Article

Polyurethane Foam Residue Biodegradation through *Tenebrio molitor* Digestive Tract. Microbial Communities and Enzymatic Activity Involvement

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Abstract: Polyurethane (PU) is a polymer widely used by humans whose recycling is highly complex due his chemical structure, being limited to incineration or accumulation in landfills. Biodegradation by enzymes and microorganisms has been studied for decades as an effective method of biological decomposition. In this study, *Tenebrio molitor* larvae (*T. molitor*) were fed with polyurethane foams, which gut enzymes and microorganisms were capable of degrading the polymer by 35% in 17 days of treatment, producing a weight loss of 14% in the mealworm. Changes in *T. molitor* gut bacterial community and diversity were observed, which may be due to colonization of species associated with PU degradation. Physical and structural biodegradation in PU by *T. molitor* compared to virgin PU, was demonstrated by Fourier Transform InfraRed spectroscopy (FTIR), Thermal Gravimetric Analysis (TGA) and Scanning Electron Microphotography (SEM).

Keywords: plastic; mealworms; insect; bacteria; gut microbiome

SYNOPSIS: Microbiota and enzymes occurred in the gut of *Tenebrio molitor* degrade residual polyurethane foams. PU diet produced higher microbial community diversity than in bran diet. *Lactococcus* was the only gut bacteria that could be associated with PU diet along the experiment.

1. Introduction

The production and consumption at world level of plastic polymers is increasing year by year, reaching around 350-400 million tons in 2019, and its reduction and elimination is a big challenge in the World [1], generating the estimated amount between 2.1 and 3.6 million tons per year in Europe [2]. Polyurethanes (PU) are a type of plastic polymers that are used as foams in automotive seats, coatings, sealants, textile industry, etc. [3]. Their production reached around 4 million tons in 2019 in the world (Plastics Europe, 2017). PU foams is synthetized by the reaction of Diisocyanate (R-N=C=O) and Polyol (R'-OH), generating an organic units called Urethane. The joined at numerous Urethane groups form a polyurethane molecule [5,6]. They are characterized to be highly resistant to both physical and biological degradation due to their chemical composition, that offers high resistance to temperatures, hydrophobicity, etc. being thus increasing its lifespan for decades [7]

Recycling of polyurethane foams (PU foams) is complicated [8], although they can be recycled through mechanical transformation processes, such as crushing and compression molding or pulverizing [9]. Also, PU foam waste are used as filler load often in products of lower value [10]. However, it is not enough, and the high amount of PU remains/wastes

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accumulation is a big environmental problem. These PU remains/wastes ends up being incinerated, whose gas emission contributes to the greenhouse effect, together with the emission of potentially toxic gases from the combustion of polyurethane; or accumulated in landfills or aquatic systems [8,11].

New approaches of PU biodegradation are being investigated using different enzymes e.g. cholesterol esterases, proteases or lipases [12,13] or microorganisms e.g. *Cladosporium pseudocladosporioides* or *Paracoccus* sp. [7,14] that attack the PU bounds, obtaining interesting degradation results. The use of insects to biodegrade plastic is a new approach hidden in nature and little known [15–17]. During the last ten years, the larvae of insects of *Tenebrio molitor* (*T. molitor*; yellow mealworms), commercially used as animal feed and as a potential alternative of food protein for human consumption [18] have been reported to capable of ingesting and degrading different types of plastics i.e. polyethylene (PE), polystyrene (PS) or less studied polyurethane (PU) [15,16,19]. Studies have shown that the ability of the insect to biodegrade plastics is in most cases dependent on its intestinal microorganisms and the ability to adapt to different foods [20,21]. Chewing by the mealworm larvae increases the specific surface area of the plastic and promotes contact with microorganisms and extracellular enzymes in the larvae digestive tract [17].

The purpose of this study was to examine: i) the feasibility of biodegradation, chemical and physical changes of PU foams by mealworms larvae of *T. molitor;* ii) the gut enzyme activities and microbiota changes associated to polyurethane feed of *T. molitor.*

2. Materials and Methods

2.1. Plastics and mealworms

The polyurethane foam (PU) used in this experiment were obtained from Interplasp S.L. (Total Carbon 540.8±15.6 g kg $^{-1}$; Total Nitrogen 43.6±1.09 g kg^{-1}; Total Phosphorous 0.0037±0.0016 g kg^{-1}; Total Potassium 0.1±0.02 g kg^{-1}). An Inductively Coupled Plasma (ICP) analysis was carry out to check the presence of heavy metals in the polyurethane composition that could be toxic to *T. molitor* larvae such as Cd, Hg or Pb [22].

Mealworm larvae of *Tenebrio molitor (T. molitor*) were purchased from Proteinsecta (Albacete, Murcia). Before starting the experiment, mealworms were fed with wheat bran and the last 24 h, they were subjected to starvation. They were fed by PU or wheat bran for 17 days where the food was incorporated at the beginning of the experiment, and they were not re-established. Three replicate containers with 100 g of randomly selected mealworms were fed with 15 g of PU foam, as PU diet (PU); three replicate containers of 100 g of randomly selected mealworms were fed with 100g of wheat bran, as control diet (Bran). Fed containers were incubated in the dark under 27±1 ºC and 80±3 % relative humidity. This provided us the possibility to determine the behaviour of the microbiota and the metabolism of mealworms when they did not obtain any further nutrient.

