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Biodegradation of Polystyrene by Plastic-Eating Tenebrionidae Larvae

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Abstract: Polystyrene (PS) is an extremely stable polymer with relatively high molecular weight and a strong hydrophobic character that makes it highly resistant to biodegradation. In this study, PS was subjected to biodegradation tests by *Tenebrio Molitor* (*T. Molitor*) and *Zophobas Morio* (*Z. Morio*) larvae. Specifically, six different experimental diets were compared: *(i) T. Molitor* fed with bran; *(ii) T. Molitor* fed only PS; *(iii) T. Molitor* fed only PS treated with H_2O_2; *(iv) Z. Morio* fed with bran; *(v) Z. Morio* fed only PS and *(vi) Z. Morio* fed only PS treated with H_2O_2. Therefore, the mass change of the larvae and the survival rate were measured periodically, while the frass collected after 15 and 30 days were analyzed by different analyses, such as spectroscopy (FTIR), spectrometry (molecular weight and polydispersity), and microscopy (scanning electron microscopy observations). The obtained results suggest that in the case of *T. Molitor* larvae, larvae feeding on bran showed the highest survival rate of ~94 % at 30 days, while in the case of the *Z. Morio* larvae, the highest survival rate was exhibited by larvae eating PS-H_2O_2. Although not strongly pronounced, the M_w and M_n of PS frass of both *T. Molitor* and *Z. Morio* larvae decreased over 30 days, suggesting the Ps biodegradation. Finally, the morphological analysis shows that PS samples isolated from the frass of *T. Molitor and Z. Morio* larvae showed completely different, rough and irregularly carved surface structures, in comparison to PS before biodegradation.

Keywords: biodegradation; polystyrene; Tenebrio Molitor; Zophobas Morio

1. Introduction

Plastic products are widely used around the world because of their low cost and ease of production. Presently, over 400 million tons (Mt) of plastics are produced yearly, with exponential growth over the past 50 years, and more than 360 million tons of polymers produced per year are of fossil based. Specifically, the main products are polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and polyvinyl chloride (PVC), materials that have very attractive characteristics, such as low density, good mechanical impact stability, resistance to chemicals and corrosion, and are commonly used for packaging, construction, automotive, agriculture, and electronic devices [1]. Increasing world population has led to increased production and consumption of plastic materials. However, the accumulation of petrol-plastic wastes in the environment has become the focus of worldwide attention because of the environmental problems caused by their improper disposal after use and decommissioning and because the natural degradation of plastics is very sluggish, resulting in the accumulation of plastic waste that poses a serious environmental threat [2–4].

The main disposal methods include landfill, incineration, chemical treatment, and recycling. Landfilling is the process of disposing and accumulating waste in a certain area. It is a traditional method of waste management, which is widely used in many parts of the world. However, major environmental issues like global warming and the increase of soil acid grow as CO_2 and other gasses emitted from landfills. This method destroys soil, affects groundwater, and cannot effectively degrade waste plastics. Incineration is the process of burning waste in an incinerator until it gets converted to ashes and gas. The process produces a large number of toxic gases, which volatilize into the air and causes harmful effects to public health, biodiversity and ecosystems. Furthermore, the cost of chemical treatment and recycling is high. Hence, these methods are not enough to successfully solve the problem of environmental pollution from plastic products [5].

Considering that much plastic waste has not been scientifically and properly treated, through a series of physical and chemical processes, microplastics are formed and dispersed into the environment [3,6]. Furthermore, organisms can interact with plastic waste: several species of vertebrates and invertebrates have been reported to ingest or become entangled in plastic as animals are unable to distinguish food from plastic in the environment, resulting in the ingestion of plastic particles [7,8].

In recent years, several studies have explored the unusual ability of some insects to consume and even biodegrade different types of plastics. While feeding, insects come into contact with a wide range of hydrocarbon polymers in their diet, and the gut of some insects contains microbial symbionts that aid in the decomposition of these polymers. Soon, various insects, such as mealworms, meal beetles, weevils or wax moths, particularly of the orders Coleoptera and Lepidoptera, were identified as having remarkable abilities to consume and degrade a wide range of synthetic polymers such as polyethylene, polyurethane, polypropylene, polystyrene, and polyvinyl chloride into lower molecular weight, simple and nontoxic molecules, which are eventually excreted as fecula [9]. Moreover, the rate of plastic consumption by these insects is higher than that of bacteria and fungi, isolated from various sources, such as soil, garbage or sewage sludge. These insects mainly include the yellow mealworm (larvae of *Tenebrio molitor*), the greater wax moth (larvae of *Galleria mellonella*), and the superworm (larvae of *Zophobas morio*) among others [10]. The complete life cycle of Lepidopterans and Coleopterans insects consists of four stages: eggs, larvae, pupae, and adults; a significant portion of their life is spent in the larval stage. It is worth noting that the length of each stage of the insect life cycle can vary according to several factors, such as temperature, humidity, nutrition and age of the parents. Interestingly, the initial larval and pupal stages of *Tenebrio Molitor,* named also yellow mealworm larvae for their color, are rich in protein and considered a popular dish in some countries. The larval period varies from 22 to 100 days, while the pupal period lasts about 8 days. *Zophobas Morio,* a synonym of *Zophobas atratus,* is commonly known as a superworm or royal worm, associated with damaged stored foods. *Z. Atratus* adult beetles look very similar to *T. Molitor*, but are larger and measure about 2-3 cm in length [11,12]. These larvae can be easily raised on fresh oats, wheat bran, or cereals with potatoes, cabbage, carrots, or apple.

Polystyrene (PS), molecular formula $[-CH(C_6H_5)CH_2-]_n$, commonly known as Styrofoam, accounted for about 5.3% (c.a. 21 Mt/year) of the total plastic consumption in 2022 [13]. Although PS is considered a durable plastic, PS products are often designed for a very short service time and one-time use because of the low cost of this material. Is an extremely stable polymer with high molecular weight and strong hydrophobic character, which makes this polymer highly resistant to biodegradation. Several soil invertebrates have also been tested to determine whether they were able to degrade PS, including earthworms, isopods, slugs, millipedes or snails [14].

In this work, the larvae of yellow mealworm *Tenebrio Molitor* and superworm *Zophobas morio,* two species of Coleopterans tenebrionidae larvae, were chosen and prepared to carry out the research. Before the tests, the larvae were placed in a polypropylene plastic container and fed their usual food, then expanded PS foam was used as raw material for the larvae of both species.

