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Biodegradation of expanded polystyrene and low-density polyethylene foams in larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae): Broad versus limited extent depolymerization and microbe-dependence versus independence

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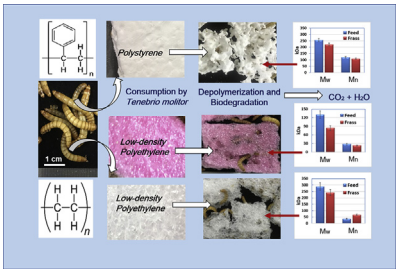
HIGHLIGHTS

- Biodegradation of PS and two LDPE with decreased M_z by *Tenebrio molitor* larvae.
- Broad depolymerization of PS and one LDPE with decreased both M_w and M_n.
- Limited extent depolymerization of LDPE with decreased M_w but increased M_n.
- The antibiotic gentamicin inhibited depolymerization of PS but not LDPE.
- Distinct microbiomes observed with three different diets bran, PS, and LDPE.

GRAPHICAL ABSTRACT



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ABSTRACT

Yellow mealworms (*Tenebrio molitor* larvae) are capable of biodegrading polystyrene (PS) and low-density polyethylene (LDPE). This study tested biodegradation of one expanded PS (EPS) with a weight-average molecular weight (M_w) 256.4 kDa and two LDPE foams with respective M_w of 130.6 kDa (PE-1) and 288.7 kDa (PE-2) in *T. monitor* larvae obtained in Beijing, China. The larvae consumed EPS and both LDPEs over a 60 day. Fourier transform infrared spectroscopy and thermogravimetric analyses of frass confirmed the formation of new oxygen-containing functional groups, as well as a change in physical property and chemical modification, indicating that biodegradation of EPS and LDPE occurred. Gel permeation chromatography analysis confirmed broad depolymerization of EPS and PE-1 (*i.e.*, a decrease in both M_w and a number-average molecular weight (M_n)) but revealed limited extent depolymerization of PE-2 (*i.e.*, increase in M_n and decrease in M_w). For all materials, the size-average

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molecular weight (M_z) was decreased. Biodegradation and oxidation of EPS and LDPE were confirmed using FTIR and TGA analysis. Depression of gut microbes by the antibiotic gentamicin resulted in significant inhibition of EPS depolymerization but did not stop LDPE depolymerization, resulting in the increase in M_n and revealing that PS biodegradation was gut microbe-dependent but LDPE biodegradation was less dependent or independent of gut microbes. Gut microbial community analysis indicated that, as expected, under different dietary conditions, the intestinal flora significantly shifted to communities associated with biodegradation of EPS and LDPE. The results indicated the complexity and limitation of biodegradation of plastics in plastics-eating *T. molitor* larvae.

1. Introduction

Plastics wastes have been a major environmental concern for decades (Gautam et al., 2007; Wu et al., 2017). In 2018 alone, the annual global plastics production reached 359 million tones (PlasticsEurope, 2019). Major plastics produced include polyethylene (PE), 29.7%; polypropylene (PP), 19.3%; polyvinyl chloride (PVC), 10.0%; polyurethane (PUR), 7.9%; polyethylene terephthalate (PET), 7.7%; and polystyrene (PS), 6.4%. PE is expressed as "$[CH_2{-}CH_2]_n$" and comprises a linear backbone of carbon atoms. Commercial PE polymers include low-density PE (LDPE), linear low-density PE (LLDPE), medium density PE, and high-density PE (HDPE). LDPE and LLDPE comprise about 17.5% of total plastic production while MDPE and HDPE account for 12.2% (PlasticsEurope, 2019). PS, another commonly used polymer ($[-CH(C_6H_5)CH_2-]_n$), contributes to about 6.4% of total plastics produced, including a) expanded PS (EPS), trade name Styrofoam, which is widely used for building insulation and packing; b) extruded PS, which is used for food containers, coffee cups, and food trays, etc.; and c) high-density PS products which are commonly used as liquid containers, toys etc. Both PE and PS are the major sources of common plastic waste, also microplastics (MPs) and nanoplastics (NPs) (Andrady 1997, 2003; Jambeck et al., 2015; Li et al., 2016; Lv et al., 2019; Wu et al., 2017; Wang et al., 2021). During last decade, significant progress in recycling of plastic postconsumer packaging waste has been achieved. For example, in Europe, 17.8 million tons of plastic wastes were collected in 2018 as 42% recycling, 39.5% energy recovery and only 18.5% landfilling (PlasticsEurope, 2019). However, compared plastic demand of 51.2 million tons in 2018, significant amount of plastic postconsumer waste may be collected and some still enters to environment as pollutants.