2.2. Analysis of PU foam biodegradation

2.2.1. Polyurethane consumption

To evaluate the PU foam biodegradation, PU and mealworms were sampled 3, 10 and 17 days after fed mealworms with PU, and wheat bran. At each sampling time, mealworms, PU and frass (faeces) were separated and stored. The equivalent of 5 g of mealworms was taken out and frozen for further analysis**.** The PU foam and the mealworms were weighted to calculate mealworm weight loss (%) and PU consumption (%).

2.2.2. Analysis of PU foam by Spectrum- of Fourier Transform InfraRed (FTIR)

Fourier Transform InfraRed spectroscopy with attenuated Total Reflectance Analysis (FTIR-ATR) (Bruker Hyperion 1000, USA) in the wavenumber range of 3100 - 400 cm^{-1} was used to analyse the changes produced in the bonds made up on PU foams (PU) at the end of the experiment (17 days), in comparison to original polyurethane foams (PU-virgin). The foams were previously washed with distilled water (x3) and they were dried in an oven for 24h at 80ºC.

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2.2.3. Analysis of PU by Thermogravimetric Analysis (TGA)

This analysis was performed by a SDT Q600 Thermogravimetric analyser (USA) in order to characterize the changes in the thermal properties of PU foams (PU) at the end of the experiment (17 days) in comparison to original polyurethane foams (PU-virgin). TGA was performed at a heating rate of 10ºC min^{-1} from 30ºC to 700°C under a nitrogen atmosphere (flow rate 25mL min^{-1}) in 2-3 mg of polyurethane foams.

2.2.4. Analysis of PU foams by Scanning Electron Microphotography (SEM)

PU foams (PU) and original PU foam (PU-virgin) after 10 and 17 days as a fed of mealworms were analysed by Scanning Electron Microscopy (SEM) using FEI, TENEO (USA). The analysis was developed according to [14]. Previously, PU foams were washed by immersion in 0.88% (w/v) sodium hypochlorite for 2 h to eliminate the possible remains of microorganisms in the foam. Later, they were washed in triplicate in 100 mL of distilled water and stirred for 2 min at 150 rpm and dried for 24 h at 80ºC and platinum coating was performed.

2.3. Gut microbiome analysis

2.3.1. Enzyme activities

The enzymatic activity of esterases, lipases, proteases and laccases, with the ability to break some of the specific bonds that form polyurethane molecules, were measured in the gut of mealworm larvae fed with PU foam and Bran after 3, 10 and 17 days. The gut was obtained by dissecting three larvae previously washed with 2 mL of distilled water and air-dried. Once dissected, the gut was immersed in 1 mL of 0.1M phosphate buffer (pH 7) and shaken in an orbital vortex at 2500 rpm for 1 minute and in a circular shaker for 3 minutes. Subsequently, the homogenate was centrifuged (Eppendorf, MiniSpin, Germany) at 14,100 x g for 10 min. The supernatant was diluted 1:10 and enzyme activity analysis was carried out (sample). All enzyme activities were analyzed on a GeneQuant 1300 spectrophotometer (United Kingdom). ***Protease activity*** was determined spectrophotometrically by hydrolysis of p-nitroaniline following the modified method of Preiser et al. [23]. 100 µL of sample was mixed with 700 µL of reaction mixture and incubated for 90 min at 37ºC. The reaction was stopped with 800 µL of 30% (v/v) acetic acid and the color change was measured at λ=410nm. The reaction mixture contained 0.05M glycine Na-OH buffer (pH 10) and 0.001M BAPNA dissolved in 1000 µL of DMSO. Controls were performed in the same way, but replacing the sample with glycine Na-OH buffer. ***Esterase activity*** was determined spectrophotometrically by hydrolysis of p-nitrophenyl acetate (p-NPA) according to the modified method of Oceguera-Cervantes et al [24]. In 1 mL of final volume, 100 µL of sample was mixed with 800 µL of sodium phosphate buffer (0.05M; pH 6.5) and 100 µL of p-NPA solution in acetonitrile (0.01M). samples were incubated at 37ºC for 20 min. The reaction was stopped by placing them in an ice bath for 5 min. After that, samples were centrifuged at 10,000 x g for 5 min and measured at λ=410nm. Controls were performed in the same way but replacing the sample with sodium phosphate buffer. ***Lipase activity*** was determined spectrophotometrically by hydrolysis of p-nitrophenyl laurate (p-NPL) using a modification of the Kilcawley et al. [13] method. In 2 mL of final volume, 100 µL of sample was mixed with 1.9 mL of sodium phosphate buffer (0.1M; pH 7). Samples were incubated at 37°C for 30 min. The reaction was stopped by placing them in an ice bath for 5 min and adding 0.85 mL of NaOH (0.5M). After that, samples were centrifuged at 10,000 x g for 5 min and measured at λ=400nm. Controls were performed in the same way, but replacing the sample with sodium phosphate buffer. ***Laccase activity*** was measured spectrophotometrically following the modified method of Dhakar and Pandey [25]. In 1mL of final volume, 100 µL of sample, 800 µL of sodium acetate buffer (0.2M/0.1M; pH 4.5) and 100 µL of ABTS (0.01M) dissolved in sodium acetate buffer (0.2M/0.1M; pH 4.5) were mixed and incubated for 1 minute. After that, samples were centrifuged at 10,000 x g for 5 min and measured at λ=420nm. Controls were performed in the same way but replacing the sample with sodium acetate buffer.

