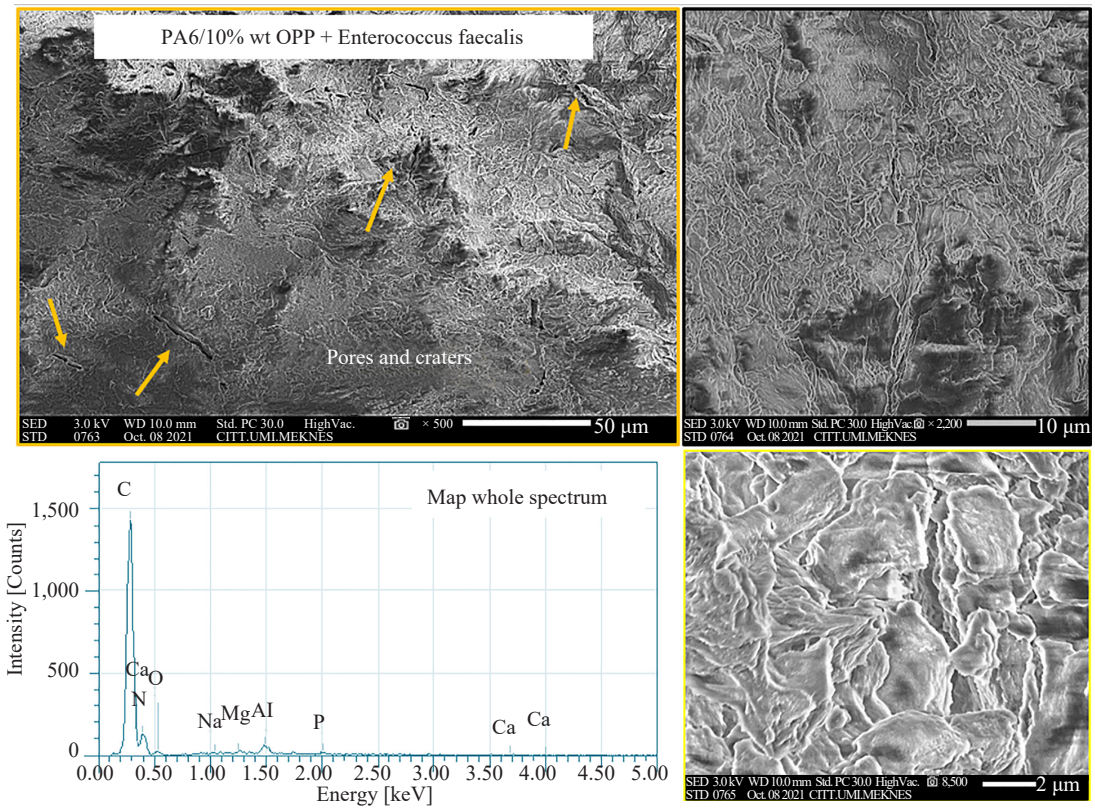

Figure 8. SEM image of the surface of a PA6/OPP in contact with *Enterococcus faecalis*

On the surface of the pellet (Figure 8), we can observe the adhesion of particles of spherical shape with others having the same shape but of small size, these small pieces were also found in the depth of the pellet thus indicating the assimilation of the battery. this spherical character was also observed by [32]. However, for the *Enterococcus faecalis* bacteria, the attack is characterized by a deterioration of the surface of the pellet which generates larger and wider voids and holes. All these SEM images confirm that in general, the two bacterial strains *Lysinibacillus sp.* (MG279144.1) and *Enterococcus faecalis* (MN12173.1) can grow and extract their basic needs for carbon and nitrogen only from the PA6/OPP composite pellet.