Since the early 1970s, tests on the biodegradation of virgin PE (unpretreated and without any additives), LDPE, and PS have been performed using various environmental microbial sources, including soils, seawater, sediments, sludge, and compost. These sources harbor a multitude of diverse microbial communities which attempt to biodegrade PE and PS into low molecular intermediates and then mineralize them into carbon dioxide and H_2O (Albertsson AC, 1978; 1988; Ohtake et al., 1998; Geyer et al., 2017; Amass et al., 1988; Jones, 1974; Kyrikou and Briassoulis, 2007; Amass 1998; Guillet, 1974; Lee, 1991). Mixed microbial cultures and isolated bacterial strains from various sources were reported (Tian et al., 2017; Gautam, 2007; Kaplan, 1979; Nakamiya, 1997; Syranidou et al., 2019; Jacquin et al., 2019; Ru et al., 2020). These studies concluded that the biodegradation of PE is extremely slow and limited in selected mixed microbial communities (Yang et al., 2014; Jacquin et al., 2019). For PS, even if the polymer was pretreated, the biodegradation efficiency by the microbial cultures remained low, counting on a basis of weeks, months and even years, likely due to the persistent macromolecular structure of the plastics (Krueger et al., 2015; Mor and Sivan, 2008; Shah et al., 2008; Sivan, 2011). The scientific consensus was that rapid PE and PS degradation would require photolytic or thermolytic cleavage of -C-C- bonds prior to biodegradation (Krueger et al., 2015; Motta et al., 2009).

Plastic waste pollution has been widely observed in terrestrial environments. MPs were found in terrestrial animals used as traditional medicinal materials such as cockroach, dragonfly, earthworm, slug, grubs, centipede etc. (Lu et al., 2020). Commonly observed soil invertebrates *e.g.,* earthworms and snails are capable of ingesting and fragmenting plastic debris in their digestive intestine (Lwanga et al., 2017; Song et al., 2019). Recent studies have shown that yellow mealworms, the larvae of *Tenebrio molitor* which belongs to darkling beetles (Coleoptera: Tenebrionidae), are capable of chewing, ingesting, and degrading expanded and extruded PS foam and LDPE foam (Yang et al. 2015a, 2015b; Yang et al., 2018a, 2018b; Brandon et al., 2018). Similar results of PS biodegradation were also reported in dark mealworms, the larvae of *Tenebrio obscurus*, and superworms (*Zophobas atratus*), which also belong to Coleoptera: Tenebrionidae (Peng et al., 2019, 2020). The intestinal tract of *Tenebrio* larvae have comprehensive microbiome with diversified microorganisms that help to break down indigestible plastics (Brandon et al., 2018; Peng et al., 2019; Yang et al., 2018b; Przemieniecki et al., 2020). Recent results also confirmed that hexabromocyclododecane (HBCD), a common flame retardant, does not negatively impact *T. molitor* larvae and accumulate in the biomass (Brandon et al., 2020). Researchers found that PS and PE foams were biodegraded via depolymerization and then biodegradation of intermediates in *Tenebrio* larvae; PS depolymerization was of gut-microbe-dependence because PS depolymerization was severely inhibited or stopped after the larvae were fed with the antibiotic *e.g.*, gentamicin (Yang et al. 2015b, 2018a, 2018b; Peng et al., 2019). However, although depolymerization and biodegradation of LDPE has been confirmed, the impact of antibiotics such as gentamicin on DLPE depolymerization and degradation has not been reported (Brandon et al., 2018).

