Biodegradation of plastics from waste electrical and electronic equipment by greater wax moth larvae (Galleria mellonella),

Ping Zhu [a], Yilin Shen[a], Xiaowei Li[a*], Xiankai Liu[a], Guangren Qian[a], John Zhou [b]

[a] *School of Environmental and Chemical Engineering, Shanghai University, 99 Shangda Road, Shanghai 200444, People's Republic of China*

[b] *School of Civil and Environmental Engineering, University of Technology Sydney, 15 Broadway, Sydney, NSW 2007, Australia*

* Corresponding author: Xiaowei Li Tel : +86- 021-66137747 E-mail: lixiaowei419@shu.edu.cn

ABSTRACT

Biodegradation of insect larva is a potential method to treat waste electrical and electronic equipment (WEEE) plastics, but the information about feeding selectivity of insect larva to WEEE plastics is lacking. Two kinds of fresh and waste plastics were fed to *Galleria mellonella* (*G. mellonella)* larvae including rigid polyurethane (RPU, a thermosetting plastics), polystyrene (PS, a thermoplastic), waste RPU (WRPU) and waste polystyrene (WPS). The larvae both preferred significantly fresh plastics to waste ones. The possible reason was that WEEE plastics contain more metals and chemical additives than fresh plastics. WRPU, WPS plastics and the corresponding frass were analyzed to explore degradation mechanisms through scanning electron microscope (SEM) and Fourier transform infrared (FT-IR). The results of SEM and FT-IR revealed the destruction of physical structure integrities and the changes in functional groups in plastics, implying the occurrence of biodegradation. A typical hard block engineering plastic, waste high impact polystyrene (WHIPS), was fed to *G. mellonella* larvae before and after grounded into powders, and the larvae showed great apparent modification selectivity. In addition, six common fresh foamed plastics were fed to the larvae using different combinations, showing obvious feeding selectivity and preference. It possibly attributed to different physical properties and chemical structures of plastics. The consumption of WRPU plastics by the *G. mellonella* larvae was higher than that by the *Tenebrio molitor* (*T. molitor*) larvae, implying that different insect larvae have different ingesting capability to plastics. The study provides new insights into the selectively of insect larva for biodegrading WEEE plastics.

Keywords: Waste Electrical and Electronic Equipment Plastics; Insect Larva; Biodegradation; Feeding selectivity; *G. mellonella*

Introduction

Waste electrical and electronic equipment (WEEE) is a mixture of materials and components of scrapped electrical and electronic appliances, typically for example, obsolete computers and cell phones (Li et al., 2019). According to the statistics, about 30 million computers are disposed in USA merely and 10 million cell phones are discarded in Europe on a yearly basis. On a worldwide scale, an estimation of 50 million tons of WEEE is produced annually (Luhar et al., 2019). Presently, research about WEEE mainly focuses on its metal recycling. However, plastics materials lack due attention. They constitute approximately 17% of WEEE stream, mainly including acrylonitrile-butadiene-styrene (ABS), high impact polystyrene (HIPS), polyurethane (PU), phenolic resin (PF), polyethylene (PE), polystyrene (PS), polypropylene (PP) and polyvinyl chloride (PVC) plastics (Yang et al., 2013). Taking typical application examples, HIPS plastics usually serve as casing, housing, and PU plastics can serve as insulation. Final disposal of WEEE plastics is reaching similar levels to municipal solid waste plastics (Wu et al., 2020, Shen et al., 2018). A lack of proper management, WEEE plastics cause environmental and health problems. Some plastics related chemicals occur in the food chain, then enter the human body. Hazardous elements in WEEE plastics, including heavy metals and chemical additives, such as lead (Pb), mercury (Hg) and flame (or fire) retardants, will have a negative impact on human health. They may damage physiological system, cause cancer and adverse hormonal effects (Petridis et al., 2017, Charitopoulou et al., 2020).

As byproducts of the booming technology, huge volume of environmentally hazardous WEEE plastics produced globally. How to dispose them has become an important and urgent issue (Akram et al., 2019). Compared to common methods of processing WEEE plastics (landfill, incineration, physical recycling and chemical recovery), biodegradation is a more noteworthy way to fight against plastic pollution for its low costs and environmentally friendly impacts (Azubuike et al., 2016). There are many studies on the biodegradation of plastics by microorganisms, including bacteria and fungi (Magnin et al., 2019). Microorganisms utilize plastics as carbon and/or nitrogen sources (Sheth et al., 2019). A fungus, *Aspergillus fumigatus* Strain S45, inoculated on specific tween 20 agar plate at 30 ℃, was able to reduce weight of PU films which exposure to UV radiation as pre-processing by 15%-20% (about 37.5 - 50 mg) in 28 days (Osman et al., 2018). A *Paenibacillus* sp. can also degrade PE after being cultured with Brain Heart Infusion (BHI), minimum salt medium (MSM) and BHI agar plates at 37℃ for 4 weeks. Cultured bacteria and PE films which pretreated with ethanol and bleach incubated in an incubator shaker for 3 months, then about 30% of PE (about 4.5 mg) was degraded (Bardaji et al., 2019). Summarily, the results about the plastics microbial degradation were gained through the simulated environment of the laboratory. Because the conditions required for microbial degradation of plastics are complex, specific culture medium, growth temperature, and pretreatment requirements for plastics are needed (Magnin et al.,2020, Yang et al., 2018). This is not conducive to the application of microorganisms to the actual treatment of waste plastics.

Insect is increasing to use for the degradation of plastics. Insect has better processing effect and stronger applicability than microorganisms (Yang et al., 2015).

Moreover, insect growth conditions are not as strict as those of microorganisms, making it more suitable for practical applications. The larvae of *Tenebrio molitor* (*T. molitor*) have been proven to ingest and biodegrade PS and PE to CO_2 and lower molecular weight compounds within their gut (Brandon et al., 2018, Wu et al., 2018). Yang et.al (Yang et al., 2018) reported that the PS and bran were fed to *T. molitor* larvae in the 32-day culture test at 20~30 °C, and up to 84 % of PS (about 1.5 g) was consumed. In another research, 47.7% of the ingested Styrofoam carbon was converted into CO_2 in 16 days under similar reaction conditions (Brandon et al., 2018). Moreover, the larvae of *Galleria mellonella* (*G. mellonella)* are also capable of chewing and ingesting PE films (Yang et al., 2014, Zhang et al., 2020). The larval homogenate smeared on PE films caused mass loss of 13% (about 92mg) in 14 hours (Bombelli et al., 2017). The plastics used in the above studied are all new thermoplastic raw material, which are not waste, not added catalyst or additives and linear low-density such as PE and PS. The performance of different insects degrading plastic is also compared (Yang et al., 2021, Peng et al., 2019, Billen et al., 2020).

However, there are many impurities in plastics from WEEE, such as metals, freon and polycyclic aromatic hydrocarbons (PAHs) in waste refrigerator plastics (Mao et al., 2020, Zhang et al., 2020, Dement'ev et al., 1991). This makes WEEE plastics more hazardous and more treatable than ordinary fresh plastics. Therefore, this paper wants to explore the difference between WEEE and fresh plastics during degradation of *G. mellonella* larvae. In addition, a variety of plastics coexist in the actual environment. There are still many types of plastics in WEEE that lack research on degradation of *G.*

mellonella larvae to co-exist plastics, such as rigid thermosetting PU, hard lumpy engineering plastics HIPS as well as PVC and PF plastics which account for a large proportion. Their physical and chemical properties such as density and functional group are very different which may affect the degradation of these plastics by *G. mellonella* larvae. When insects face different plastics, there may be a certain preference and selectivity under the action of many factors. As far as we know, there is little information about how insect larvae deal with different plastics in WEEE. In addition, the performance of different insects in the face of the same WEEE plastics is also worth exploring.