The degradation of polystyrene treated with hydrogen peroxide and subjected to microwaves irradiation was also studied. The use of microwaves for polymer degradation is an excellent alternative to conventional thermal heating, offering increased reaction rates, reduced reaction times

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and energy savings. Typically, polymers such as PS have poor dielectric properties and are unable to absorb enough microwave energy to achieve the necessary temperature for degradation. Consequently, in order to increase absorption, it is necessary to use solvents such as hydrogen peroxide that can absorb microwave energy to achieve the required temperature [15–17].

Six different experimental diets were compared: *(i) T. Molitor* fed with bran; *(ii) T. Molitor* fed only PS; *(iii) T. Molitor* fed only PS treated with H_2O_2; *(iv) Z. Morio* fed with bran; *(v) Z. Morio* fed only PS and *(vi) Z. Morio* fed only PS treated with H_2O_2. The change of the larvae mass and the survival rate were measured periodically. Furthermore, a morphological analysis of frass was performed by Scanning Electron Microscopy (SEM), Mn, Mw and polydispersity (PD) were determined by Size Exclusion Chromatography (SEC) and additional characterization of the residual polymer was obtained using Fourier Transform Infrared Spectroscopy (FTIR) to identify chemical modifications resulting from PS digestion. There has been investigated an innovative aspect regarding the treatment of PS with H_2O_2 in order to facilitate PS biodegradation by Tenebrionidae larvae.

2. Materials and Methods

2.1. Test Materials

Tenebrio Molitor (yellow mealworms) and *Zophobas Morio* (superworms) at the larval stage were purchased from a local supplier (Palermo, Italy) and tanged in length from 2 to 3 cm and 4 to 8 cm, respectively. Expanded PS foam waste of electronic equipment boxes was collected and used as a feedstock for the larvae of both species. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of PS were 133,174 Da and 236,693 Da, respectively. Hydrogen peroxide (36 volumes 100 ml) was purchased from a local supplier (Palermo, Italy). For microwave oxdidation, a block of PS was placed in a beaker with hydrogen peroxide H_2O_2 for 5 minutes and then microwaved into a commercial microwave oven at 800 Watt for 3 minutes, which caused the initial breakage of the polystyrene chains.

2.2. Feeding Tests

To compare the PS consumption and biodegradation between the larvae of the two species, six treatments were prepared based on feeding conditions. A group of *T. molitor* and *Z. Morio* larvae (30 as a group) were reared on PS foam (~0.4 g) and PS foam treated with H_2O_2 (PS H_2O_2) (~0.4 g) as exclusive diet in a polypropylene plastic container. As a control, other group of larvae (30 as a group) were reared on normal diet of wheat bran and fruits. Prior to the commencement of the feeding experiment, the larvae were subjected to a 48 h starvation period to empty their intestines. All containers were maintained in the climatic chamber under the controlled condition i.e., 25 ± 1°C, 60 ± 2% humidity, for a period of 30 days.

2.3. Frass Collection and Characterization

The mealworms were fed with polystyrene blocks (PS) and polystyrene treated with H_2O_2 and microwave irradiation (PS-H_2O_2) as their sole diet for 30 days. Figure 1a shows the difference in length of the *Tenebrio Molitor* larva before and after transformation into a pupa. As mealworm pupae are not mobile, removing the dead larvae and pupae from the containers during the experiment was performed to prevent them from being eaten and to protect them from cannibalism by the remaining larvae.

Frass samples were collected from the container after 15 and 30 days of the experiment and Figure 1b shows the difference between *Tenebrio Molitor* frass and *Zophobas Morio* frass.

Figure 1c shows the evolution of *T. Molitor* from larva to pupa/ beetle for more than ca. 30 days, using real images of *T. Molitor* evolution stages. It is important to highlight that for more than ca. 30 days, all larvae turn into pupae/beetles and the experimental analysis, concerning the nutritional diet with bran or plastic, was inevitably interrupted.

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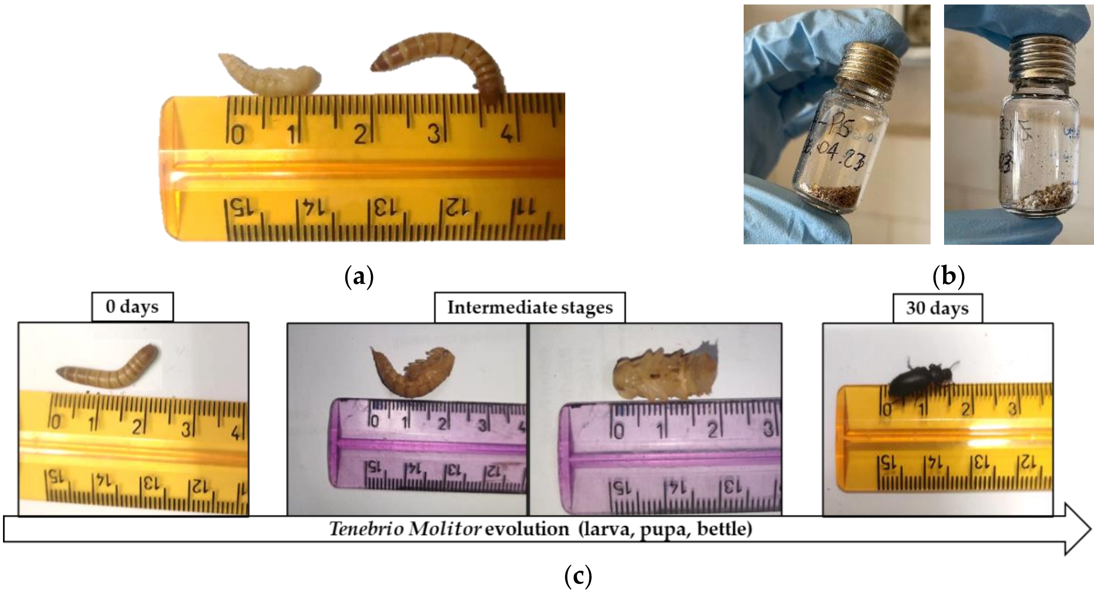


Figure 1. (a) Length measurement of the pupa and larva of T. Molitor; **(b)** Frass excreted by the *T. Molitor* and *Z. Morio* larvae after being fed with polystyrene and (c) *T. Molitor* evolution from larvae to pupa/ beetles for more than ca. 30 days (there are shown real images).