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The calculations of each one of the activities were carried out using the molar extinction coefficients. Protease activity was calculated using the molar extinction coefficient at λ=410nm ε= 8.8 $mM^{-1}cm^{-1}$ [26]; esterase activity at λ=410nm by ε= 18.5 $mM^{-1}cm^{-1}$ [27]; lipase activity by λ=400nm ε= 14.8 $mM^{-1}cm^{-1}$ [13] and laccase activity at λ=420nm by ε= 36 $mM^{-1}cm^{-1}$ [25]. All activities were expressed in $\mu M^{-1}\, g^{-1}\, min^{-1}$.

2.3.2. Gut DNA extraction and amplicon sequencing

DNA from the gut of mealworms fed with PU foam (PU) and wheat Bran (Bran) after 3, 10 and 17 days were extracted from four gut mealworms from the same fed container pooled to eliminate individual variability. Guts were harvested and added to a tube with 100µl of phosphate buffer (0.1M). DNA was extracted using the Dneasy PowerSoil kit (Qiagen, Germany). The quantity and quality of DNA extracts were quantified using Qubit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, USA). Samples for sequencing were performed with Illumina Nextera barcoded two-step PCR libraries (V4, ITS2), and sequenced on Illumina MiSeq, v3, 2 x 300 bp. Demultiplexing and trimming of Illumina adaptor residuals and Trimming of locus specific primer sequences (Microsynth AG, Switzerland).

2.3.3. Bioinformatic analysis

The analysis of the sequences was carried out as follows: the samples were received in fastq format and entered in QIIME2 [28], denoising through dada2 algorithm [29] taking into account the replicates of each treatment to avoid the small variations between them. Once the ASV (Amplicon Sequence Variant) was obtained, the taxonomic classification was carried out using consensus-vsearch with the Silva 132 database (released 2020) as reference. Eukaryotes, archeas, mitochondria, chloroplasts as well as non-assignments were eliminated before the statistical analysis. Finally, normalization was done through rarefaction, bringing all samples to the same value, using the value of the last sample that conserved a minimum of 3000 readings. Sequences were deposited in the ENA database with the accession code PRJEB54959.

2.4. Statistical analysis

Results were analyzed using Statgraphics 18 and Sigmaplot v12.0. Correlations were performed using Spearman's correlation test and statistical significance was determined using a two-way analysis of variance (ANOVA) with post-hoc Least Significant Difference (LSD) Fisher Test. To study the microbial community a NMDS was performed using Bray-Curtis distance through vegan package (Oksanen et al., 2019). The graphs were made using ggplot2 package (Wickham 2016).

3. Results and Discussion

3.1. PU foam consumption and its effect on mealworms

To our best knowledge there are only a few reports about the efficiency of PU utilization by mealworms. PU consumption by mealworm larvae was linear showing a lower slope (y_1=2.995+1.893x) compared to Bran consumption (y_2=16.424+8.049x). After 17 days, PU consumption was 35% and the mealworm weight loss was 14%, compared to Bran that showed 100% of Bran consumption and no weight loss (Table 1). Peng et al. [15] observed that T. molitor mealworms fed with wheat bran provides enough nutrients to complete their life cycle. Our results indicated that PU as the only carbon source does not provide sufficient nutrients to support growth, already observed by Lou et al. [30] with polyethylene (PE) and polystyrene (PS) as mealworm feed, or may be, due to the energy cost necessary to eliminate toxic substances derived from the degradation of the PU foam [21]. Bulak et al. [22] observed after 58 days not the whole ingested plastic was assimilated by the mealworms and PU consumption was 45% and mealworm weight losswas 26-28%.. If our experiment had had the same duration as those carried out in the study of Bulak et al. [22] may be, we could reach those data, although such consumption would perhaps

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not be reached due to the death of the larvae or metabolic exhaustion [21]. Although, Yang et al. [31] showed that mealworm fed with polystyrene did indeed have a lower fat content than those who ingested a conventional diet. Amazingly, they were still capable of successful pupation, which indirectly proves that digestion and assimilation occurred after 32 days.

Results showed that PU is a feed no such palatable for mealworms as bran, although it has been proved that they can decompose it [32]. It could be possible that a mix of PU and bran could improve the percentage of degradation as other authors observed with other plastics i.e. Peng et al. [15] observed higher degradation with a mix of bran or corn flour and polystyrene.

Table 1. Mealworm larvae weight loss and feed consumption by mealworms larvae.