In this study, we compared depolymerization and biodegradation of a commercial EPS and two LDPE foams with different molecular weights in *T. monitor* larvae in order to assess the depolymerization and biodegradation, examine the role of microbes in depolymerization with antibiotic gentamicin, and analyze the intestinal microbes of *Tenebrio molitor* under different feeding conditions by using high-throughput sequencing technology. The results confirmed that the larvae are capable of biodegrading both EPS and LDPE but that the depolymerization patterns of LDPE were different. EPS and LDPE with lower molecular weight were broadly depolymerized while depolymerization of LDPE with higher molecular weight was limited; gentamicin depressed EPS depolymerization but did not do so for PE depolymerization. Furthermore, microbial community structures were changed under different feedstock conditions.

2. Materials and methods

2.1. Test materials and Tenebrio monitor larvae

Expanded PS foam was obtained from Yixing Plastic Factory (Beijing, China). Two LDPE foams were used in this study. LDPE foam (PE-1), which contains pink color additives, was purchased from the Yixing Plastic Factory (Beijing, China); LDFE film (PE-2), which is colorless without additives, was purchased from the Foam Factory (Macomb, MI, the USA). PE-2 was the same material tested previously at Stanford University (Brandon et al., 2018). The EPS foam had a number-average molecular weight (M_n) of 120.5 ± 2.6 kDa, and a weight-average molecular weight (M_w) of 256.4 ± 2.7 kDa (n = 2, mean ± standard deviation). The PE-1 foam had a M_n of 27.9 ± 1.4 kDa, and a M_w of 130.6 ± 5.5 kDa. The PE-2 foam density of 0.023 ± 0.009 g cm^{-3}, a M_w of 288.7 ± 9.8 kDa, and an M_n of 35.8 ± 0.9 kDa. Their size-average molecular weights (M_z) were 2-3 times of M_w (Table 1). No extra additives or catalysts were added, according to the manufacturer.

Tenebrio monitor larvae (approx. weight 70–80 mg each) were purchased from the Zizhuqiao Flower and Bird Fish Market (Beijing, China). They were fed with wheat bran prior to tests.

The tetrahydrofuran (THF) (GC grade purity ≥99.9%), 1,2,4-trichlorobenzene (1,2,4-TCB) (purity≥99%, Alfa Aesar, Haverhill, MA, the USA), gentamicin sulfate, and trypticase soy agar (TSA), and wheat bran were purchased from Ruizhihanxing Company (Beijing, China).

2.2. Biodegradation of PS and LDPE

Two experiments were conducted. In the first test, the larvae were kept unfeeding for 48-h to allow to egest frass from previous feedstock, then they were fed with three respective diets: EPS foam, LDPE foam (PE-1) and bran. To assess the survival rate (SR) and plastic mass loss, 300 larvae were reared in each incubator. The SR was recorded based on the number of living larvae divided by the initial number of larvae added. The larval number and the plastic mass loss were recorded every 5 days. Dead larvae and molted exoskeletons were removed from containers to prevent the living larvae from eating them. The test lasted 60 days. All treatments were conducted in duplicate.

The second test was conducted with PE-2 to verify LDPE depolymerization/biodegradation in the larvae from the same source in Beijing. The procedures were the same as that above except that EPS was not tested.

2.3. Analytical methods

The methods and procedures for the collection and analysis of the residual polymers in the frass of the larvae were similar to those previously reported (Yang et al., 2018a, 2018b; Brandon et al., 2018). The *T. molitor* larvae fed with respective diets were moved into clean PP containers overnight while frass was collected and then returned to their original incubators. The collected frass was stored at −80 °C prior to analysis.