2.3.1. Weight Variation and Survival Rate

The weight of the larvae was monitored by weighing them every 5 days and ended on day 30. Since not all larvae were the same size, the weight was normalized to the number of individuals and the NW normalized weight (grams of total larvae/number of larvae) was calculated. The tests were conducted in triplicate.

2.3.2. FT-IR Analysis

A Fourier Transform Infrared Spectrometer (Spectrum One, Perkin Elmer, Shelton, CT, USA) was used to record IR spectra using 16 scans at a resolution of 4 cm^{-1} in the range 4000–450 cm^{-1}, using air as background. For KBr pelleting sample preparation, a press is used to make KBr pellets of the powder. The frass samples were mixed with KBr at a ratio of 1:100 and ground uniformly in a mortar and pestle. The mixture was then pressed with a hydraulic press (Mini Pellet Press, Specac, Orpington, UK) to form a 7 mm disc pellet. The mixture was then pressed into a 7 mm pellet using a hydraulic press (Mini Pellet Press, Specac, Or-pington, UK).

2.3.3. Morphological Analysis

The morphology of the surfaces of PS, PS-H2O2 and frass samples were investigated using a Scanning Electron Microscope (SEM FEI Quanta 200 FEG) equipped with an X-ray energy dispersive spectrometer (EDS). Prior to examination, samples were placed onto a conductive stub and then gold sputtered to avoid electrostatic charging effect.

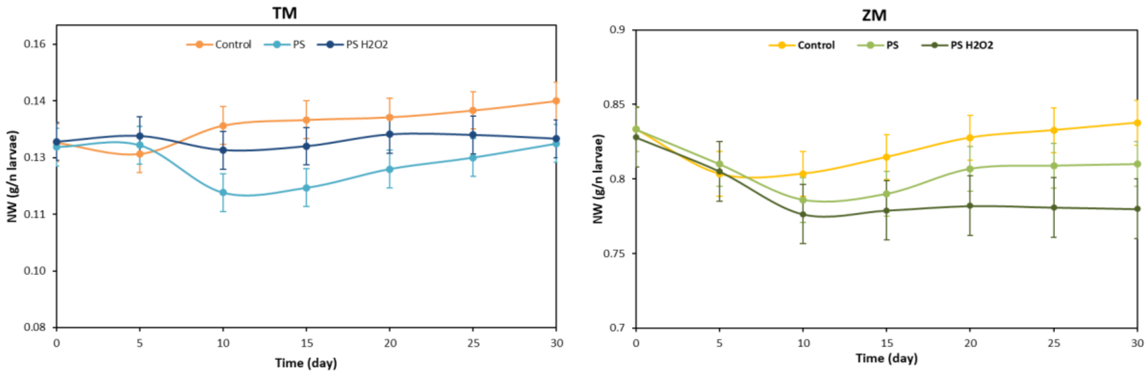
2.3.4. Size Exclusion Chromatography (SEC)

The changes of molecular weight of the polymer were followed by SEC. The detection was carried out using a differential refractometer. THF was the mobile phase with a flow rate of 1 ml min^{-1}. Calibration was performed with polystyrene standards (from 20250 to 470000 g mol^{-1}). Preparative SEC analyzes were performed in THF with Azura GPC Knauer apparatus equipped with four TSKgel Guard Super columns using an RDI 2.1 L differential refractometer. Sixty microliters of a polymer solution (3 mg ml^{-1}) were injected and eluted at a flow rate of 1 ml min^{-1}.

3. Results and Discussion

3.1. Weight Variation and Survival Rate of T. Molitor and Z. Morio Larvae

The weight of the larvae was monitored every 5 days during the test and Figure 2a,b show the normalised weight (NW) of the *T. Molitor* and *Z. Morio* larvae over 30 days of testing.



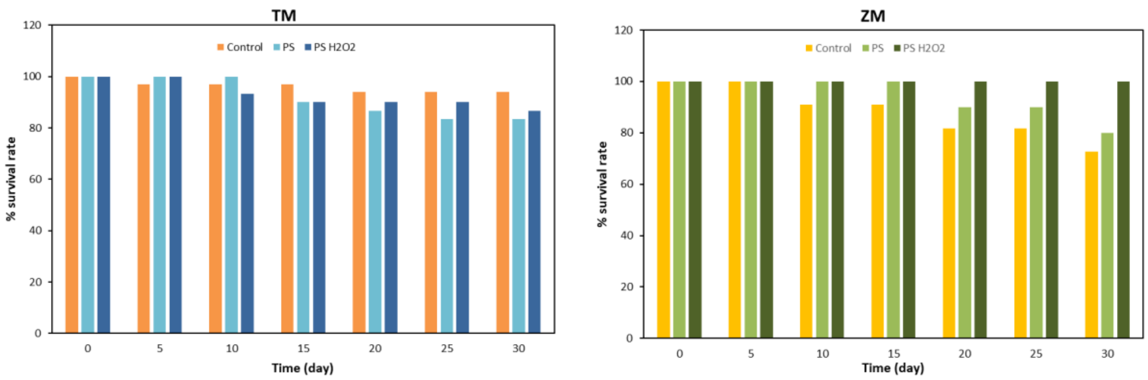
(**a**) (**b**)

Figure 2. Weight variation during the time: (**a**) NW of *T. Molitor* and (**b**) NW of *Z. Morio* fed with bran (control), foam PS and foam PS-H_2O_2.

The NW of *T. Molitor* larvae eating bran slightly decreased during the first 5 days, see Figure 2a The NW then slowly increased over the further 25 days, reaching a final increment of ~8.5 % with respect to the initial NW value. The NW of *T. Molitor* larvae eating PS remained almost constant during the first 5 days, while it decreased at 10 days. The NW then increased during the remaining 20 days with a final NW value similar to the initial value. *T. Molitor* larvae eating PS treated with H_2O_2 maintained similar NW value to the initial NW value all over the 30 days, suggesting that larvae ate the PS-H_2O_2 foams more easily than the untreated ones. Results are in line with data available in the literature. Peng et al. [18] reported a mass decrease of ~8% of *T. Molitor* larvae eating PS; while the same authors observed a mass increase of ~14 % when feeding the larvae with PS and bran at the same time.