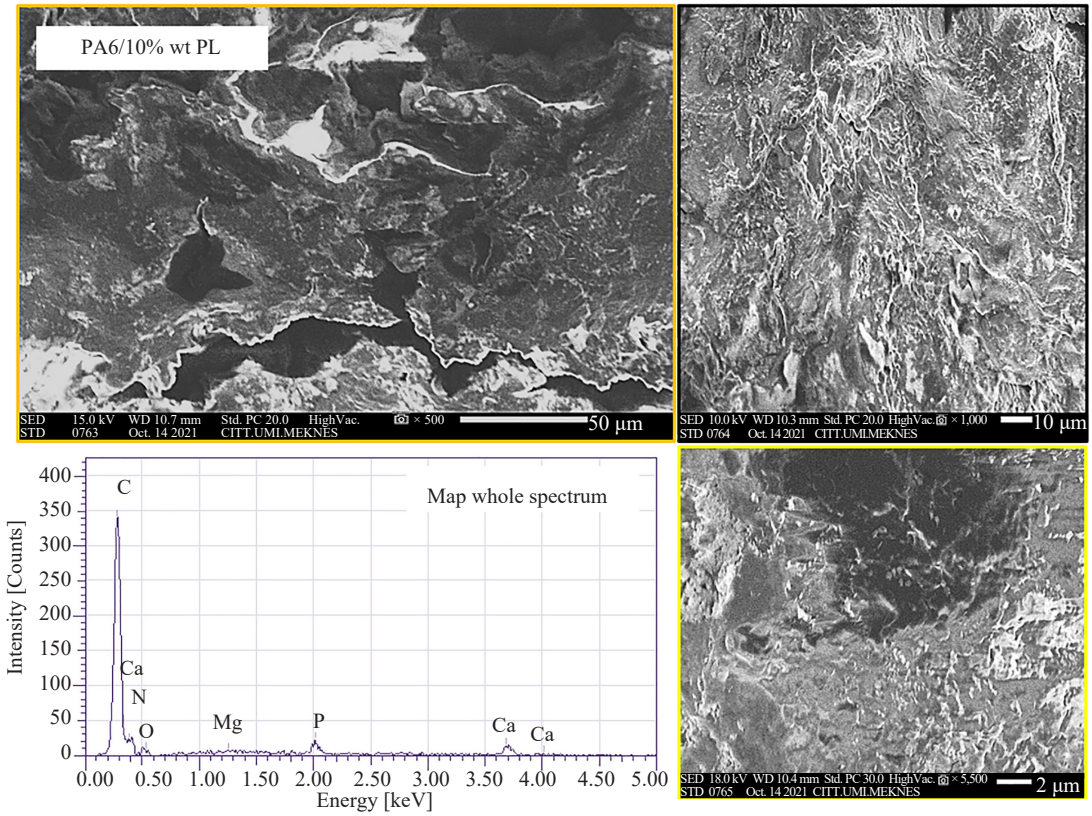

Figure 9. SEM-EDX of the surface of a PA6/PL without contact by bacteria

The scanning electron microscopy (SEM) analysis of the PA6/PL composite reveals a robust surface devoid of voids or pores, as depicted in Figure 8. Upon incubation of the PA6/PL composite with *Lysinibacillus sp*. and *Enterococcus faecalis* bacteria (Figure 10-11), extensive damage is evident. Notably, the surface exhibits an accumulation of modified microspheres, leading to the formation of cracks. Consequently, observable sections undergo cutting, and wider, deeper craters emerge, signifying the profound impact of bacterial activity on the composite structure.