Gel permeation chromatography (GPC, Waters Breeze, Waters, U.S.A.) was applied to analyze molecular weights of PS polymers. The procedures of PS sample preparation with THF extraction were similar to those described previously (Yang et al., 2018a, 2018b). PS was extracted from EPS feedstock (1.0 g) and frass samples (1.0 g) of EPS-fed larvae. THF solution was mixed with the sample on a magnetic stirrer with gentle heating (60 °C), then settled and filtered via a 0.22 μm PVDF filter. The THF extracted solution was concentrated to 5 mL in volume to achieve about 5 mg polymer ml^{-1}. The extract (20 μL in volume) was injected into the GPC analyzer, with a flow rate of 0.8 mL/min.

For LDPE samples, M_n, M_w and M_z were determined by using high-temperature gel permeation chromatography (HT-GPC, PL Q220 , PL Instruments, U.S.A.) as described previously (Brandon et al., 2018). Frass samples (50 mg) were gently crushed in a mortar with a pestle prior to a 2-h extraction in 2 mL dichloromethane (≥99.9%, Thermo Fisher Scientific Inc., Pittsburgh, PA, the USA) solvent aliquots with gentle heating (placed on a hot plate on the lowest setting). After 2 h, the solution was filtered via a 0.22 μm PVDF filter (Thermo Fisher Scientific Inc., Pittsburgh, PA, the USA) and transferred into a clean glass vial. The extracted PE polymer in the filtered solution was concentrated by using rotary evaporation, and the residual polymer was weighed to determine the extractable fraction. Residual polymer was then dissolved in (1,2,4-TCB) to obtain a final concentration of approximately 5 mg mL^{-1}. The sample (100 μL) was injected into the GPC analyzer at 150 °C with an eluent (1, 2, 4-TCB) flow rate of 1.0 mL min^{-1}.

The functional groups of the extracted residual PS or PE polymers from the frass were characterized by Fourier transform infrared spectroscopy (FTIR) (Thermo Scientific Nicolet iS5 FTIR Spectrometer, Pittsburgh, PA, the USA), ranging from 4000 to 500 cm^{-1} in comparison with feedstock EPS or LDPE foams. The method of sample preparation was that same as that described in previous reports (Brandon et al., 2018; Yang et al., 2018a, 2018b). The EPS and PE-fed frass samples were analyzed using the ATR method (Thermo Fisher IS10). Prior to analysis, the samples were dried by a freeze dryer to 50 °C for at least 36 h and then milled with potassium bromide (KBr) to prepare homogenous pellets for scanning (Thermo Fisher iS5).

Characterization of thermal changes from PS to frass was performed by using thermogravimetric analysis (TGA) with TA Q500 (TA Instruments, USA.). The specific test conditions are similar to previous reports (Peng et al., 2019). The heating procedure included two different atmospheres—nitrogen and air—under which to study the pyrolysis of the sample. The sample (5 mg) was heated from 40 °C to 800 °C at a rate of 20 °C min^{-1} under a high purity nitrogen atmosphere (99.999%). It was then cooled to 500 °C and finally heated at 800 °C under an air atmosphere.

2.4. Antibiotic suppression tests

The effects of antibiotic on EPS and LDPE (PE-1 and PE-2) depolymerization/biodegradation were tested using gentamicin sulfate (CAS:1405-41-0) which was selected based on previous results with *T. monitor* larvae (Yang et al. 2015b, 2018a, 2018b). The gentamicin suppressive groups (100 larvae) were fed with bran and gentamicin sulfate at 100:3 (w/w) for 3 days and then fed with EPS or LDPE feedstock for four days prior to sampling. This feeding procedure was repeated. On day 7, day 14, and day 21, frass samples from the gentamicin suppressive groups were collected for GPC analysis. On day 0 (prior to feeding PS), 7, 14, and 21 , *T. molitor* larvae (10 worms) were also randomly selected from each group of larvae to prepare a gut suspension to count gut microbes as described previously (Yang et al., 2018b). The larval samples were decontaminated using 80% ethanol, and then rinsed with Milli-Q water. The larval bodies were cut open to collect the guts. Subsequently, the gut contents were extracted and suspended in 5 mL sterile saline water, serially diluted (10^{-1} to 10^{-9}) and cultivated on nonselective TSA plates at 37 °C for 24 h. Finally, the number of colonies were counted as described elsewhere (Peng et al., 2019; Yang et al., 2015b; Yang et al., 2018a; Brandon et al., 2018).