The NW of the *Z. Morio* larvae eating bran showed a similar trend to that of the *T. Molitor* ones, see Figure 2b. However, a lower final NW value of ~0.5% is achieved at 30 days. Conversely, the NW of the *Z. Morio* larvae eating treated and untreated PS foams always decreased during the first 10 days of ~6%. The NW of larvae eating PS then slightly increased, while that of larvae eating PS-H_2O_2 remained almost constant. The final NW values of larvae fed with untreated and treated PS were ~2.8 % and ~5.8 % lower than the initial value (day 0).

The survival rates of the larvae of *T. Molitor* and *Z. Morio* eating bran (control), PS foam and PS-H_2O_2 foam are shown in Figure 3 and summarized in Table 1.



(**a**) (**b**)

Figure 3. Survival rate of (**a**) *T. Molitor* and (**b**) *Z. Morio* larvae fed with bran (control), foam PS and foam PS-H_2O_2.

In the case of *T. Molitor* larvae, see Figure 3a, larvae feeding on bran showed the highest survival rate of ~94 % at 30 days. Conversely, larvae fed PS reported the lowest value of ~83 %. Larvae fed with PS-H_2O_2 foam had a survival rate of ~87 %. In the literature, Peng et al. [18] reported survival rates of ~90 % for *T. Molitor* larvae eating PS, while Yang et al. [14] observed values of ~86%. In addition, Yang et al. [14] measured survival rates of ~85 % also for larvae eating bran. The different, although similar, survival rates of *T. Molitor* larvae can be attributed to the genetic differences between mealworm populations around the globe [19].

In the case of the *Z. Morio* larvae, the highest survival rate was exhibited by larvae eating PS-H_2O_2. The lowest value of ~73 % was found for *Z. Morio* larvae eating bran, while larvae fed with PS had a survival rate of ~80 %. These results are in contrast with the NW behaviour of *Z. Morio* larvae, indicating that, although larvae lost weight during the tests, they had a more suitable environment where live. The lower survival rates of the *Z. Morio* larvae with respect to that of the *T. Molitor* ones were somehow expected. An et al. [3] reported a survival rate of ~70 % of PS foam-eating and normal diet (bran)-eating Zophobas atratus larvae, which belong to the family of Zophobas larvae.

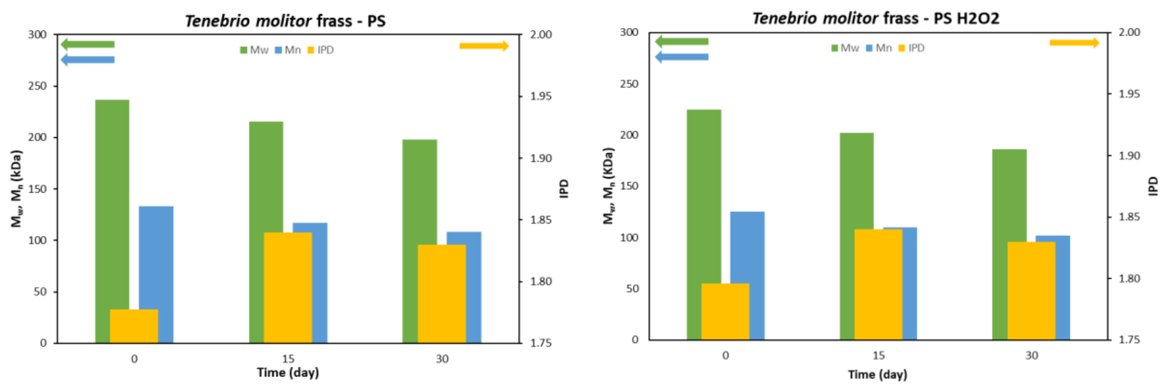
Table 1. Summary of PS biodegradation by *T. Molitor* and *Z. Morio* larvae.

Worm source	Initial weight (g)	Feedstocks	Survival rate (%)	M_w of frass (kDa)	M_w reduction (%)	M_n of frass (kDa)	M_n reduction (%)	PDI
T. Molitor	3.8	Bran	94	n.d.	n.d.	n.d.	n.d.	n.d.
		PS	83	197.8	16.4	108.4	18.6	1.83
		PS-H_2O_2	87	186.0	17.3	101.8	18.7	1.83
Z. Morio	8.3	Bran	73	nd	nd	nd	nd	nd
		PS	80	221.9	6.3	122.3	8.1	1.81
		PS-H_2O_2	100	208.5	7.3	116.2	7.1	1.79

Note: n.d. means not determined.

3.2. SEC analysis

SEC analysis was conducted to characterize the depolymerization and biodegradation of ingested PS [20]. Ponderal molecular weight (M_w), numerical molecular weight (M_n) and polydispersity index (IPD) of frass of *T. Molitor* and *Z. Morio* after 15 and 30 days are shown in Figure 4a–d, respectively. M_w, M_n and IPD of PS and PS-H_2O_2 foams analysed at day 0 are also reported.



(**a**) (**b**)

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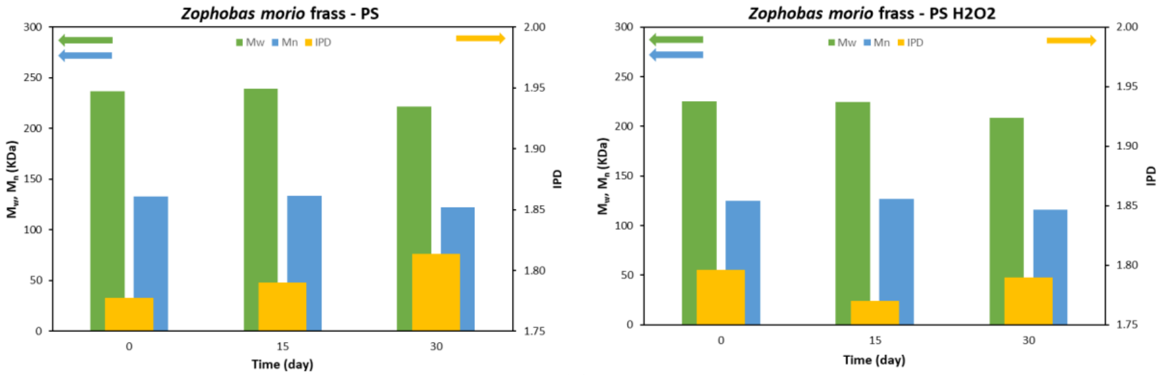


(c) (d)

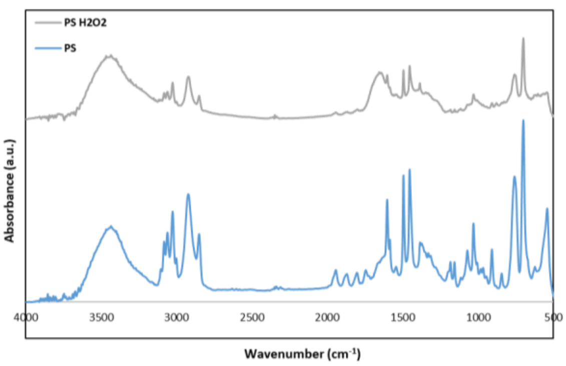
Figure 4. Data related to the changes of ponderal molecular weight (M_w), numerical molecular weight (M_n) and polydispersity (PD) of PS and PS-H_2O_2 (0 days), and frass of **(a, b)** TM and **(c, d)** ZM after 15 and 30 days.