Days	Mealworm weight loss PU diet (%)	Mealworm weight loss Bran diet (%)	PU consumption (%)	Bran consumption (%)
0	100	100	0	0
3	97.95 ± 0.2	102.82 ± 1.35	8.27 ± 0,5	41.13 ± 1.04
6	95.32 ± 0.63	106.4 ± 1.12	14.35 ± 0.57	63.75 ± 5.35
10	93.53 ± 0.78	105.73 ± 4.36	22.73 ± 2.44	97.33 ± 0.79
17	85.84 ± 1.61	97.18 ± 7.82	34.78 ± 2.48	99.95 ± 0.05

3.2. Evidence of biodegradation of PU foams by mealworm

Evidence of changes in functional groups of the PU used to fed mealworm compared to PU-virgin was provided by FTIR analysis at the end of the incubation period (17 days) (Figure 1). Changes were mainly observed in pick intensity more than appearing or disappearing picks (functional groups). It was observed an intensity decrease of the spectrum peak from PU compared to PU-virgin, corresponding to peaks 1090-1099 cm^{-1} C-O-C bond, 1220-1225 C-N bond [33], 1630-1736 cm^{-1} CO bond or the 3288 cm^{-1} corresponding to -OH bond [32]. It was also showed a decrease on the 1642, 1536 and 1220-1225 cm^{-1} peaks corresponding C=O urea, N-H and C-N bond respectively (Figure 1). These peak intensity decrease would be able to probably attributed to their correspondence bond broken down by enzymatic activities produced by bacteria from mealworm gut [34]. The peak 2916 cm^{-1}, corresponding to CH_2 bond was the only one on the spectrum broken and new CH_2 bond were formed [35] (Figure 1).

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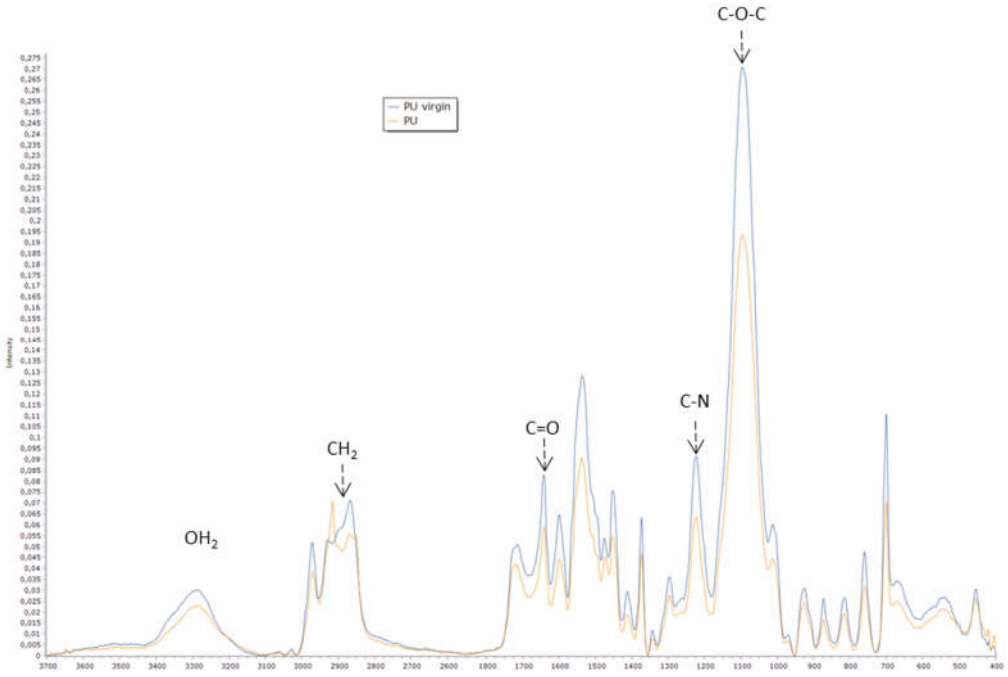

Figure 1. Fourier transform infrared spectroscopy (FTIR) analysis of PU foam at the end of the experiment (17days) (PU) and original PU foam (PU-virgin).

The TGA analysis provided also evidence of modification in the structure of PU used to fed mealworm compared to PU-virgin after 17 days (Figure 2). The weight loss of PU was higher (96%) than on PU-virgin (94%). These would be attributed to the lower amount of soft and hard segments that would be found in the PU with higher thermic degradation resistance [7]. The weight loss occurred in three phases: a first stage, from 220 to 280ºC, where about 30% of weight loss occurred, it could be due to the release of volatiles organic compounds [35,36]; a second stage, from 300 to 400ºC occurred a weight loss of 60% corresponding of dissociation of urethane hard and soft segments [37]; and a third stage, from above 400 ºC where the lower fraction of PU around 10% was degraded corresponding to organic residue decomposition [36] (Figure 2).