In this study, *G. mellonella* larvae were fed with nine types of plastics commonly found in WEEE, including three waste plastics, i.e. waste HIPS (WHIPS), waste rigid PU (WRPU), waste PS (WPS), and six fresh plastics, i.e. rigid polyurethane (RPU), PF, PE, PS, PP, PVC. Selection considers representative, these plastics basically include the most content plastics in WEEE. The influences of plastics and insect types on degradation, feeding selectivity of insect larvae were investigated and analyzed. X-ray fluorescence spectrometer (XRF) was performed to analyze the element composition between the fresh and WEEE plastics. Scanning electron microscope (SEM) and Fourier transform infrared (FT-IR) were performed to characterize the changes in morphology and functional groups of the plastic feedstock and *G. mellonella* larvae's frass. Besides, as the most typical thermosetting WEEE plastics, WRPU plastic was selected to compare the differences between *G. mellonella.* and *T. molitor* larvae.

2. Materials and Methods

2.1 Materials

The WRPU, WHIPS and WPS plastics (Fig. S1a, b, c) were gained from an environmental protection company in Shanghai, China. WRPU is insulation material from the waste refrigerator. WHIPS is engineering plastic from the shell of a WEEE. WPS is insulation material from a WEEE. WRPU and WPS are also foamed plastics. Fresh RPU, PF, PE, PS, PP and PVC plastics were uniformly selected to compare feeding selectivity of insect larvae to different plastics. Fresh plastics were all foamed plastics and purchased from a plastic supplier in Shanghai, China. The plastics were cut into cubes with a side length of 1 cm, typically like PS (Fig. S1d).

G. mellonella larvae and *T. molitor* larvae (Fig. S1e, f) were purchased from a breeding farm in Yancheng, Jiangsu, China. *G. mellonella* was mature larvae of about 6th instar, with high activity, 25-30 mm in length, and 310-370 mg / worm mass. *T. molitor* was mature larvae of about 5-6th instar, with high activity, 20-25 mm in length, and 55-110 mg / worm mass. All larvae were experienced starvation treatment for 3 days before the experiment.

2.2 Methods

2.2.1 Plastics feeding Insect Larvae tests

According to the pre-experiment of *G. mellonella* larvae ingesting plastics, 2.0 g of WHIPS, WPS, WRPU, PS, RPU plastics and honeycomb (HC) as the only carbon source were fed to 40 *G. mellonella* larvae in incubators of 30 °C and full darkness for

7 days, respectively. Remaining mass of plastics was weighed, the generated frass was collected and survival rate of the larvae was measured every day.

In the experiment exploring the feeding selectivity of *G. mellonella* larvae to six fresh foamed plastics, 40 *G. mellonella* larvae were as a group. Each group was fed with 30 pieces of different kinds of fresh foamed plastics, including RPU, PS, PP, PVC, PF and PE. One kind (30 pieces for each), two (15 pieces for each), three (10 pieces for each) or six kinds (5 pieces for each) of plastics were combined differently. Other experimental condition was the same as the above.

G. mellonella and *T. molitor* larvae were selected to compare the behavior of feeding WRPU. According to the same total weight, 40 *G. mellonella* (or 100 *T. molitor*) larvae were as a group, respectively. Each group was cultured for 20 days, the situation of the larvae was observed and recorded every 2-4 days. Other experimental condition was the same as the above.

2.2.2 Analysis and Characterization Methods

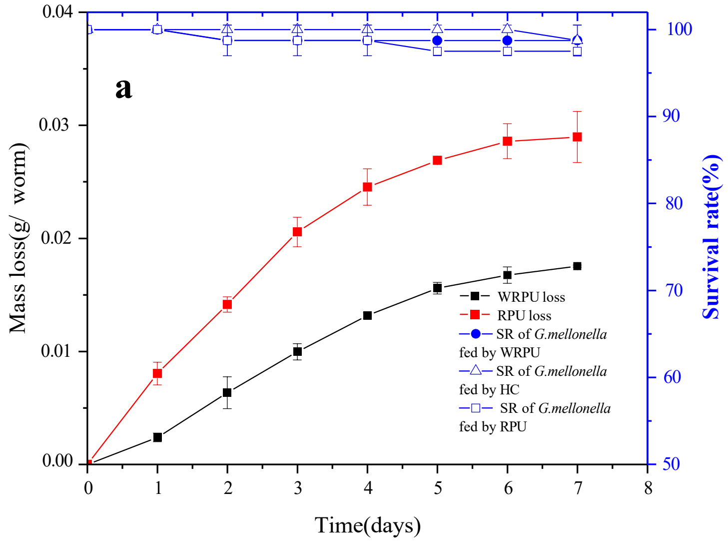
Fresh and WEEE plastics were measured by XRF (XRF-1800, Shimadzu Ltd, Kyoto, Japan), to explore the element composition. SEM and FT-IR were used to characterize the changes in surface topography and functional groups of the feedstock and frass of WEEE plastics, to explore the effect of ingestion on the plastic properties. The samples were crushed, sieved through 200 mesh, and then dried at 80°C for 24 hours before analysis. SEM images were determined using a JSM-6700F microscope (Royal Dutch Philips Electronics Ltd, Eindhoven, Netherlands) at 30 volts. FT-IR spectra were gained using a NICOLET 6700 FT-IR Spectrometer (Thermo Fisher Scientific Corporation, Massachusetts, USA) over the range of 400-4000 cm^{-1}, resolution of 4 cm^{-1} and scanning step of 1 cm^{-1}. All tests were repeated in triplicate.

3. Results and Discussion

3.1 Feeding Selectivity of *G. mellonella* Larvae to Kinds of Fresh and WEEE Plastics

Some research on insects degrading plastics focused on PS (Peng et al., 2019, Billen et al., 2020). As the representative of thermosetting plastics and thermoplastics, RPU, PS, WRPU and WPS foamed plastics from WEEE were selected as the sole carbon source respectively, to investigate the difference and selectivity of *G. mellonella* larvae being fed fresh and WEEE plastics.

The consumption and survival rate of RPU, WRPU, PS, WPS plastics and HC by *G. mellonella* larvae are measured (Fig.1). After one week, the consumption of RPU and WRPU plastics are about 1.16 g and 0.70 g, respectively. And the average consumption of RPU and WRPU plastics are about 4.14 mg / worm / d and 2.53 mg / worm / d, respectively (Fig. 1a). The consumption of PS and WPS plastics are about 0.33g and 0.27 g, respectively. And the average consumption of PS and WPS plastics are about 1.18mg / worm / d and 0.96 mg / worm / d, respectively (Fig. 1b). The consumption of *G. mellonella* larvae to the WRPU and WPS plastics is lower than the corresponding fresh plastics, implying that the larvae prefer the fresh plastics to the WEEE plastics. There are presumably two reasons for the difference. One reason is that waste plastics have strong smell and migration of heavy metals, etc (Mao et al., 2020, Liu et al., 2019). The other reason is the contents of metals and additives in waste and fresh plastics are different. As shown in Table S1a, there is chlorine in WRPU, which is probably from refrigerator foaming agent-Freon (Dement'ev et al., 1991). The metal contents in WPS is much higher than that in PS (Mao et al., 2020) (Table S1b). The strong smell and metals migrating to the surface can reduce the appetite of larvae ingesting waste plastics. The chlorine and metals can poison the gut microbes in the larvae (Caravelli et al., 2004, Jacquioda et al., 2018).