The M_w and M_n of PS frass of *T. Molitor* larvae decreased over 30 days. Specifically, M_w was 237, 215 and 197.8 kDa at 0, 15 and 30 days, respectively, with a final reduction of 16.4 %. M_n values decreased from 133, 117 to 108.4 kDa, showing again a decrease of 18.6 %. In the literature, Yang et al. [14] reported similar M_w and M_n percentage reductions. Peng et al. [18] also observed a M_n decrease of ~20 %, however, the authors reported a M_w reduction of ~60 %. IPD increased in digested samples, as a consequence of the depolymerization and degradation action of the larvae producing lower molecular weight fragments [14,21]. PS-H_2O_2 foams had already lower initial values of M_w and M_n with respect to those of pristine PS ones due to the action of the H_2O_2, which also caused an increase in the IPD index. M_w and M_n decreased in frass of *T. Molitor* larvae eating PS-H_2O_2 foam due to the action of the larvae digestion, showing similar reduction percentages as those of the PS foam case, see Figure 4b.

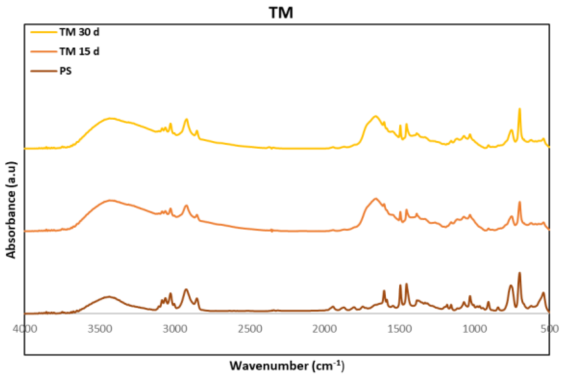
A lower degradation action was observed by *Z. Morio* larvae. M_w and M_n values remained almost constant during the first 15 days in both frass of PS and PS-H_2O_2 foams, while only at 30 days a decrease of ~7 % was achieved, see Figure 4c,d. The results indicate the superior depolymerization and biodegradation activity of *T. Molitor* larvae compared to *Z. Morio* larvae.

3.3. Spectroscopy Analysis

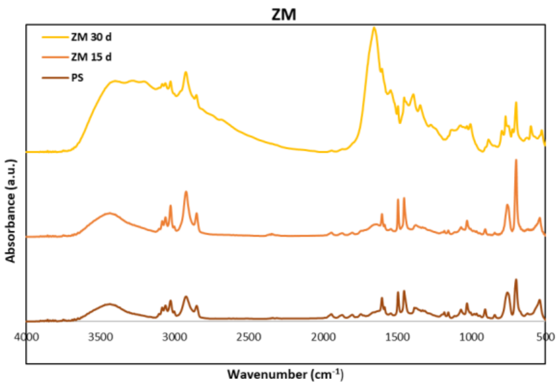
FTIR spectra of neat PS and PS-H_2O_2 foams before and after *T. Molitor* and *Z. Morio* larvae degradation are shown in Figure 5. Specifically, Figure 5a illustrates the FTIR spectra of neat PS and PS-H_2O_2 foams; Figure 5b,c refer to the frass collected after 15 and 30 days from *T. Molitor* and *Z. Morio* larvae eating PS foams; Figure 5d,e report the comparison between FTIR spectra of frasses collected at 30 days from *T. Molitor* and *Z. Morio* larvae eating PS and PS-H_2O_2 foams.



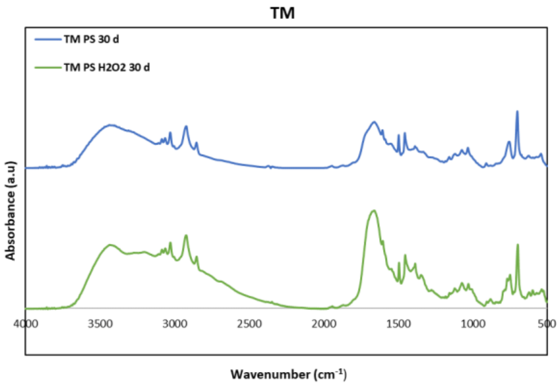
(a)



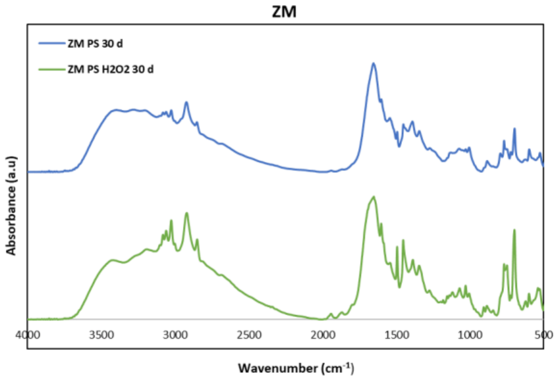
(**b**)



(**c**)



(**d**)



(**e**)

Figure 5. FTIR spectra of **(a)** neat PS and PS-H_2O_2 before the experiments (0 days) and FTIR of **(b)** frass of TM and **(c)** frass of ZM for the larvae fed with PS and PS-H_2O_2 after 15 and 30 days. **(d)** and **(e)** are the comparison of the spectra after 30 days for frass of TM and ZM feeding with PS and PS-H_2O_2, respectively.