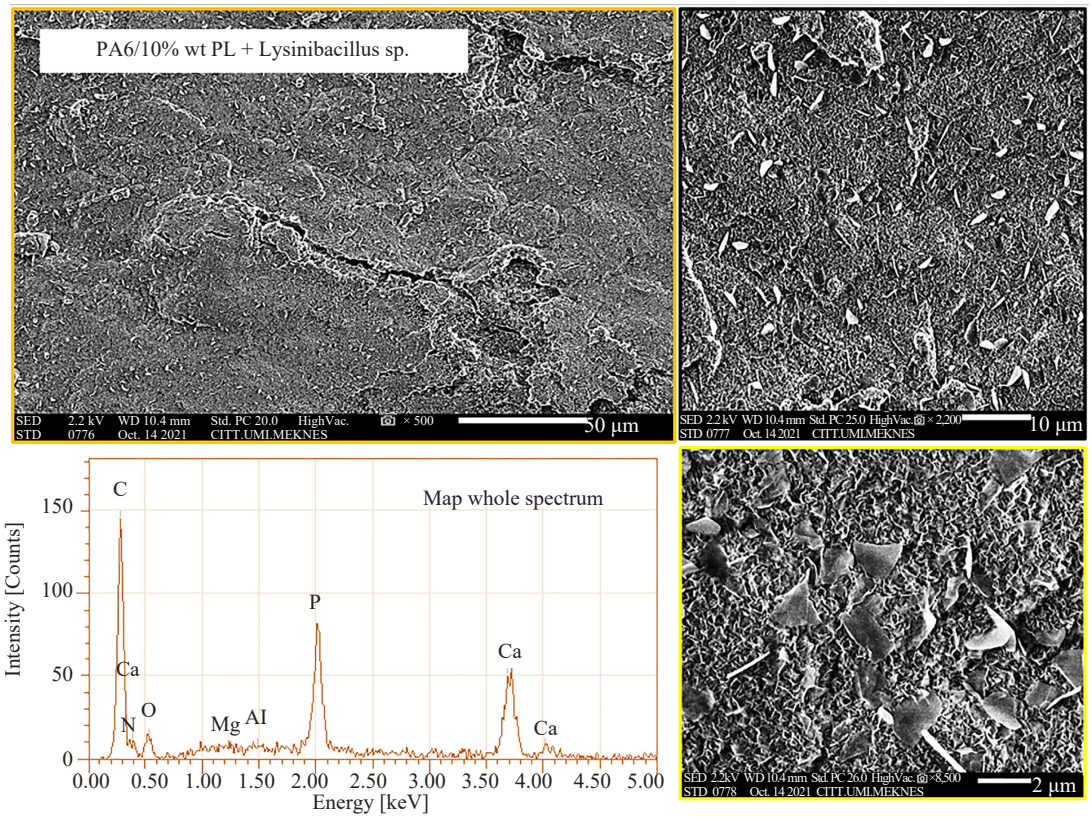

Figure 10. SEM-EDX of the surface of a PA6/PL in contact with *Lysinibacillus sp*.