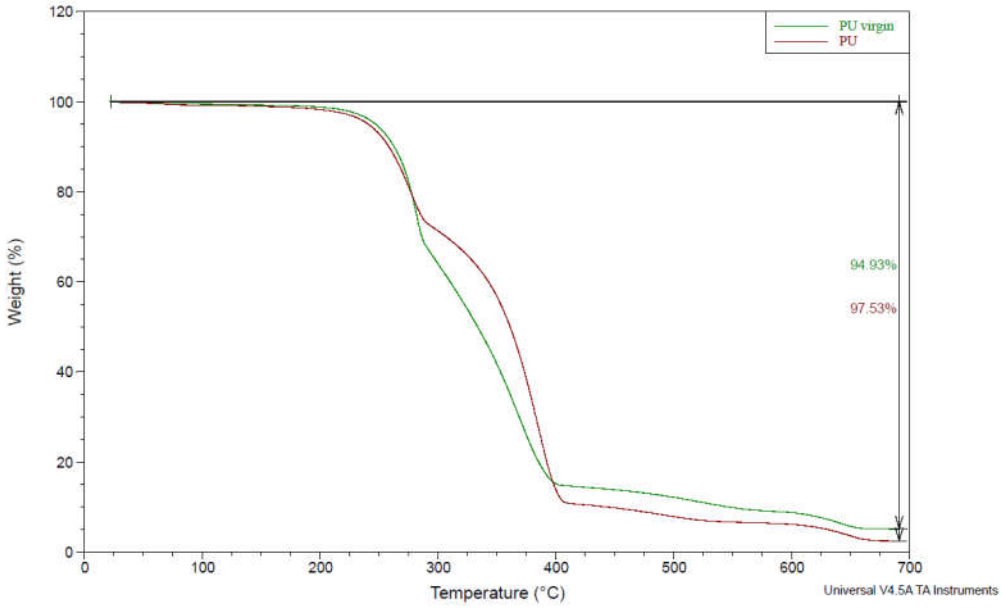

Figure 2. Thermogravimetric analysis (TGA) of PU foam at the end of the experiment (17days) (PU) and original PU foam (PU-virgin).

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SEM also demonstrated physically degradation of PU after 10 and 17 days compared to PU-virgin (Figure 3). Surface from the PU-virgin showed smooth edges with no apparent breaks (Figure 3A) while the ones from PU used to fed mealworms showed wrinkled edges and pits, cracking and erosion, that it would be attributed to mealworm chewing to be ingested and obtained energy and nutrients [38] (Figure 3 B, C and D). Different authors showed similar results of roughness e.g. Khan et al. [39] on PU films exposed to the fungi Aspergillus tubingensis or Bulak et al. [22] on PU foams as feed of T. molitor larvae.

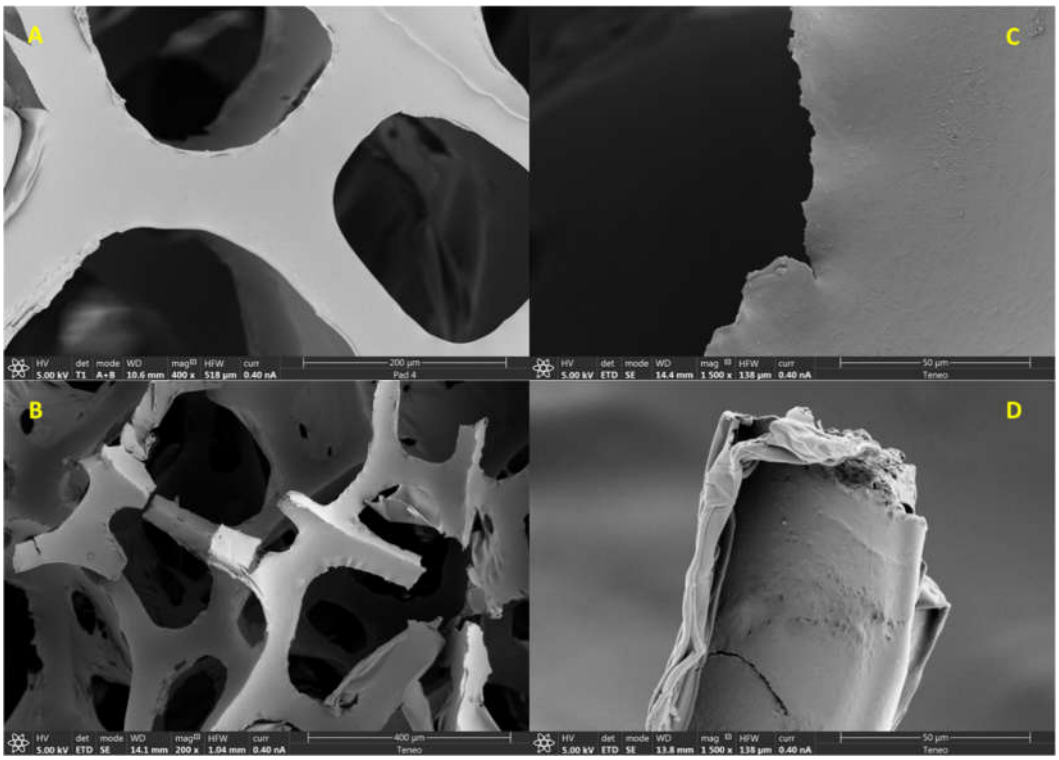

Figure 3. Scanning Electron Microphotography (SEM) of different PU foams: PU-virgin (A); PU after 10 days (B); PU after 17 days (C and D).

3.3. Mealworm gut on PU consumption: enzyme activity and its microbial community effect

3.3.1. Enzyme activity from mealworm gut

The analysis of the different enzymatic activities of polyurethanases such as lipases, esterases, proteases and laccases related to the hydrolysis of polyurethane bonds [40] were measured in the gut of mealworms larvae from both diets, PU and Bran (Figure 4). These polyurethanes enzymes showed a significant ($p \leq 0.001$) interaction between the type of diet and the sampling time. Enzyme activities in the Bran diet were significantly higher than those in the PU along the experiment (Figure 4). Enzyme activities on Bran diet showed two phases (Figure 4). An initial phase from 1-6 days of the experiment where the values were constant, and a second phase, from 6-17 days of the experiment, where the measured enzymatic activities increased. This could be due to bran depletion from mealworm consumption, thus having less nutrient availability and greater need for enzyme synthesis to obtain nutrients from the scarce food available. However, on diet PU the behavior was different (Figure 4). The values of the enzyme activities were lower and mostly constant along the experiment, that could be due to the low availability of nutrients (necessary for their life) and for the synthesis of digestive enzymes capable of degrading polyurethane [41]. Esterase and lipase enzymes showed higher values (Figure 4A and B), probably due to they are the ones that produce the greatest degradation of PU [11]. Also, the lower enzyme activities observed could also be due to the synthesis of corona protein that inhibits the absorption of nanoparticles by intestinal cells such as nanoplastics [42] or the synthesis of enzymes and molecules related to the immune system [43,44].