Peaks at 625-970 cm^{-1} (ring-bending vibration) were visible in all FTIR spectra, although peak intensity was slightly damped after larvae digestion. The characteristic peaks known to represent the PS benzene ring (1550-1610 cm^{-1} and 1800-2000 cm^{-1}) were visible in neat PS samples, while they were considerably dampened in frass, providing evidence of ring cleavage. In addition, evidence of degradation was the appearance of carbonyl groups (1700 cm^{-1}) in frass. The broadening of peaks at 2500-3500 cm^{-1} in the FTIR of frass was also associated with the hydrogen bond of hydroxyl groups and/or carboxylic acid groups, suggesting a shift from hydrophobic to more hydrophilic surface properties [12].

Frass of *T. Molitor* larvae showed PS degradation already after 15 days, Figure 5b. Conversely, frass of *Z. Morio* larvae after 15 days had the same FTIR spectrum of that of neat PS foams. The H_2O_2 treatment already caused a damping effect of peaks at 2500-3500 cm^{-1}, as well as of those at 1550-1610 cm^{-1} and 1800-2000 cm^{-1}, indicating the degradation of the PS material [15,17]. Treatment of the PS with H2O2 promoted the degradation action of the larvae. A more intense peak at 1700 cm^{-1} and a wider peak at 2500-3500 cm^{-1} were, in fact, reported in FTIR spectra of frass collected from larvae eating PS-H_2O_2 foams with respect to those collected from PS eating one, see Figure 5d.

3.4. Scanning Electron Microscopy

The surface erosion of the plastic by the larvae was evaluated by SEM analysis of the frass. Firstly, the action of the H_2O_2 and microwave irradiation on the PS foam was investigated. Figure 6 shows the SEM images of the original PS samples and those treated with PS-H_2O_2 at different magnifications, i.e., 150x, 1200x and 20,000x.

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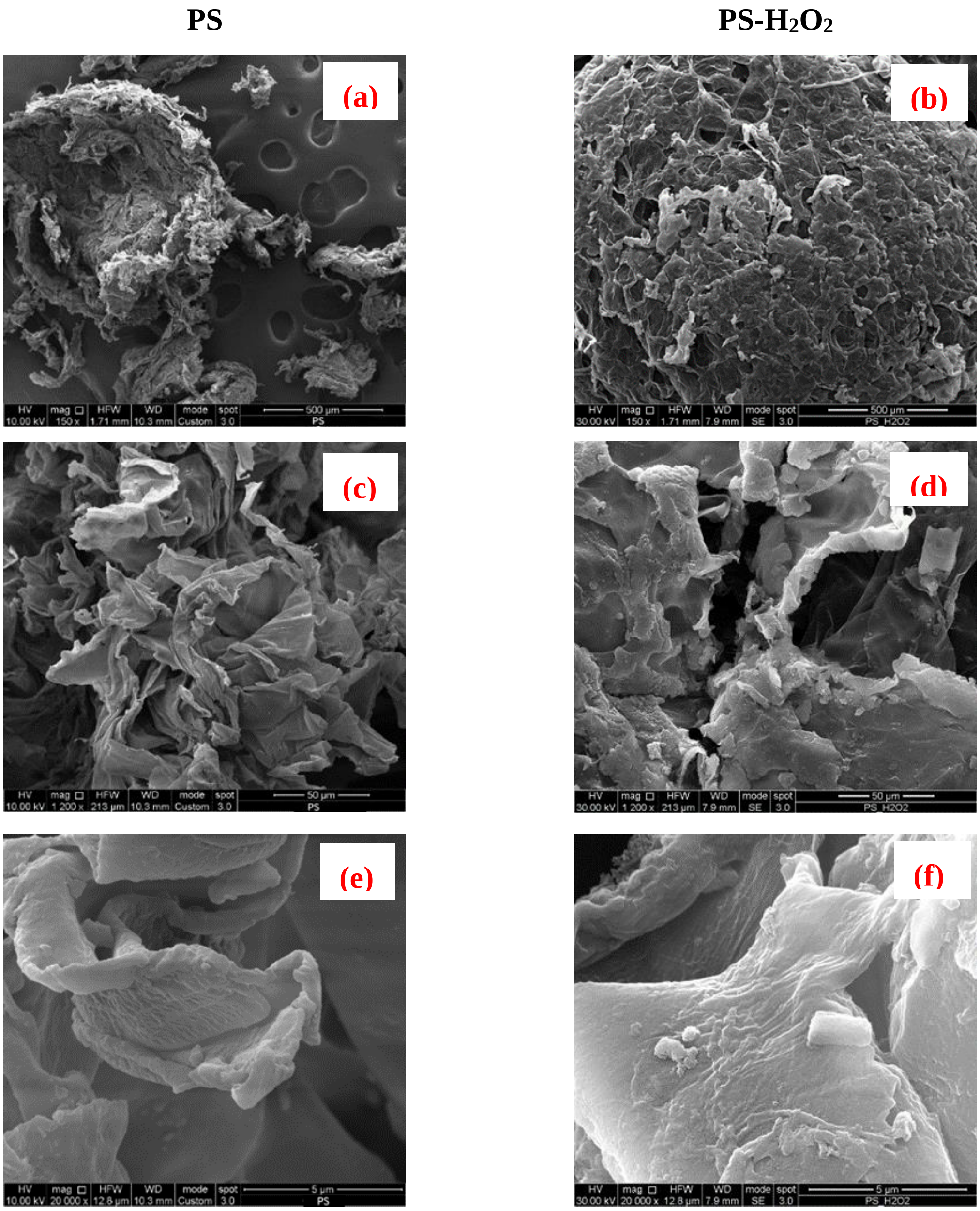

Figure 6. SEM observations at different magnifications, i.e., 150x, 1200x and 20000x, of PS (a, c, e) and PS-H_2O_2 (b, d, f) frass samples, respectively.

A small sphere of PS foam can be seen in Figure 6a, which probably imploded due to the high vacuum pressure applied during the gold spatter process. At higher magnification, in Figure 6d,g, the smooth surfaces of the PS material can be clearly seen, as typically reported in the literature [19]. Similar structures and smooth surfaces can also be observed in the PS-H_2O_2 samples, Figure 6b,d,f. This indicates that the action of H_2O_2 did not affect the surface of the PS foam. Based on this result, only SEM images of the frond collected after 15 and 30 days from *T. Molitor* and *Z. Morio* larvae that fed on PS foam will be discussed below.

Figure 7a,c,d are SEM images of *T. Molitor* frass after 15 days, while Figure 7b,d,f refer to SEM images of *T. Molitor* frass after 30 days.