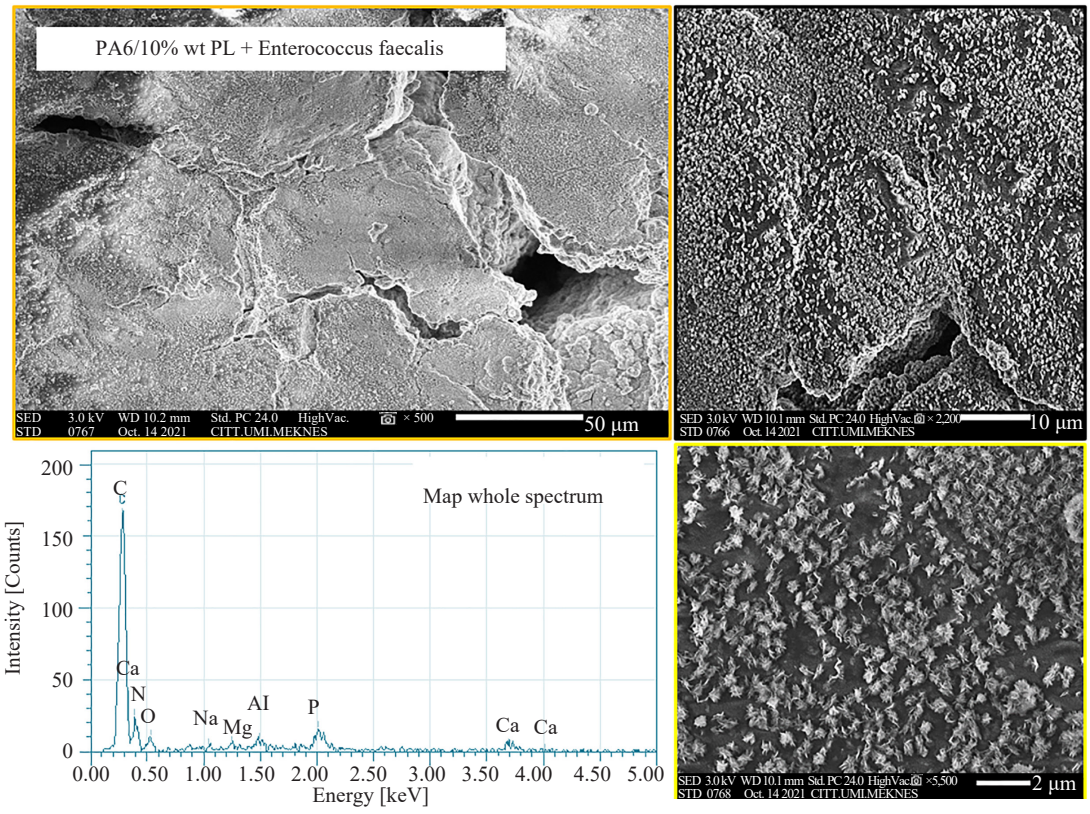

Figure 11. SEM-EDX of the surface of a PA6/PL in contact with *Enterococcus faecalis*

SEM images were performed on PA6/ PSP pellets incubated without (Figure 12) and in contact with the bacteria *Lysinibacillus sp*. and *Enterococcus faecalis*. In Figure 12, a PA6/PSP pellet was incubated for 48 days in the minimum medium without any bacteria to see the behavior of this on the morphology of the composite. We can observe that this incubation generated a deterioration of the pellet, with the appearance of superimposed layers that appear over the entire wrinkled surface. In contact with the bacterial strain *Lysinibacillus sp*., the pellets underwent fascinating modifications, in fact, by admiring Figure 13, we can see the diffusion of the spherical particles over the entire surface, and these billets were stuck on top of each other creating both voids and pores, having a diameter of 0.9 µm, this spherical character was also observed by [32], others observed the shape of this bacteria more closely, they found that it is rather slender in shape [33]. For the *Enterococcus faecalis* strain (Figure 14), craters and cracks were noted with the appearance of particles adhered to the surface having a round shape and which appear spiny, can present aggregations of the microorganisms, causing holes scattered over the entire surface.

All these observations undoubtedly confirm the propagation of the bacterial strains *Lysinibacillus sp*. (MH717277.1) and *Enterococcus faecalis* (KR137541.1) roughly throughout the incubated pellet. This multiplicity also remains proof of the growth of these bacteria, by deriving their basic need for carbon and nitrogen from the PA6/ PSP material put in place. Similar results were recorded by Esmaeili et al. [15] using a culture medium combining the action of the three bacteria *Lysinibacillus*, xylanilyticus, and *Aspergillus niger* on low-density PE. The bacteria *Enterococcus faecalis* had a good impact on the degradation of PA6 [9].