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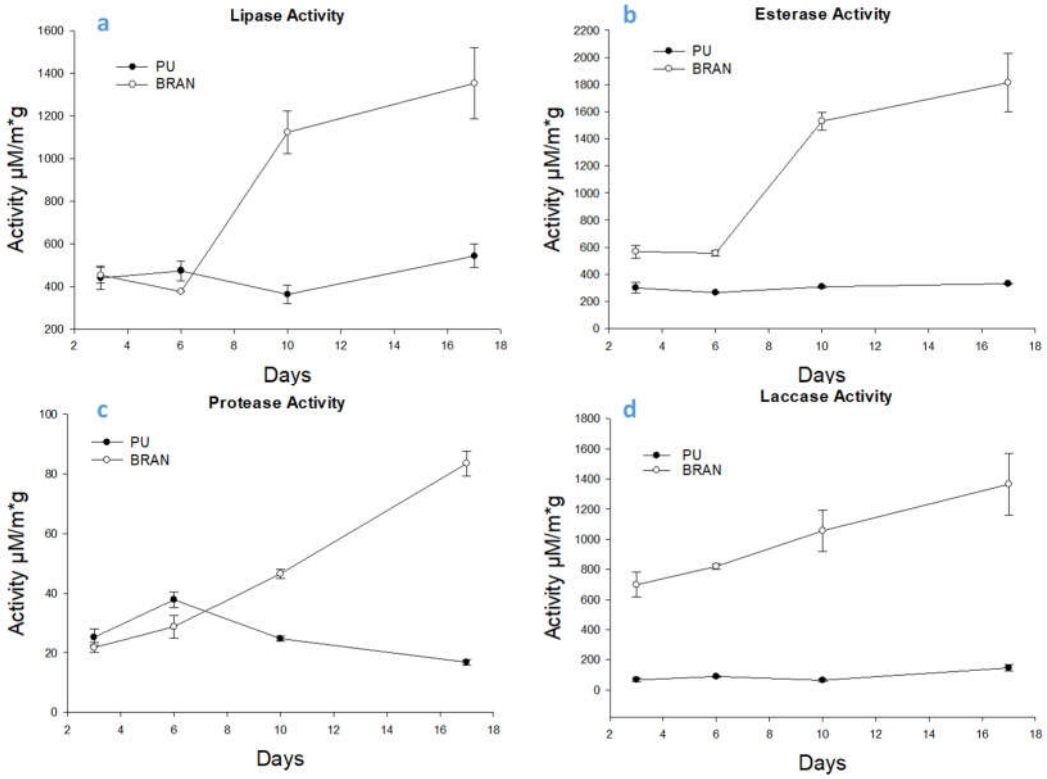

Figure 4. Polyurethanes activities along the experiment of both diets PU and Bran a) Lipase activity; b) Esterase activity; c) Protease activity; d) Laccase activity. For all enzyme activities Two-Ways ANOVA (Pdiet<0.001); (Ptime<0.001).

Insects living on nutrient poor diets, such as the one found with PU diet, where essential compounds such as amino acids are very scant, Genta et al., (2006) demonstrated the benefit to establish bacterial symbiosis due to their rapid ability to adapt tod it changes of the host by altering easily the population profiles and induction of essential enzymes [45]. It has been shown that one of the survival mechanisms of some insects in situations of stress or nutritional deficit is to consume their reserves, of a lipid nature [46]. In addition, it is known that an increase in proteases inside insects only occurs as a last resort in extreme stress situations, since this would lead to protein biodegradation, the last to degrade [47].

3.3.2. Microbial community of mealworm gut

Illumina MiSeq analysis of PCR amplified 16S rRNA fragments was used to assess the changes of the gut microbiome community of mealworms with PU along the experiment, considering that the gut microbiome of insects has an important role to play in their digestion process [48]. The mealworm gut microbiome diversity in PU diet was higher (average 3.23) than in Bran diet (average 2.90) (Figure 5) similar to results observed by Wang et al. [32] or Peng et al. [15]. Shannon diversity in PU diet slightly increase along the experiment, probably due to change the proportion of microorganisms capable of degrading PU while in Bran diet decrease slightly.