***T. Molitor* frass after 15 days** **T. Molitor frass after 30 days**

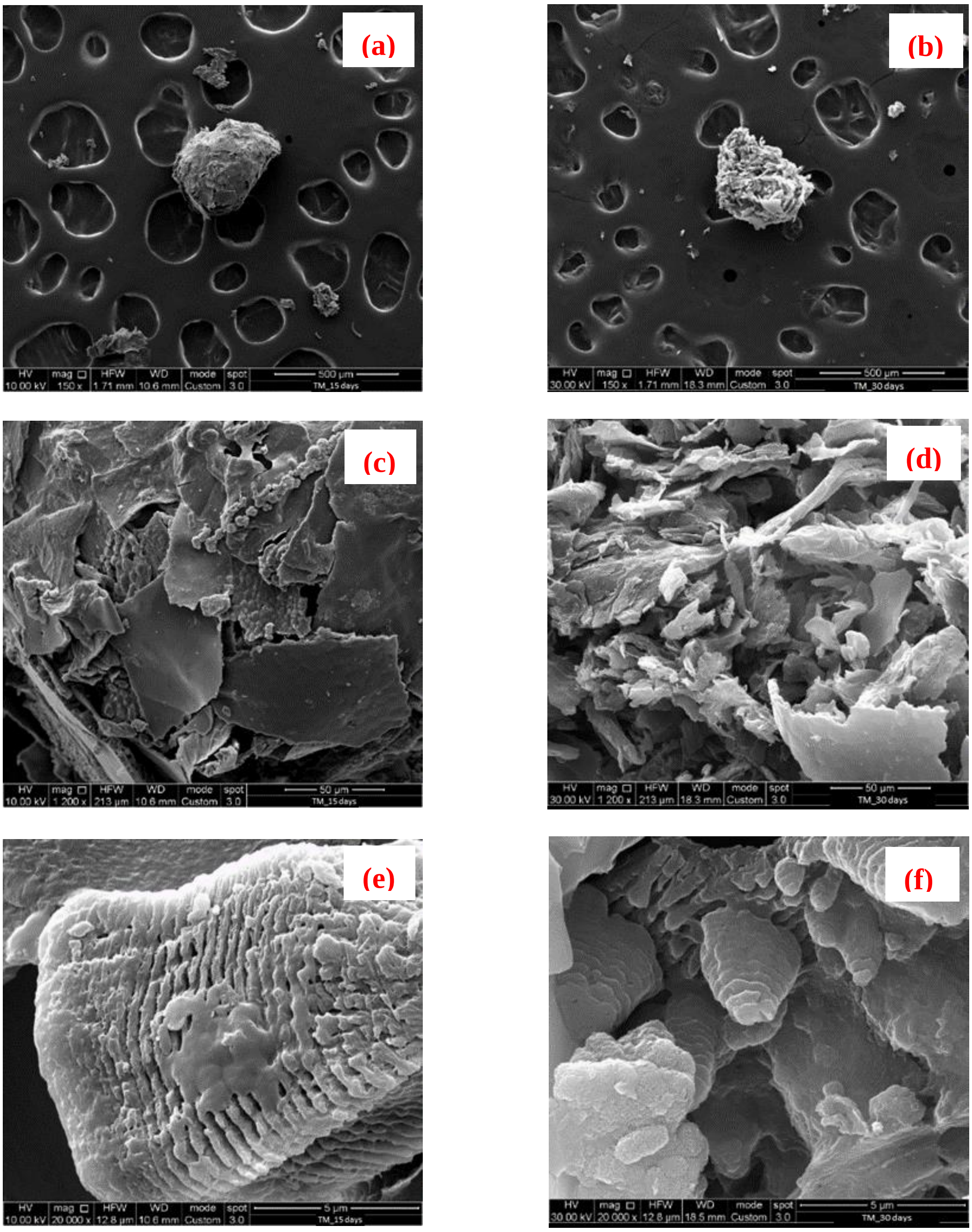

Figure 7. SEM observations at different magnifications of frass of *T. Molitor* larvae fed with PS foam.

PS samples isolated from the frass of *T. Molitor* larvae showed completely different, rough and irregularly carved surface structures. PS foam, see Figure 6.

PS flaskes can be observed at magnification of 1200 x, Figure 7c,d. In addition, pores can be identified at a magnification of 20000 x on PS surface, Figure 7e,f, demonstrating the degradation action of the larvae. Surface alterations of the plastics are characteristic of the aging processes occurring under the influence of microorganisms present in the intestines of insects or, more specifically, enzymes secreted by them. The folding of the previously smooth surface of the polymer and the formation of pitting was observed during the biodegradation of the polymers [19].

SEM observations of frasses collected from *Z. Morio* larvae after 15 and 30 days are shown in Figure 8.