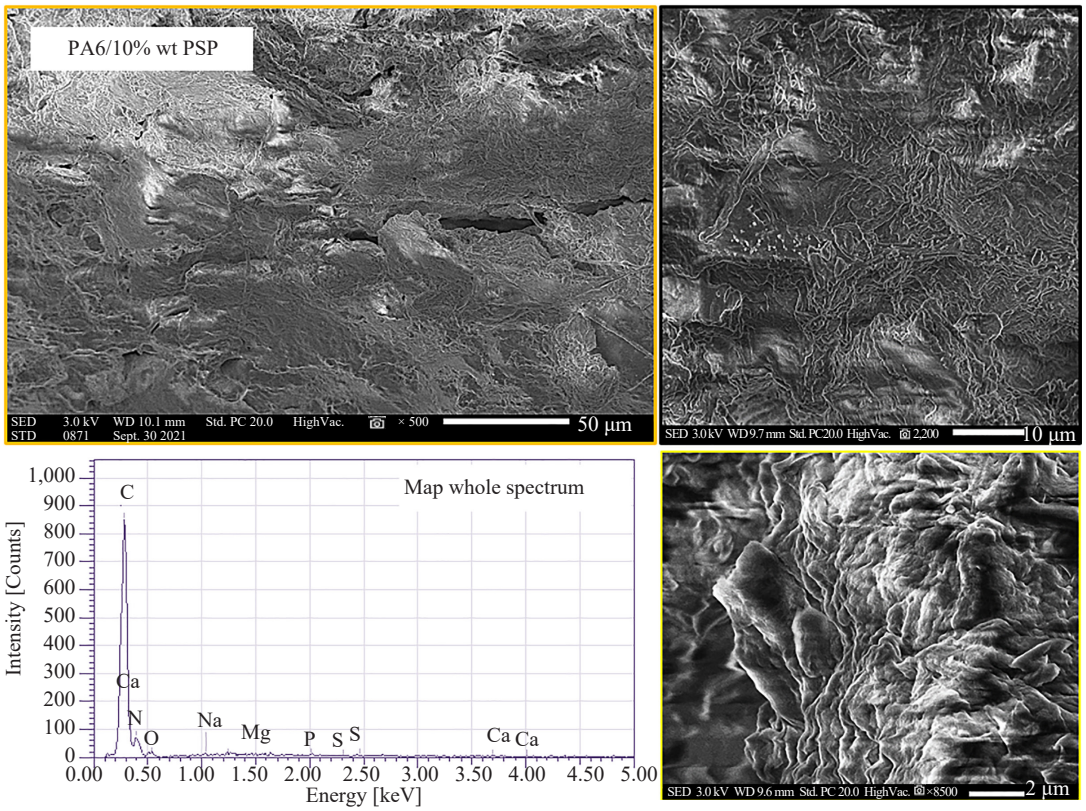

Figure 12. SEM image of PA6/PSP composite without contact with bacteria

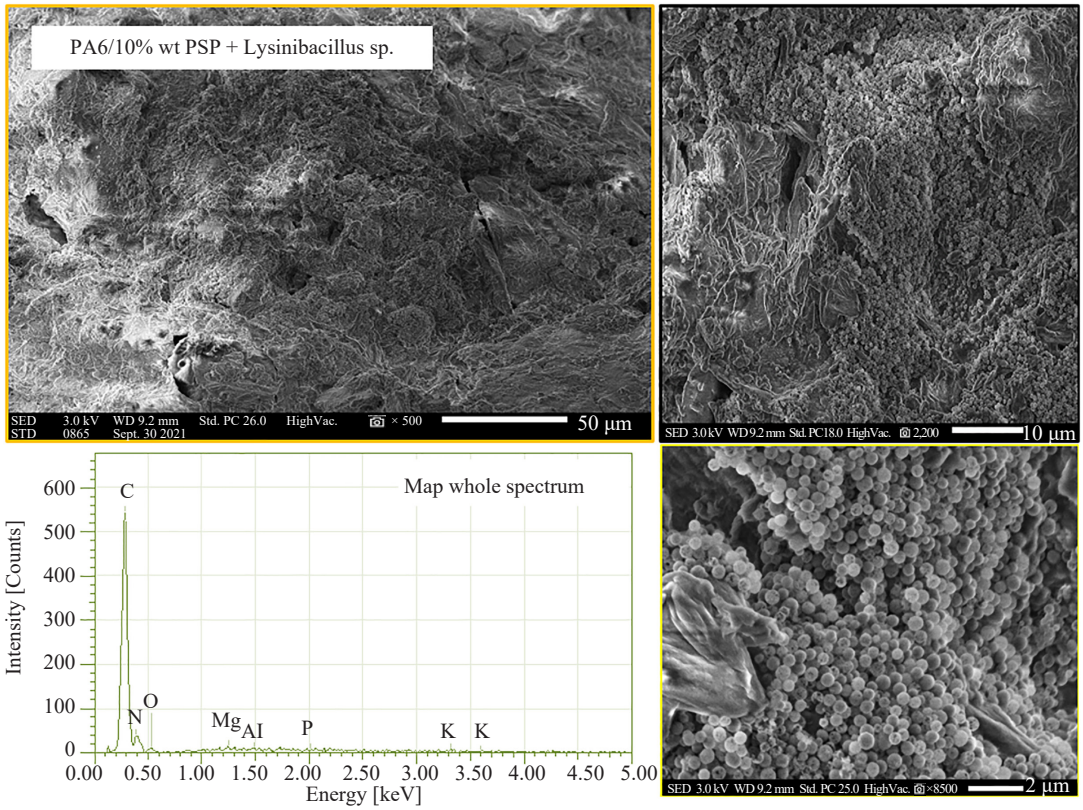

Figure 13. SEM image of the PA6/PSP composite in contact with *Lysinibacillus sp*.

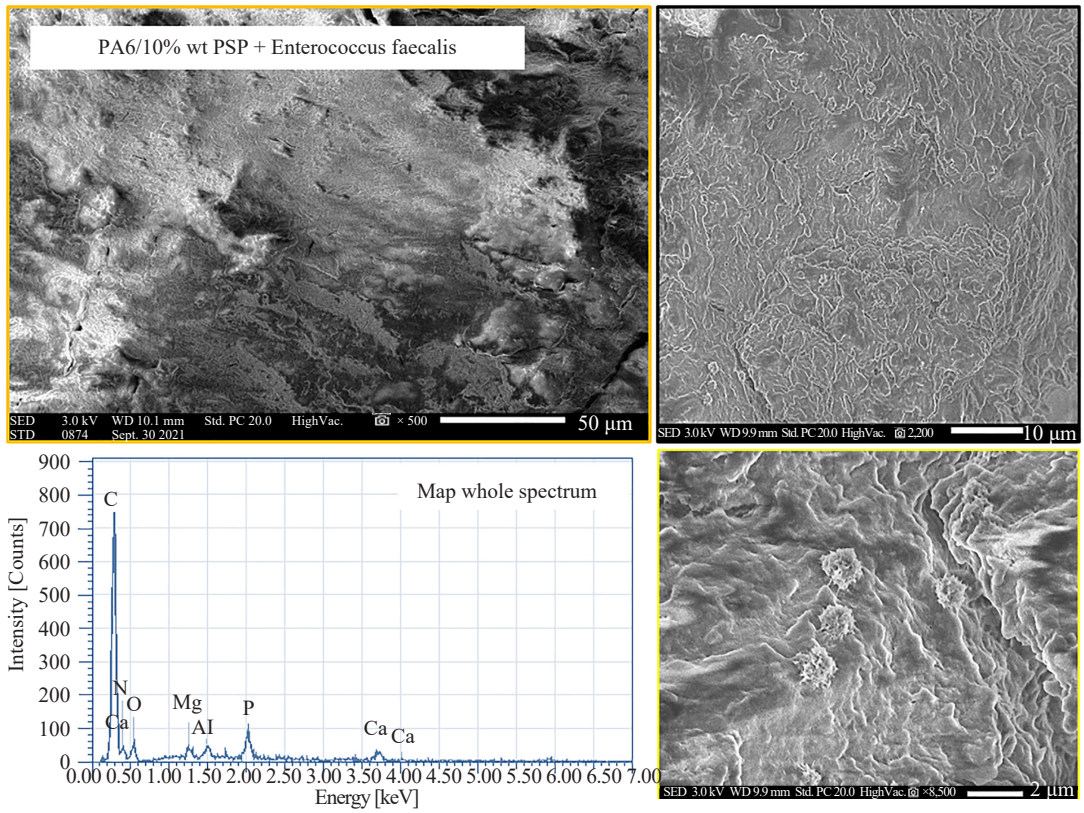

Figure 14. SEM image of the PA6/PSP composite in contact with *Enterococcus faecalis* bacteria

The investigation into the degradation of polyamide 6 (PA6) composites by bacteria, such as *Lysinibacillus sp*. and *Enterococcus faecalis*, offers valuable insights into the compositional changes undergone by these materials during the degradation process. Polyamide 6 composites, reinforced with peanut shell powder (PSP), olive pomace powder (OPP), and plaster (PL), have been subjected to microbial degradation, providing a unique opportunity to explore the alterations in their chemical composition. Understanding the compositional variations post-degradation is crucial for assessing the efficacy of these bacterial strains in breaking down the polymer matrix and reinforcing agents. This study delves into the structural transformations and elemental changes in PA6/PSP, PA6/OPP, and PA6/PL composites, shedding light on the microbial impact on their composition and potential implications for environmentally sustainable material utilization.