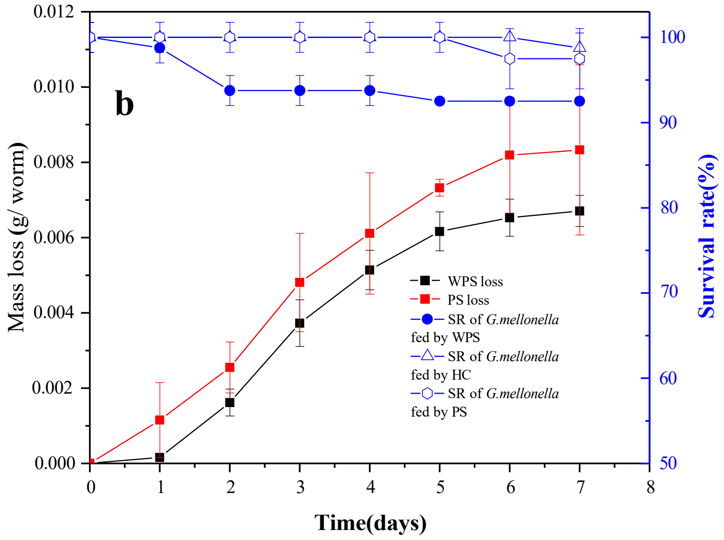


Fig. 1. Consumption and survival rate of *Galleria mellonella* larvae fed with HC, RPU and WRPU (a) or PS and WPS (b). HC, honeycomb; RPU, rigid polyurethane; PS, polystyrene; WRPU, waste rigid polyurethane; WPS, waste polystyrene.

Moreover, the consumption of *G. mellonella* larvae to the WPS and PS are much lower than that of the WRPU and RPU. The SR of the WRPU group is similar to that of the RPU and HC group (Fig. 1a), and the SR of the WPS group is significantly lower than that of the PS and HC group (Fig. 1b). It is inferred that compared with WPS and PS, WRPU and RPU contains not only carbon source but also nitrogen and less metals (Peng et al., 2019). The metal contents may have a greater impact on the *G. mellonella* larvae feeding on WPS. But the SR of plastic-fed *G. mellonella* larvae all remains above 90% in one week, suggesting that the SR of *G. mellonella* larvae is not significantly affected by WEEE plastics in the experiment period.

3.2 Analysis of the Frass Produced by *G. mellonella* Larvae Ingesting WEEE Plastics

After the WRPU and WPS plastics were ingested, it deserves to investigate whether they are biodegraded by *G. mellonella* larvae according to the physical and chemical changes of the feedstock and frass of WEEE plastics. Fig. S2a shows that the plastics produce holes and even fragments after being gnawed by the larvae, implying the *G. mellonella* larvae have a strong gnawing effect on the block-shaped WRPU plastics. SEM images of Fig.2 observe that microscopic morphology changes from honeycomb-like cellular structure of the WRPU plastics (Fig. 2a) to eroded sheet-like structure of the WRPU frass which has generated many small fragment (Fig. 2b). It indicates that *G. mellonella* larvae destroy the physical structure of WRPU plastics and produce an erosion effect in the intestine.

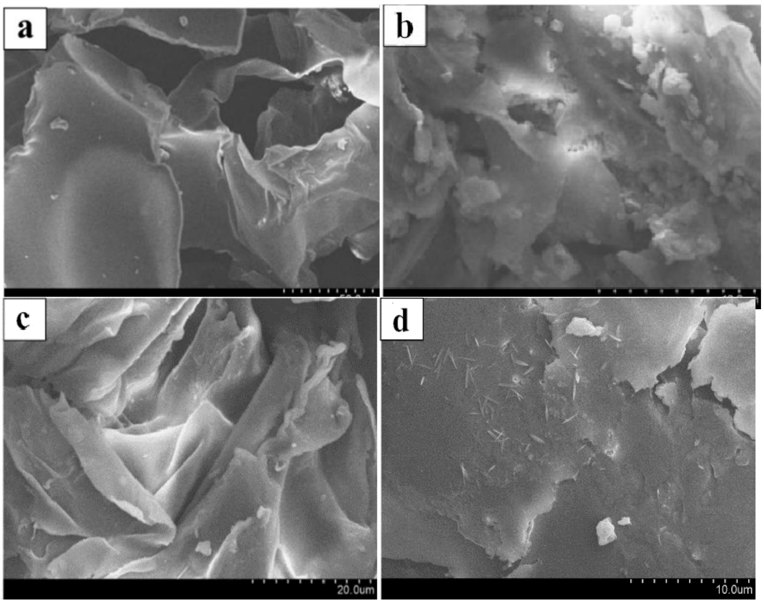
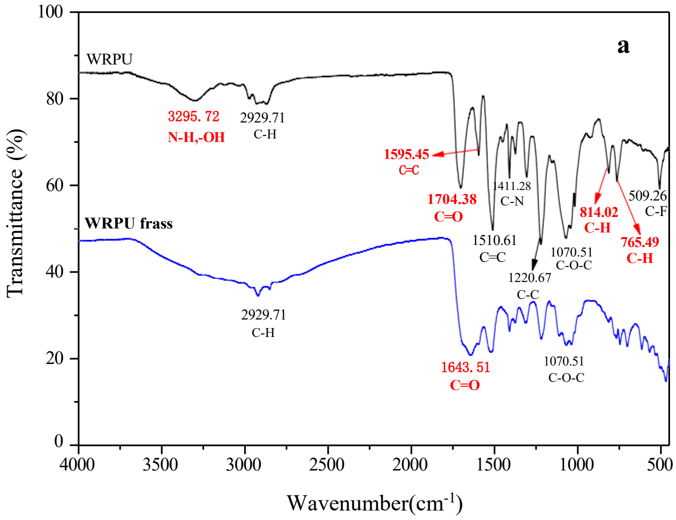


Fig. 2. SEM of WRPU feedstock (a) and frass (b), and WPS feedstock (c) and frass (d). WRPU, waste rigid polyurethane; WPS, waste polystyrene.

Fig. S2b shows that the WPS plastics are less damaged than the WRPU plastics. The result seems to confirm the finding in Fig.1 that the consumption of *G. mellonella* larvae to the WPS is lower than that of the WRPU. SEM images of Fig.2 observe that microscopic morphology changes from sheet-like wrinkled structure of the WPS plastics (Fig. 2c) to flatter, cracks and small needle-like particles structure of the WPS frass through the function of the intestine of *G. mellonella* larvae. (Fig. 2d). These phenomena indicate that the feeding process of *G. mellonella* larvae on WRPU and WPS plastics is to gnaw the plastic into granules and then enter the intestine for digestion. However, the products of digestion-frass have different forms.