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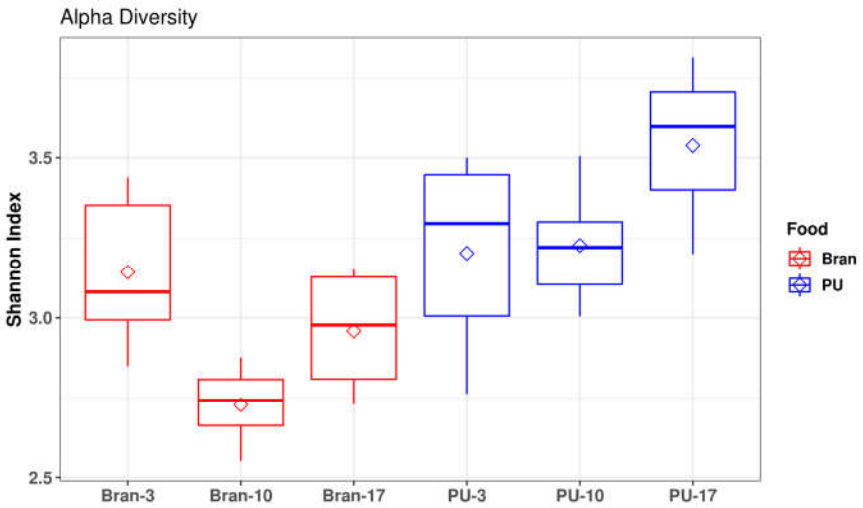

Figure 5. Shannon diversity index of the gut microbiome of PU and Bran diet along the sampling time (3, 10 and 17 days).

A non-metrical multidimensional scaling (NMDs) exhibited differences in the gut microbiome of PU diet and Bran diet principally after 10 and 17 days (Figure 6). The principal phylum observed in both diets (PU and Bran) were Tenericutes, Protobacteria and Firmicutes (Figure 7). Similar results have been obtained by other authors with different types of plastics such as polystyrene [15–17]. Tenericutes was the dominant phylum, reaching the highest proportion in the Bran diet (63%), while in PU diet reached 51%. These proportions decreased throughout the experiment, reaching at the final of experiment (17 days) the opposite trend being higher in PU diet around 30% and 17% in Bran diet. Nevertheless, the relative abundance of Firmicutes along the experiment increased 18% on PU diet compared to 10% on Bran diet at the end of the experiment (17 days). Bacteroidetes showed the lower abundance principally in PU diet [49].

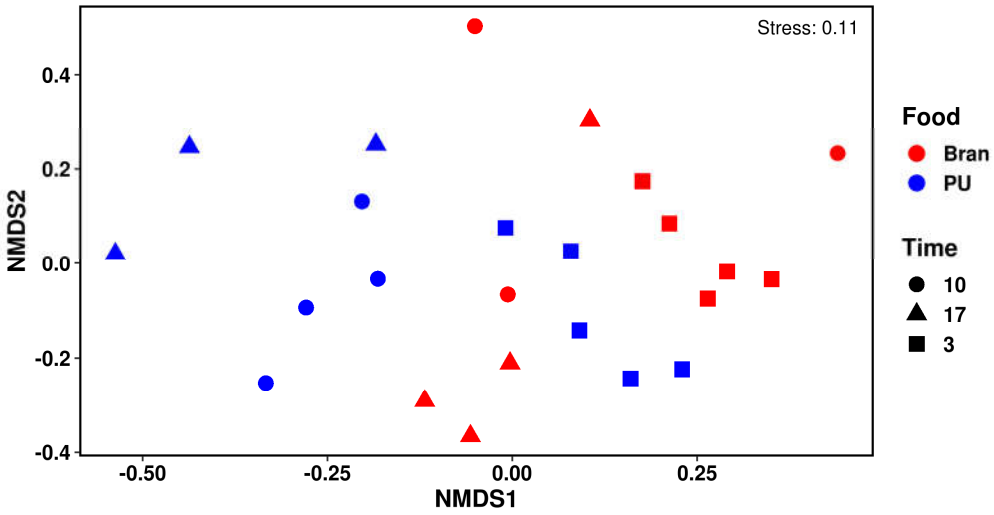

Figure 6. NMDs of gut microbial community of both diets (PU and Bran) at different sampling times (3, 10 and 17 days).

Analysis of relative abundance at genus level indicated four dominant genera in both diets Spiroplasma (average 66.5%), Enterococcus (11.08%), Lactococcus (9.75%), and Pediococcus (5.32%) (Figure 7). Spiroplasma (Tenericutes) decreasing along the experiment on Bran diet (7%) and on PU diet (44%), being at the end of the experiment, more abundant in Bran (69.49%) than PU (38.62%). Surprisingly, Spiroplasma is generally regarded as a

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pathogen or male-killing bacterium in insects but in the mealworm gut is not harmful [50]. It has also observed in many studies with different type of plastics [16,20].