For an estimation of the atom and mass compositions of carbon and nitrogen remaining after biodegradation by the microorganisms *Lysinibacillus sp*. and *Enterococcus faecalis*, an area was selected and an EDX analysis was carried out.

The results in Figures 15, 16, and 17 show that the composition of the pellets is slightly affected by the bacterial attack, especially in carbon, this may be due to the elements composing the OPP and PSP knowing that it is rich in cellulosic material and in phenol which plays a role as an antibacterial agent. This remains only an estimate derived from the area selected during the analysis and which may not present the overall state of the pellet. It can be concluded that the structure and composition are extremely affected by these bacterial strains, similar results appear in the review [29].

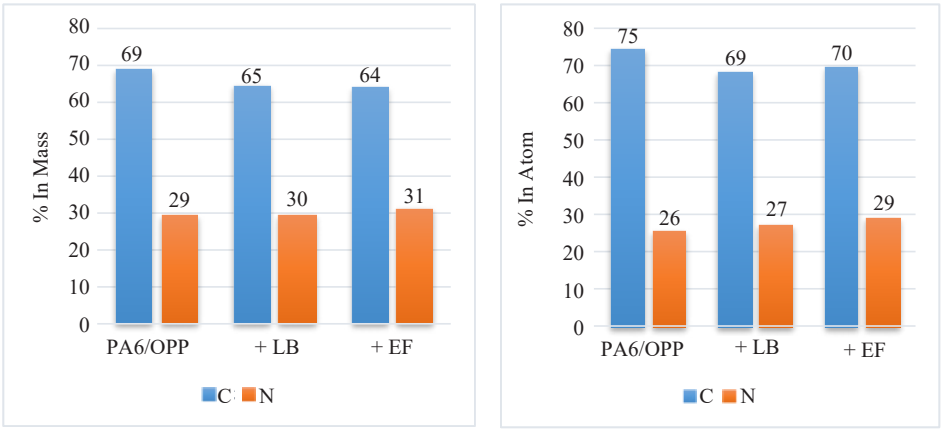

Figure 15. Evolution of the chemical composition by mass of the PA6/OPP composite without and in contact with bacteria: *Lysinibacillus (LB)* and *Enterococcus faecalis (EF)*

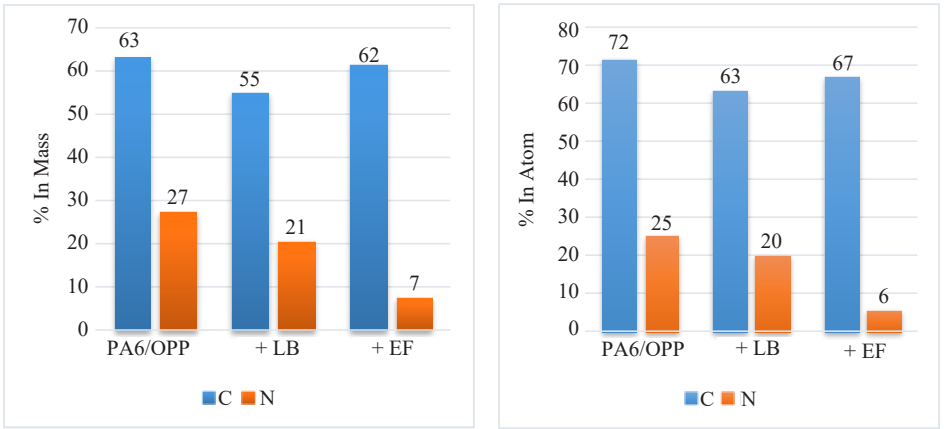

Figure 16. Evolution of the chemical composition by mass of the PA6/PL composite without and in contact with bacteria: *Lysinibacillus (LB)* and *Enterococcus faecalis (EF)*