Subsequently, chemical structures of the WRPU, WPS plastics and the corresponding frass were analyzed by FT-IR. Fig. 3a shows the spectra of WRPU frass have the significant changes in three regions: 3250-3330, 1550-1710 and 500-900 cm^{-1}. These changes indicate that some functional groups bonds are decomposed and transformed to the new bonds. *G. mellonella* larvae mainly acts on the hard segments of WRPU mainly composed of urethane bonds (Oprea et al., 2010). The absorption peaks of 3296 cm^{-1} gradually broaden until they merge with those at 2930 cm^{-1} to form the wider absorption peak. This can be presumably attributed to the H- bonded N-H and -OH of the biodegradation products. The increase in the hydroxyl suggests a possibility of conversion from hydrophobicity to hydrophilicity of polyurethane (Oprea et al., 2018). The rupture of the urethane bond in the main chain of WRPU at 1704 cm^{-1}, the formation of hydrogen bonded carbonyl at 1644 cm^{-1}, and disappearance of C=C and C-H peaks of benzene rings at 1595, 814 and 765 cm^{-1}, all indicate that WRPU has been not only ingested, but also degraded. In addition, no significant change occurs in ether bonds (1071 cm^{-1}), because the soft segment of WRPU dominated by the ether bond is more bio-resistant.



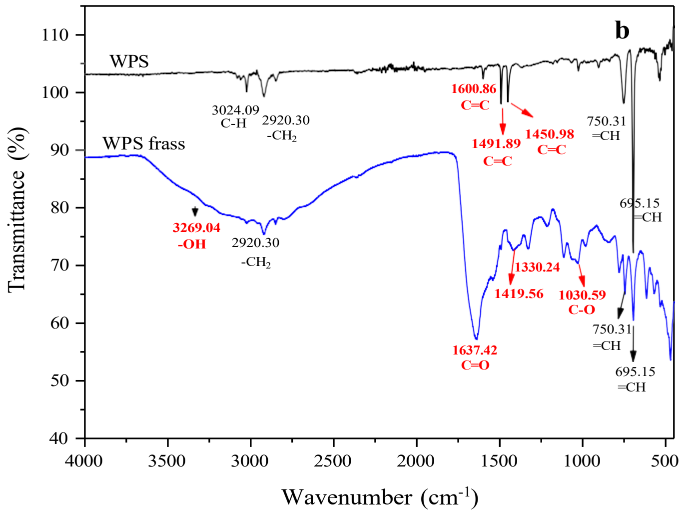


Fig. 3. FT-IR spectra of WRPU feedstock and frass (a), and WPS feedstock and frass (b). WRPU, waste rigid polyurethane; WPS, waste polystyrene.

FT-IR spectra of the WPS frass have significant changes in two regions: 2500-3500 and 1400-1710 cm^{-1}, compared with the WPS (Fig. 3b). Like Fig. 3a, the changes in 3269 cm^{-1} absorption peaks representing the WPS benzene ring (C=C stretch, 1400-1710 cm$^{−1}$) are dampened in the frass samples, indicating the ring cleavage (Yang et al., 2018). Further evidence of degradation is the observed decrease in intensities of peaks characteristic for WPS and the appearance of -C=O- (1637 cm$^{−1}$) (Yang et al., 2014, Sekhar et al., 2016). The broadening of peaks at 2500-3500 cm$^{−1}$ is associated with the hydrogen bond of hydroxyl groups and/or carboxylic acid groups, suggesting a shift from hydrophobic to more hydrophilic surface properties (Lou et al., 2020).

In other words, *G. mellonella* larvae changes not only the physical structure but also chemical structure and composition of the WRPU and WPS plastics. As shown in Fig. 4, the biodegradation process of WRPU and WPS by *G. mellonella* larvae undergoes the hydrolysis or oxidation. According to the analysis of chemical groups, the urethane bonds in WRPU are broken due to the hydrolysis. However, the carbon chain of WPS is mainly attacked, and oxygen-containing functional groups are generated due to the oxidation reaction. Depolymerization of high molecular weight substances occurs, and low molecular weight products are formed, which may be further assimilated or mineralized. The different degradation products of the two WEEE plastics are attributed to the differences in not only chemical composition but also structure of WRPU and WPS. The molecular configuration of the WRPU is a cross-linked body structure, while the molecular structure of the WPS is linear (Yuan et al., 2020).

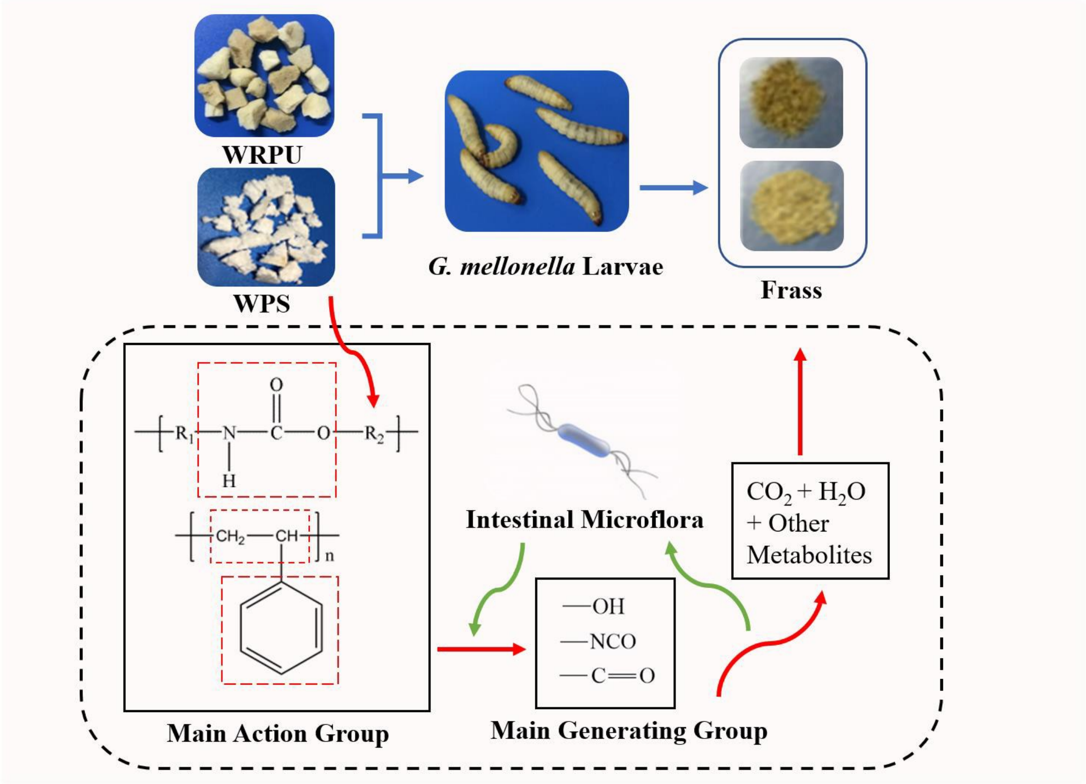


Fig. 4. Mechanism diagram of *G. mellonella* larvae biodegrading WRPU and WPS. WRPU, waste rigid polyurethane; WPS, waste polystyrene.