2.5. Microbial community analysis

After DNA was extracted from the gut suspension of the larvae,

the DNA was detected using Nanodrop. PCR was performed using TransGen AP221-02: TransStart Fastpfu DNA Polymerase. All samples were processed according to the formal experimental conditions, and each sample was repeated 3 times. The PCR products of the same sample were mixed and detected using 2% agarose gel electrophoresis and recovered by AxyPrep DNA gel. Referring to the preliminary quantitative results of electrophoresis, the PCR products were quantified by QuantiFluor TM-ST Blue Fluorescence Quantification System (Promega), and then mixed according to the sequencing amount of each sample. Phased amplicon sequencing was used to sequence the V3–V4 region of the 16 S rRNA gene. Purified amplicons were pooled in equimolar and paired-end sequencing on an Illumina MiSeq platform (Allwegene, Beijing, China) according to standard protocols (Hale and Crowley, 2015; Zhang et al., 2014).

2.6. Statistical analysis

Students' test was used for pairwise comparison to evaluate the differences in molecular weights as described previously (Brandon et al., 2018; Peng et al., 2019). All p-values were adjusted. Sequencing data was demultiplexed, quality filtered on Trimmomatic, and combined according to criteria. The Operational Taxonomic Units (OTU) were clustered using UPARSE (Version 8.1) with an OTU recognition threshold of 97%, after which the monomer and chimeric sequences were removed. UPARSE was used to cluster the operating taxonomic unit (OTU) with the 0.97 identity threshold. UCHIME was used to identify and remove the chimeric sequences. The classification of each 16 S rRNA gene sequence was analyzed by the RDP classifier against the Silva 16 S rRNA database with a confidence of 70%. Finally, the free online platform of the Allwegene Cloud platform (Beijing, China) was used to determine microbial community composition, hierarchical cluster analysis, principal coordinate analysis (PCoA), and ternary analysis(Fan et al., 2017).

3. Results and discussion

3.1. EPS and LDPE consumption and biodegradation

The test of biodegradation of EPS and LDPE (PE-1) foams lasted 60 days (Fig. 1). The larvae ate all plastic foam as expected (Fig. 2A). The sequence of survival rates (SRs) of *T. molitor* larvae fed with different diets was: bran > PE-1 ≈ EPS > unfed (Fig. 1A and Table 1), which is similar to what was observed by Brandon et al. (2018), who tested biodegradation of EPS and LDPE foams in *T. monitor* larvae obtained from a USA source. Pupariation can be used as an indication to describe the health and development of the larvae. Initially, the pupation rates of the larvae fed with different diets were similar. After 20 days, only the larvae fed with bran continued pupation while the pupation of others (unfed group, and LDPE- and EPS-fed groups) stopped (Fig. 1B). This indicated that *T. molitor* larvae under starvation or feeding with only LDPE or EPS lacked the necessary nutrients to complete their life cycle. Only when they were fed with bran they were supported to complete their life cycle of growth and development. The lack of the necessary nutrients also had negative impact on their symbiotic microorganisms which performed biodegradation of LDPE and EPS. In previous studies, cannibalization was observed when the larvae were fed with EPS only and unfed (Yang et al., 2018a, 2018b). In this study, we found that cannibalization occurred in all treatment groups when food source become limited. The sequence of cannibal rates (%) over a 60-day period was: bran fed (14.9%) < EPS only (16.0%) < LDPE only (23.0%) < unfed (37.2%) (Fig. 1C). The unfed group had the highest cannibal rate of up to 37.2%, indicating that the stronger larvae killed the weaker larvae for food and nutrients for their survival. The same cannibalization was also observed in the groups fed on EPS or LDPE foam only since both polymers do not contain necessary nutrients for the growth (Yang et al., 2018a). On the other hand, the cannibal rate of the larvae fed with bran was lower although we did not supply efficient bran to them (Fig. 1C). When EPS and LDPE were supplied as the sole diet, the larvae consumed EPS than LDPE continously over a 60-day period (Fig. 1D).