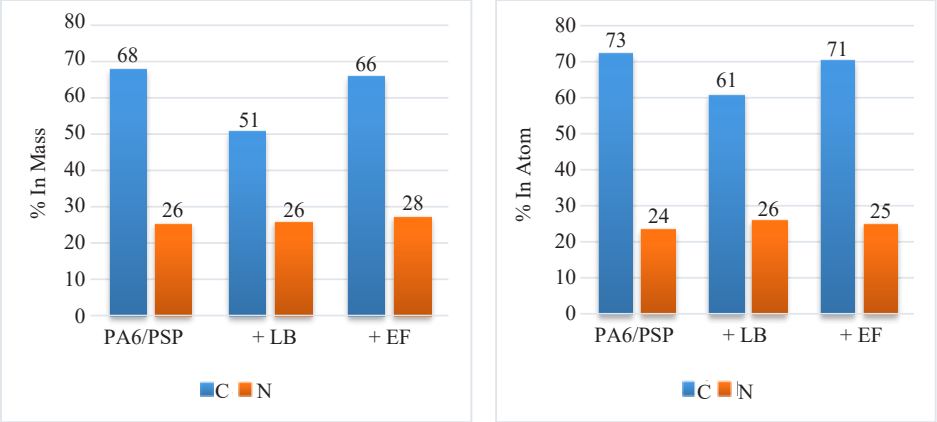

Figure 17. Evolution of the chemical composition by mass of the PA6/PSP composite without and in contact with bacteria: *Lysinibacillus (LB)* and *Enterococcus faecalis (EF)*

4. Conclusion

The investigation into polymer degradation, particularly polyamide (PA), has yielded compelling findings, showcasing the remarkable efficacy of bacterial strains *Lysinibacillus sp*. (MH717277.1) and *Enterococcus faecalis* (KR137541.1). The study elucidated their proficiency in utilizing PA6 both as a substrate for biofilm formation and as a rich source of carbon and nitrogen. Notably, the accelerated degradation of PA6/PL, PA6/OPP, and PA6/PSP composites within 48 days at 37 °C highlighted the dual role of PA6, demonstrating its significant impact on the microbial breakdown process.

Specifically, the PA6/PSP composite exhibited a rapid degradation rate, with the optical density (OD) decreasing by 45% and a mass loss of 30% within the 48-day period. This was further accentuated by the observable deterioration of pellets, emphasizing the role of peanut shell powder (PSP) as an effective reinforcement.

These findings are consistent with previous research where PA6/PSP, PA6/OPP, and PA6/PL composites were incubated with the bacteria *Alcaligenes faecalis*. It was found that the addition of PSP improved the biodegradation of PA6 (38% weight loss), unlike OPP, which delayed it (19% weight loss) due to its richness in polyphenols known for their inhibitory activity. Plaster's incorporation caused a similar degradation to that of raw PA6. Overall, the previous study demonstrated the potential of the *Alcaligenes faecalis* strain (KM222194.1) to effectively biodegrade PA6 and its composites. Together, these studies highlight the influence of different bacterial strains and natural reinforcements on the biodegradation of PA6 composites. The current findings with *Lysinibacillus sp*. and *Enterococcus faecalis*, along with previous results using *Alcaligenes faecalis*, underscore the potential applications of these bacterial strains in advancing environmentally friendly waste management practices. The enhanced degradation observed in these synthetic polymer composites holds promise for sustainable solutions in waste treatment and underscores the pivotal role of microbial activity in shaping the future of polymer materials.

Acknowledgments

The author would like to thank Dr. Jane Smith for her invaluable guidance and support, and the laboratory team, including John Doe and Emily Brown, for their assistance with experiments and data analysis. The author also appreciates the valuable suggestions from the peer reviewers, which greatly improved this manuscript.

Conflict of interest

The authors declare there is no conflict of interest at any point with reference to research findings.

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