3.3 Feeding Selectivity of *G. mellonella* Larvae to Apparent Modification of WEEE Plastics

Most EEE plastics are lumpy and hard, such as ABS for TV shell and HIPS for printer shell. So we investigated the effect of changing the appearance of WEEE plastics-WHIPS on the feeding selectivity of *G. mellonella* larvae. Fig. S3a shows that there are no signs of damage on the appearance of the lumpy WHIPS plastics. However, after the lumpy WHIPS plastics are physically modified to grind into powders (Fig. S3b), the powders are basically ingested by *G. mellonella* larvae and the corresponding frass are generated (Fig. S3c). The result reflects that the *G. mellonella* larvae cannot ingest WHIPS hard lumpy engineering plastics, but have a feeding behavior to the powdered WHIPS plastics (Fig. 5). In fact, grinding WHIPS into powder only changes its particle size and does not change its chemical properties. The above results indicate that the appearance of plastics have a great effect on *G. mellonella* larvae ingesting the plastics.

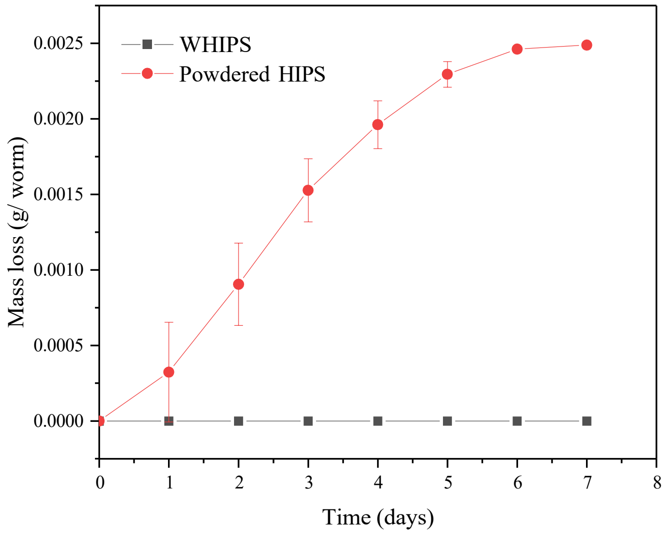


Fig. 5. Consumption of *G. mellonella* larvae to the WHIPS and powdered WHIPS. WHIPS, waste high impact polystyrene.

3.4 Feeding Selectivity of *G. mellonella* Larvae to Different Plastic Combinations

In the actual environment, a variety of plastics usually coexist. Therefore, it deserves to investigate feeding selectivity of *G. mellonella* larvae in the coexistence of multiple plastics. In the study, fresh foamed plastics is collected to eliminate the interference of plastic form and residual substances in WEEE.

Fig. 6a shows the consumption of *G. mellonella* larvae to the plastics fed solely. The result presents a decreasing order as following: RPU>PF>PE>PP>PS>PVC. Lou et al. (Lou et al., 2020) also reported that the consumption of the larvae to PE is higher than PS. Table S2 outlines the physical and chemical properties of these plastics. Minimal mass loss of PVC in all of the plastics is possibly resulted from its excessive density (291 kg/m^3) preventing the larvae from breaking it. Poor abrasion resistance and toughness of PF and RPU make them easy to decompose (Yang et al., 2018). Compared with PF and RPU, PE, PP, and PS have relatively higher abrasion resistance and toughness, causing their much lower mass loss. Research reported that the chemical structures and functional groups of the plastics also affect their degradation (Magnin et al.,2020) . Another reason that PE, PP, and PS are difficult to be biodegraded is their stable carbon chain structure and simplified chemical groups. However, RPU and PF contain urethane groups and hydroxyl groups, respectively, that are easily biodegradable, supplying another explanation why the larvae prefer them (Tang et al., 2020, Liu et al., 2019). In addition, the survival rate (SR) of *G. mellonella* larvae in each group remain above 90% (Fig. 6b), indicating that *G. mellonella* larvae can feed a variety of plastics, and the plastics have no obvious toxic effect on them in the experiment period.

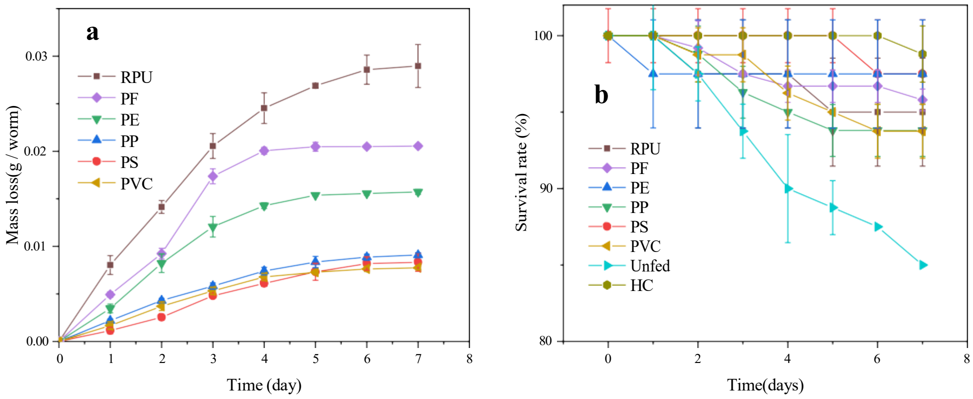


Fig. 6. Consumption (a) and survival rate (b) of G. *mellonella* larvae fed with fresh RPU, PS, PP, PE, PF, PVC plastics, HC and unfed, respectively. RPU, rigid polyurethane; PS, polystyrene; PP, polypropylene; PE, polyethylene; PF, phenolic resin; PVC, polyvinyl chloride; HC, honeycomb; unfed, without any food.

The consumption of *G. mellonella* larvae to the plastics in different plastic combinations is shown in Fig. 7. Interestingly, no matter how the six fresh foamed plastics were combined to feed the *G. mellonella* larvae such as a group of six (Fig. 7a), three (Fig. 7b) or two kinds of plastics (Fig. 7c), they select to ingest some of each plastics instead of one plastics. Even if the plastics (PS or PE) was mixed with beeswax or bran as reported in the literature (Lou et al., 2020), the *G. mellonella* larvae did not only select beeswax or bran to ingest. And the consumption order of *G. mellonella* larvae to the plastics in different combinations is similar to that of fed by one kind of plastic alone (Fig. 6). The result indicates that there is no difference in the feeding selectivity of *G. mellonella* larvae in different plastic combinations. In addition, although the consumption of *G. mellonella* larvae to each plastics in the different combination groups is less than that of the plastics fed alone, the total consumption of *G. mellonella* larvae is similar to the individual consumption of the plastics that the larvae prefer. It is different from the literature (Lou et al., 2020) reported that the consumption of *G. mellonella* larvae in the combination of the plastics and beeswax or bran was lower than that in only the plastics (PS or PE). The food consumption of *G. mellonella* larvae is limited, when beeswax or bran exist, the larvae prefer them and eat less plastics.