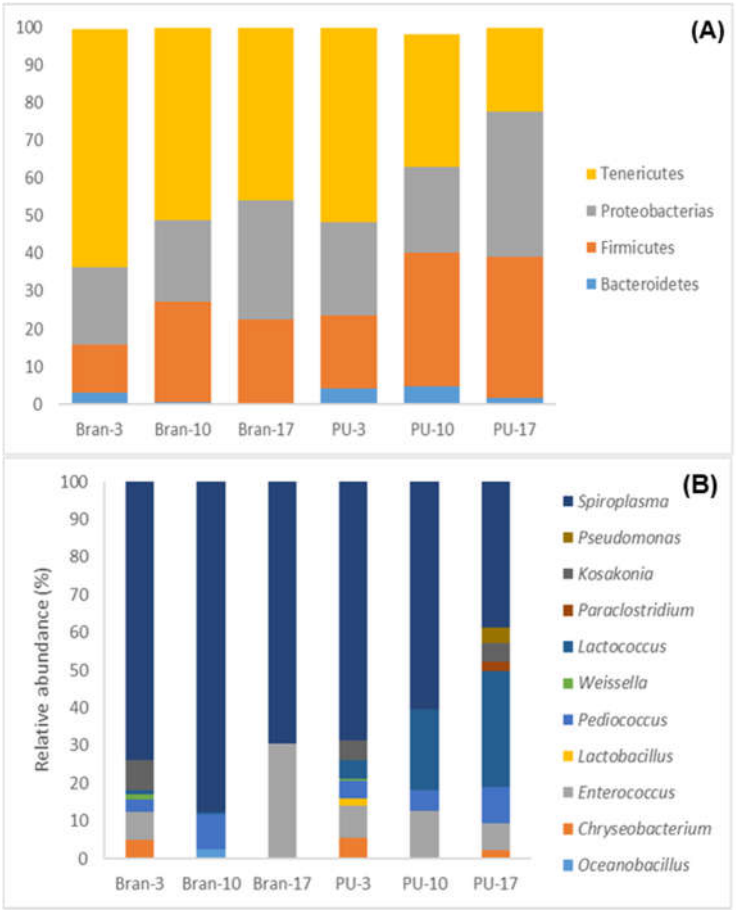

Figure 7. Bar plot of community analysis of gut mealworm of PU diet (PU) and Bran diet (Bran) on phylum (A) and Genus (>1%) (B) .

Lactococcus was the only bacteria that could be associated with PU diet along the experiment (Figure 8). Lactococcus and Pediococcus (Firmicutes) (associated to PU diet at 3-17 days) are lactic acid bacteria, which may have contributed to adjusting and maintaining the health of the gut microbiome (Figure 8) [51]. Also, Lactococcus and Enterococcus (Firmicutes) (associated to PU diet at 3-17 days) (Figure 8) are common insect gut associated bacteria and are known member of the T. molitor gut microbiome [20,52]. According to Lou et al. [30] understanding the approximate parasite sites of different bacteria (Lactococcus is present in every part of the gut, while Enterococcus was absent in the foregut and anterior midgut [52] is good to infer possible degradation pathways on the mealworm gut, being Enterococcus related to PU degradation (Wang et al.) [38].

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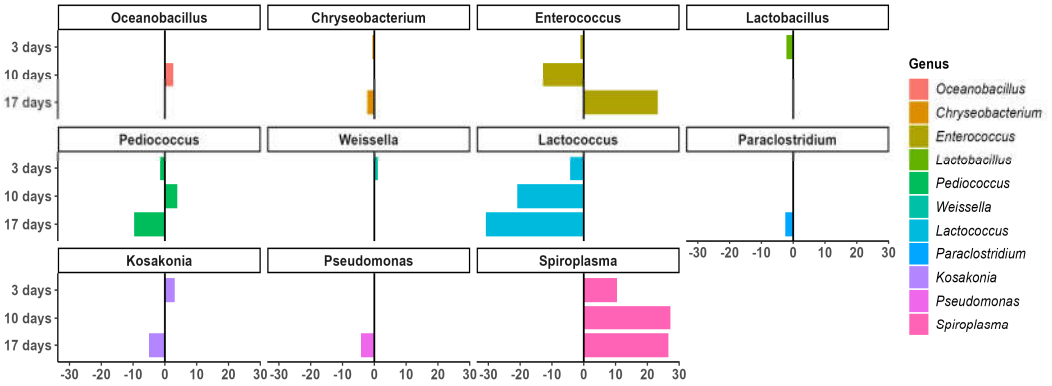

Figure 8. Levels and differential abundance analysis of gut microorganism between both diets.

At the end of the experiment (17 days) in addition to Lactococcus, Pediococcus and Enterococcus different bacteria could be also associated to PU diet (Figure 8). Bacteria such as Paraclostridium (Firmicutes) or Chryseobacterium (Bacteroidetes) this last already observed in mealworm gut on different PS diets [53] and Kosakonia and Pseudomonas (Proteobacteria). Kosakonia member of Enterobacteriaceae, a family known to contain PE degrading bacteria (Enterobacter asburiae YT1) [54] that can use oxygen for biodegradation, already observed in PE and PS degradation [55,56]. Pseudomonas were also associated to plastic (PS) biodegradation [16,57]. The digestion process occurred in the intestine of mealworms is more complex than it seems and the role of whole microbiota and synergic interactions are important in the PU degradation as Urbanek et al. [16] observed for PS.

From our study, it can be concluded that larvae of T. molitor showed the capability of PU consumption of around 35%, being demonstrated through FTIR, TGA and SEM analysis that the PU remains showed structural and physical changes. Biodegradation could be due to different microorganisms and enzyme activities, where Lactococcus was the only bacteria that could be associated with PU diet along the experiment; however, the changes on bacterial community and diversity over time could indicate the growth of others microorganisms capable of using PU as energy source, such as Paraclostridium (Firmicutes), Chryseobacterium (Bacteroidetes), Kosakonia and Pseudomonas (Proteobacteria).

Author Contributions: All authors read, revised, and approved the final manuscript. J.A.P and M.R carried out the experiments for this study. J.M.O; M.T and J.P. performed the FTIR, TAG and SEM analysis to demonstrate the PU biodegradation evidence. J.M.O also carried out the enzymatic assay and M.R and J.C performed the microbial community assay. J.M.O and M.R wrote the manuscript with contributions from J.A.P and J.P.

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