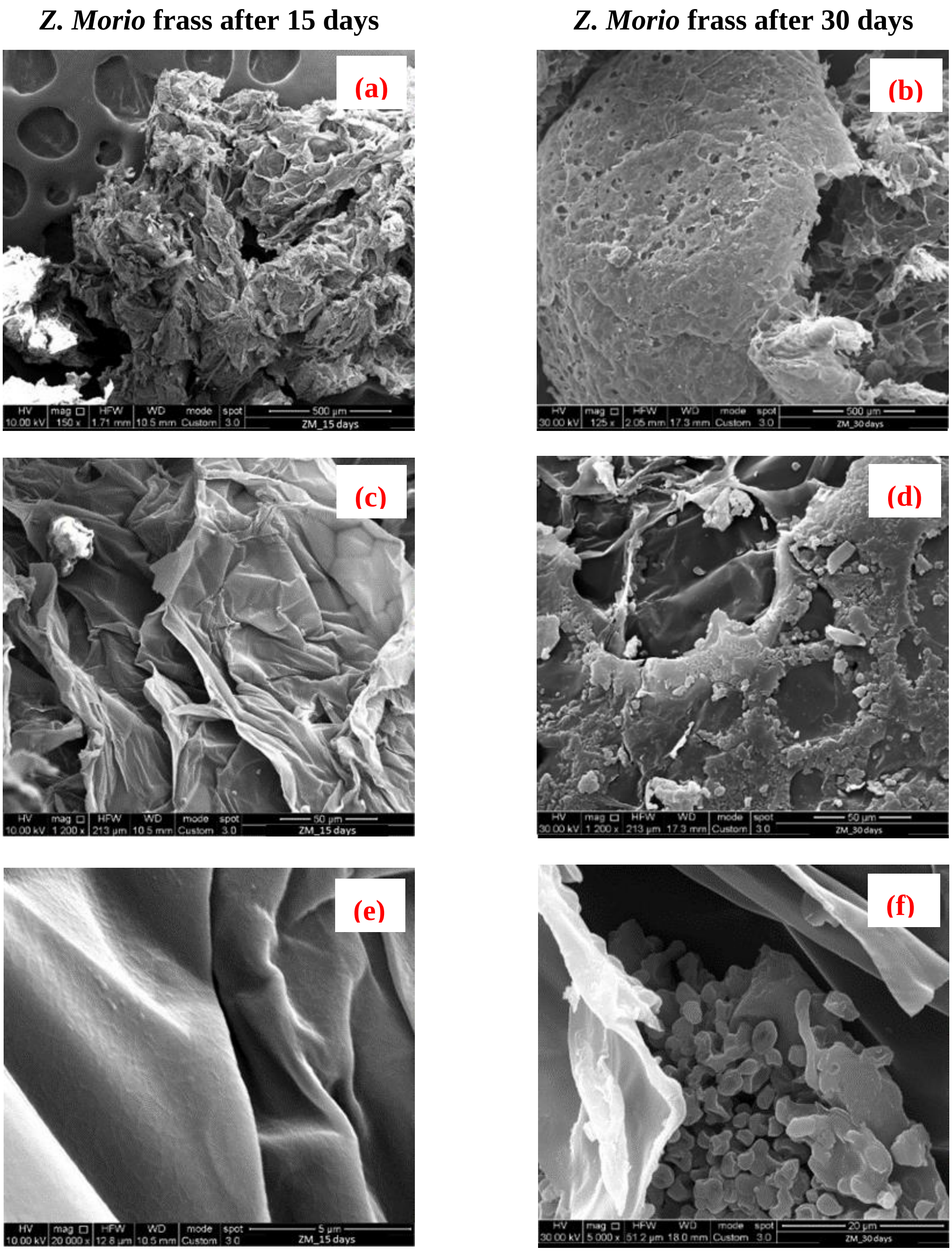

Figure 8. SEM observation at different magnifications of the frass of *Z. Morio* larvae fed with PS foam.

SEM images of PS samples collected from the frass of the *Z. Morio* larvae after 15 days, Figure 8a,c,e, showed a very similar structure with respect to that of the PS one, Figure 6. This indicates that *Z. Morio* larvae did not interact with PS material during the first 15 days of tests, as also discussed in Sections 3.1–3.4.

PS degradation can be noticed only after 30 days, namely PS shreds and holes can be observed Figure 8b,d,f. However, comparing Figures 7b and 8b, it can be always observed that large pieces of PS materials are still found in trass of *Z. Morio* larvae confirming the lower degradation action of the *Z. Morio* larvae with respect to that of the *T. Molitor* ones. Interestingly, traces of microorganisms were observed in frass of Z. *Morio* larvae as shown in Figure 9.

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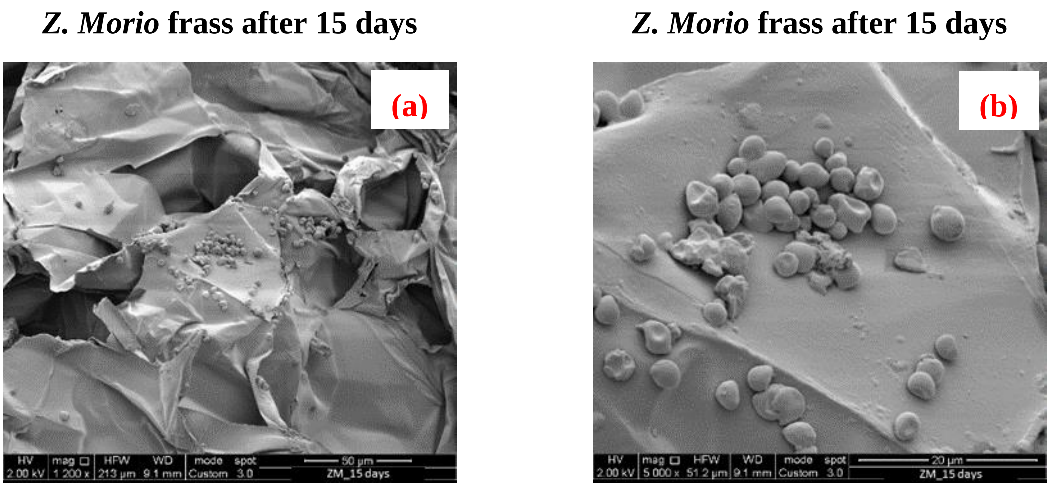

Figure 9. (a, b) SEM observation at different magnifications of organic matter in frass of *Z. Morio* larvae fed with PS foam.

Spherical cocci bacteria are observed. The presence of cocci bacteria is in accordance with evidence reported by Yang et al. [22]. The authors attributed the ability of the *Z.morio* larvae to depolymerize PS to the presence of microbiota, such as the cocci, in their gut. Cocci bacteria were also found in frass after 30 days of tests, here omitted for the sake of brevity.

4. Conclusions

In this work, the biodegradation of PS by *T. Molitor* and *Z. Morio* has been investigated, considering also treatment of PS with H_2O_2 in order to facilitate PS degradation. As discussed before, six different experimental diets were compared: *(i) T. Molitor* fed with bran; (ii) *T. Molitor* fed only PS; *(iii) T. Molitor* fed only PS treated with H_2O_2; *(iv) Z. Morio* fed with bran; *(v) Z. Morio* fed only PS and *(vi) Z. Morio* fed only PS treated with H_2O_2. The change of the larvae mass and the survival rate were monitored periodically and obtained results suggest that both *T. Molitor* and *Z. Morio* larvae are able to biodegrade the PS and their mass changes and survival rate are similar to that of larvae fed with bran. The analysis of *T. Molitor* and *Z. Morio* frass after 15 and 30 days show slightly decreased PS molecular weight and increase of polydispersity, suggesting the reduction of PS chain weight and length. Further, the data coming from FTIR analysis support this thesis highlighting the changes of PS compositions after the digestion.

Therefore, all these results highlight the ability of both larvae to survive using as fed plastic rather than bran.

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