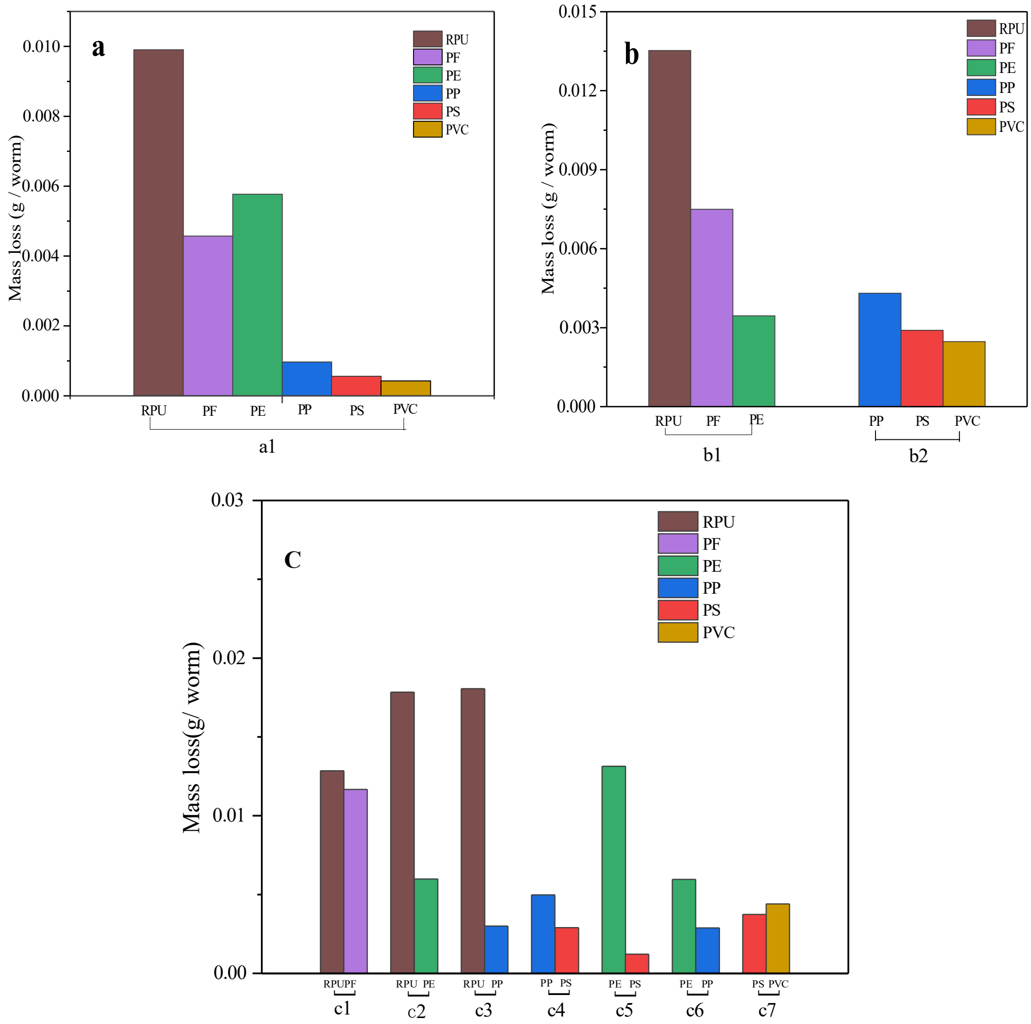


Fig. 7. Feeding preferences of *G. mellonella* larvae co-fed with six kinds (a), three kinds

(b), two kinds (c) of different foamed plastics.

3.5 Selectivity of Two Insect Larva (*G. mellonella* vs *T. molitor*) being fed WRPU

The above results show that *G. mellonella* larvae show feeding selectivity to different plastics. Meanwhile, it deserves to investigate the difference in two insect larvae feeding to the plastics. After 8 days, the WRPU in the group of *G. mellonella* larvae possesses many holes and even fragment (Fig. S4a), but in the group of *T. molitor* larvae is not obviously damaged (Fig. S4b). As shown in Fig. 8, the plastic loss in the *G. mellonella* larvae group is 0.66 g, while in the *T. molitor* larvae group is only 0.1 g after 20 days. The result also indicates that the feeding activity of *G. mellonella* larvae is higher than that of *T. molitor* larvae. It is no accident that *G. mellonella* larvae has a better effect on ingesting WRPU plastics than the *T. molitor* larvae. The literature (Billen et al., 2020) also showed that the consumption of *G. mellonella* larvae to the PS or PE is higher than that of *T. molitor* larvae (mealworms).

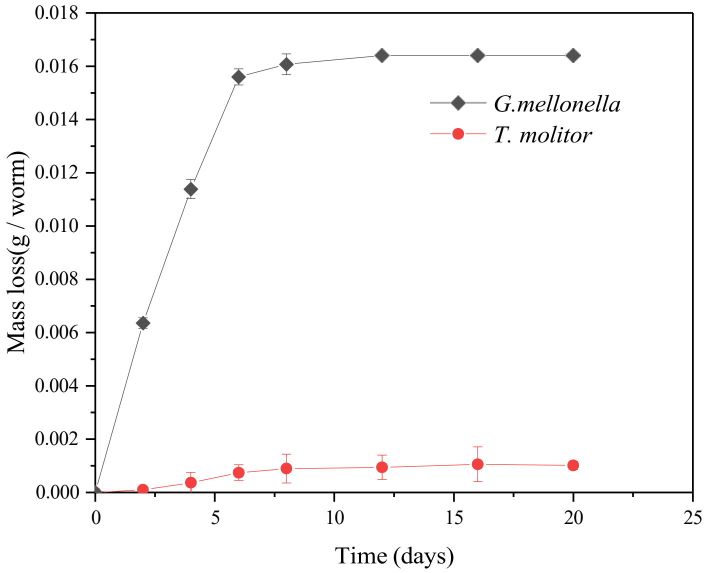


Fig. 8. WRPU consumption of *G. mellonella* and *T. molitor* larave. WRPU, waste rigid

polyurethane.

The possible reason is that *G. mellonella* larvae has larger volume, high activity, high food intake and well-developed gut system (Cassone et al., 2020, Wojda, 2017). These factors will affect the survival and diet of insects (Matyja et al., 2020). The mass loss of plastics in the *G. mellonella* larvae group increases rapidly during the first 8 days, and tends to stable after 8 days. Because the larvae of *G. mellonella* in the early stage are very active and ingest a large amount of plastics, and then gradually pupate and no longer ingest after 8 days.

Conclusions

In this study, the selectivity of *G. mellonella* larvae to the WEEE plastics in the biodegradation process was explored. Fresh and powder plastics were preferred to ingest by *G. mellonella* larvae due to less metals and additives than WEEE plastics. The consumption of *G. mellonella* larvae to the plastics presents the decreasing order as following: RPU>PF> PE>PP>PS>PVC regardless of feeding alone or in combination. SEM and FT-IR analyses of WRPU and WPS frass complement and confirm the biodegrading of the plastics. Simultaneously, *G. mellonella* larvae to WRPU showed higher feeding activity than that of *T. molitor* larvae.

All the results indicate that the consumption of *G. mellonella* larvae to WEEE plastics is lower than that of fresh plastics, but the larvae can ingest a variety of WEEE plastics gnawed suitably. *G. mellonella* larvae has different degradation effects on different WEEE plastics, and the degrading effect is related to the chemical and physical properties of plastics. Different insect larvae have different capacity to degrade WEEE plastics. The possible reason is that the intestinal microorganism of the *G. mellonella* larvae have different effects on different plastics. The microorganisms preferring different plastics might be screened out from the intestinal microflora of the insects. Therefore, the mechanism of the action of insects on plastics needs to further investigate through analyzing systemically the functional characteristics of the microbe.

Acknowledgements

The authors are grateful for support of National Key R&D Program of China (2019YFC0408204, 2018YFC1903201 and 2018YFC0213605), Shanghai Committee of Science and Technology (19DZ1204702) and National Natural Scientific Foundation of China (52070126).

Reference:

Ahmad et al., 2020 N. Ahmad, N. Ahmad, I. M. Maafa, U. Ahmed, P. Akhter, N. Shehzad, U. E. S. Amjad, M. Hussain, Thermal conversion of polystyrene plastic waste to liquid fuel via ethanolysis, Fuel, 279(2020) 118498.

Akram et al., 2019 R. Akram, Natasha, S. Fahad, M. Z. Hashmi, A. Wahid, M. Adnan, M. Mubeen, N. Khan, M.I. A. Rehmani, M. Awais, M. Abbas, K. Shahzad, S. Ahmad, H. M. Hammad, W. Nasim, Trends of electronic waste pollution and its impact on the global environment and ecosystem, Environmental Science and Pollution Research, 26(2019) 16923-16938.

Azubuike et al., 2016 C. C. Azubuike, C. B. Chikere, G. C. Okpokwasili, Bioremediation techniques-classification based on site of application: principles, advantages, limitations and prospects, World Journal of Microbiology & Biotechnology, 32(2016) 180.

Bardaji et al., 2019 D. K. R. Bardaji, J. P. R. Furlan, E. G. Stehling, Isolation of a polyethylene degrading *Paenibacillus* sp. from a landfill in Brazil, Polymer Degradation and Stability, 201(2019) 699-704.

Billen et al., 2020 P. Billen, L. Khalifa, F. Van Gerven, S. Tavernier, S. Spatari, Technological application potential of polyethylene and polystyrene biodegradation by macro -organisms such as mealworms and wax moth larvae, Science of The Total Environment, 735(2020) 139521.

Bombelli et al., 2017 P. Bombelli, C. J. Howe, F. Bertocchini, Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*, Current Biology, 27(2017) R292-R293.

Brandon et al., 2018 A. M. Brandon, S. H. Gao, R. M. Tian, D. L. Ning, S. S. Yang, J. Z. Zhou, W. M. Wu, C. S. Criddle, Biodegradation of Polyethylene and Plastic Mixtures in Mealworms (Larvae of *Tenebrio molitor*) and Effects on the Gut Microbiome, Environmental Science & Technology, 52(2018) 6526-6533.

Caravelli et al., 2004 A. Caravelli, L. Giannuzzi, N. Zaritzky, Effect of chlorine on filamentous microorganisms present in activated sludge as evaluated by respirometry and INT-dehydrogenase activity, Water Research, 38 (2004) 2395–2405.

Cassone et al., 2020 B. J. Cassone, H. C. Grove, O. Elebute, S. M. P. Villanueva, C. M. R. Lemoine, Role of the intestinal microbiome in low-density polyethylene degradation by caterpillar larvae of the greater wax moth, *Galleria mellonella*, Proceedings Biological Sciences, 287(2020) 20200112.

Charitopoulou et al., 2020 M. A. Charitopoulou, K. G. Kalogiannis, A. A. Lappas, D. S. Achilias, Novel trends in the thermo-chemical recycling of plastics from WEEE containing brominated flame retardants, Environmental Science and Pollution Research, 2020. DOI: 10.1007/s11356-020-09932-5

Chen et al., 2020 R. Y. Chen, X. K. Xu, Y. Zhang, S. X. Lu, S. M. Lo, Characterization of ignition and combustion characteristics of phenolic fiber-reinforced plastic with different thicknesses, Journal of Thermal Analysis and Calorimetry, 140(2020) 645-655.

Dement'ev et al., 1991 A. G. Dement'ev, T. K. Khlystalova, A. I. Demina, P. A. Zinger. Structural-physical properties of foam polyurethanes with various foaming agents, Polymer Science U.S.S.R. 33(1991) 2125-2134.

Jacquioda et al., 2018 S. Jacquioda, V. Cyriaque, L. Riber, W. A. Al-soud, D. C. Gillan, R. Wattiez, S. J. Sørensen, Long-term industrial metal contamination unexpectedly shaped diversity and activity response of sediment microbiome, Journal of Hazardous Materials, 344 (2018) 299–307.

Ji et al., 2020 M. X. Ji, L. Chen, J. J. Que, L. L. Zheng, Z. Z. Chen, Z. S. Wu, Effects of transition metal oxides on pyrolysis properties of PVC, Process Safety and Environmental Protection, 140(2020) 210-220.

Li et al., 2019 X. Li, Q. Ren, X. L. You, Y. Q. Yang, M. W. Shan, M. X. Wang, Material flow analysis of discarded refrigerators from households in urban and rural areas of China, Resources Conservation and Recycling, 149(2019) 577-585.

Liu et al., 2019 Y. Liu, H. H. Luo, Y. J. He, Studies on synthesis and characterization of waterborne polyurethane from epigallocatechin-3-gallate, International Journal of Polymer Analysis and Characterization, 24(2019) 597-609.

Liu et al., 2019 S. Y. Liu, Y. Z. Chen, P. Chen, D. W. Xu, X. H. Xiong, J. Wang, Properties of novel bismaleimide resins and thermal ageing effects on the ILSS performance of their carbon fibre-bismaleimide composites, Polymer Composites, 40(2019) 1283-1293.

Lou et al., 2020 Y. Lou, P. Ekaterina, S. S. Yang, B. Lu, B. F. Liu, N. Q. Ren, P. F. X. Corvini, D. F. Xing, Biodegradation of Polyethylene and Polystyrene by Greater Wax Moth Larvae (*Galleria mellonella* L.) and the Effect of Co-diet Supplementation on the Core Gut Microbiome, Environmental Science & Technology, 54(2020), 2821−2831.

Luhar et al., 2019 S. Luhar, I. Luhar, Potential application of E-wastes in construction industry, Construction and Building Materials, 203(2019) 222-240.

Magnin et al., 2019 A. Magnin, L. Hoornaert, E. Pollet, S. Laurichesse, V. Phalip, L. Averous, Isolation and characterization of different promising fungi for biological waste management of polyurethanes, Microbial Biotechnology, 12(2019) 544-555.

Magnin et al.,2020 A. Magnin, E. Pollet, V. Phalip, L. Averous, Evaluation of biological degradation of polyurethanes, Biotechnology advances, 39(2020) 107457.

Mao et al., 2020 S. H. Mao, W. H. Gu, J. F. Bai, B. Dong, Q. Huang, J. Zhao, X. N. Zhuang, C. L. Zhang, W. Y. Yuan, J. W. Wang, Migration of heavy metal in electronic waste plastics during simulated recycling on a laboratory scale, Chemosphere, 245(2020) 125645.

Matyja et al., 2020 K. Matyja, J. Rybak, B, Hanus-Lorenz, M. Wrobel, R. Rutkowski, Effects of polystyrene diet on *Tenebrio molitor* larval growth, development and survival: Dynamic Energy Budget (DEB) model analysis, Environmental Pollution, 264(2020) 114740.

Nassar et al., 2021 M. M. M. A. Nassar, K. I. Alzebdeh, T. Pervez, N. Al-Hinai, A. Munam, F. Al-Jahwari, I. Sider, Polymer powder and pellets comparative performances as bio-based composites, Iranian Polymer Journal, (2021). DOI: 10.1007/s13726-020-00888-4.

Oprea et al., 2010 S. Oprea, Dependence of fungal biodegradation of PEG/castor oil-based polyurethane elastomers on the hard-segment structure, Polymer Degradation and Stability, 95(2010) 2396-2404.

Oprea et al., 2018 S. Oprea, V. O. Potolinca, P. Gradinariu, V. Opera, Biodegradation of pyridine-based polyether polyurethanes by the Alternaria tenuissima fungus, Journal of Applied Polymer Science, 135(2018) 46096.

Osman et al., 2018 M. Osman, S. M. Satti, A. Luqman, F. Hasan, Z. Shah, A. A. Shah, Degradation of Polyester Polyurethane by *Aspergillus* sp. Strain S45 Isolated from Soil, Journal of Polymers and Environment, 26(2018) 301-310.

Peng et al., 2019 B. Y. Peng, Y. M. Su, Z. B. Chen, J. B. Chen, X. F. Zhou, M. E.

Benbow, C. S. Criddle, Wei-Min Wu, Y. L. Zhang, Biodegradation of Polystyrene by Dark (*Tenebrio obscurus*) and Yellow (*Tenebrio molitor*) Mealworms (Coleoptera: Tenebrionidae), Environmental Science Technology 53(2019) 5256−5265.

Petridis et al., 2017 K. Petridis, N. Petridis, E. Stiakakis, P. Dey, Investigating the factors that affect the time of maximum rejection rate of e-waste using survival analysis, Computers & Industrial Engineering, 108(2017) 15-26.

Sekhar et al., 2016 V. C. Sekhar, K. M. Nampoothiri, A. J. Mohan, N. R. Nair, T. Bhaskar, A. Pandey, Microbial degradation of high impact polystyrene (HIPS), an e-plastic with decabromodiphenyl oxide and antimony trioxide, Journal of Hazardous Materials, 318(2016) 347-354.

Shen et al., 2018 Y. F. Shen, X. M. Chen, X. L. Ge, M. D. Chen, Thermochemical treatment of non-metallic residues from waste printed circuit board: Pyrolysis vs. combustion, Journal of Cleaner Production, 176(2018) 1045-1053.

Sheth et al., 2019 M. U. Sheth, S. K. Kwartler, E. R. Schmaltz, S. M. Hoskinson, E. J. Martz, M. M. Dunphy-Daly, T. F. Schultz, A. J. Read, W. C. Eward, J. A. Somarelli, Bioengineering a Future Free of Marine Plastic Waste, Frontiers in Marine Science, 6(2019) 624.

Tang et al., 2020 X. J. Tang, Z. H. Chen, J. Y. Liu, Z. Y. Chen, W. M. Xie, E. Fatih, B. Musa, Dynamic pyrolysis behaviors, products, and mechanisms of waste rubber and polyurethane bicycle tires, Journal of Hazardous Materials, 402(2020) 123516.

Wang et al., 2019 H. Wang, Y. S. Zhang, C. Q. Wang, Surface modification and selective flotation of waste plastics for effective recycling-a review, Separation and Purification Technology, 226(2019) 75-94.

Wojda, 2017 I. Wojda, Immunity of the greater wax moth *Galleria mellonella*, Insect Science, 24(2017) 342-357.

Wolfel et al., 2020 B. Wolfel, A. Seefried, V. Allen, J. Kaschta, C. Holmes, D. W. Schubert, Recycling and Reprocessing of Thermoplastic Polyurethane Materials towards Nonwoven Processing, Polymers, 12(2020). DOI:10.3390/polym12091917.

Wu et al., 2018 Q. Q. Wu, H. C. Tao, M. H. Wong, Feeding and metabolism effects of three common microplastics on *Tenebrio molitor L*., Environmental Geochemistry and Health, 41(2018) 17-26.

Wu et al., 2020 X. Y. Wu, J. Li, L. P. Yao, Z. M. Xu, Auto-sorting commonly recovered plastics from waste household appliances and electronics using near-infrared spectroscopy, Journal of Clean Production, 246(2020) 118732.

Yang et al., 2013 X. N. Yang, L. S. Sun, J. Xiang, S. Hu, S. Su, Pyrolysis and dehalogenation of plastics from waste electrical and electronic equipment (WEEE): a review, Waste Management, 33(2013) 462-473.

Yang et al., 2014 J. Yang, Y. Yang, W. M. Wu, J. Zhao, L. Jiang, Evidence of Polyethylene Biodegradation by Bacterial Strains from the Guts of Plastic-Eating Waxworms, Environmental Science & Technology, 48(2014) 13776-13784.

Yang et al., 2015 Y. Yang, J. Yang, W. M. Wu, J. Zhao, Y. L. Song, L. C. Gao, R. F. Yang, L. Jiang, Biodegradation and Mineralization of Polystyrene by Plastic-

Eating Mealworms: Part 1. Chemical and Physical Characterization and Isotopic Tests, Environmental Science & Technology, 49(2015) 12080-12086.

Yang et al., 2018 S. S. Yang, W. M. Wu, A. M. Brandon, H. Q. Fan, J. P. Receveur, Y. R. Li, Z. Y. Wang, R. Fan, R. L. McClellan, S. H. Gao, D. L. Ning, D. H. Phillips, B. Y. Peng, H. T. Wang, S.Y. Cai, P, Li, W. W. Cai, L.Y. Ding, J. Yang, M. Zheng, J. Ren, Y. L. Zhang, J. Gao, D. F. Xing, N. Q. Ren, R. M. Waymouth, J. Z. Zhou, H. C. Tao, C. J. Picard, M. E. Benbow, C. S. Criddle, Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of *Tenebrio molitor Linnaeus* (Coleoptera: *Tenebrionidae*), Chemosphere, 212(2018) 262-271.

Yang et al., 2018 S. S. Yang, A. M. Brandon, J. C. A. Flanagan, J. Yang, D. L. Ning, S. Y. Cai, H. Q. Fan, Z. Y. Wang, J. Ren, E. Benbow, N. Q. Ren, R. M. Waymouth, J. Z. Zhou, C. S. Criddle, W. M. Wu, Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor Linnaeus*): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle, Chemosphere, 191(2018) 979-989.

Yang et al., 2021 S. S. Yang, M. Q. Ding, L. He, C. H. Zhang, Q. X. Li, D. F. Xing, G. L. Cao, L. Zhao, J. Ding, N. Q. Ren, W. M. Wu, Biodegradation of polypropylene by yellow mealworms (*Tenebrio molitor*) and superworms (*Zophobas atratus*) via gut-microbe-dependent depolymerization, Science of the Total Environment, 756 (2021) 144087.

Yuan et al., 2020 J. H. Yuan, J. Ma, Y. R. Sun, T. Zhou, Y. C. Zhao, F. Yu, Microbial degradation and other environmental aspects of microplastics/plastics, Science of the Total Environment, 715(2020) 136968.

Zhang et al., 2020 J. Q. Zhang, D. L. Gao, Q. H. Li, Y. X. Zhao, L. Li, H. F. Lin, Q. R. Bi, Y. C. Zhao, Biodegradation of polyethylene microplastic particles by the fungus Aspergillus flavus from the guts of wax moth *Galleria mellonella*, Science of the Total Environment, 704(2020) 135931.

Zhang et al., 2020 Y. Zhang, F. Li, N. J. Peng, Environmental impact assessment of air-permeable plastic runway production in China, Science of the Total Environment, 730(2020) 139073.