Test results using PE-2 showed a similar pattern, *i.e.*, results indicated higher SR and lower cannibal rates among larvae fed on PE-2 compared with those unfed. The consumption rates of PS, PE-1, and PE-2 were 4.27 ± 0.09, 3.33 ± 0.02, and 3.45 ± 0.04 mg 100 larvae^{-1} d^{-1}, respectively. The rates were lower than a range reported previously, which varied from 8 to 40 mg 100 larvae^{-1} d^{-1} (Yang et al., 2019b; Brandon et al., 2018). The relatively lower consumption rates were likely dependent on the size of larvae used.

3.2. Depolymerization of EPS and LDPE foams

Using GPC analysis, M_n provides information about the lowest molecular weight portion of the sample, M_w is the average closet to the center of molecular distribution curve, and M_z(the size average molecular weight) represents the highest molecular weight portion of the sample. M_n and M_w have been widely used as parameters for plastic biodegradation (Ohtake et al., 1998; Albertsson et al., 1998; Yang et al., 2015a). In this study, frass samples were taken on day 32 to characterize depolymerization and biodegradation of EPS and LDPE foams using previously established methods (Yang et al., 2018a; Brandon et al., 2018). PS depolymerization was characterized using GPC analysis. The results confirmed the ubiquity of PS depolymerization in *T. molitor* as reported previously (Yang et al., 2018b). The M_n of the PS residues extracted from the frass was reduced at a rate of 14.7% compared to raw PS foam, decreasing from 120.0 to 109.5 kDa. Meanwhile, the M_w comparatively decreased at a rate of 9.2%, reducing from 256.4 to 220.7 kDa. After ingestion and passage via intestinal tract, the molecular weight distribution (MWD) shifted towards the lower molecular side. This is a typical depolymerization pattern observed during previous research on biodegradation of PS in *T. molitor* larvae (Brandon et al., 2018; Yang et al. 2015a, 2015b; Yang et al., 2018a, Yang et al., 2018b). The M_z of EPS was also reduced from 569.1 to 474.6 kDa, showing reduction of the highest molecular weight portion (Table 1).

The change in M_n and M_w of LDPE (PE-1 and PE-2) after passage via the larval gut showed different patterns. The M_w of the residue PE polymer of PE-1 was reduced by 35.7%, decreasing from 130.6 to 84.0 kDa; the M_n of the PE-1 residue declined by a slight 16.5%, going from 27.9 to 23.3 kDa; and the M_z declined from 461.1 to 188.7 kDa. When the larvae were fed with PE-2, M_w decreased from 288.7 to 241.5 kDa; M_n increased from 35.8 to 66.8 kDa (Fig. 2B and C), and M_z decreased from 930.4 to 577.8 kDa (Table 1). The LDPE depolymerization pattern of PE-1was similar to that of PS in this study and that of LDPE biodegradation reported previously by Brandon et al. (2018), showing broad depolymerization. PE-2 showed a different depolymerization pattern, i.e., there was a decrease in M_w and increase in M_n along with limited extent depolymerization when PE-2 was fed. However, M_z values were reduced during all above tests, indicating the reduction of the highest molecular weight portion.

3.3. Evidence of biodegradation and oxidation of PS and LDPE

The biodegradation of PS and PE in the larvae was confirmed by analysis of the frass of the larvae fed on PS and PE-1 using previously established methods involving FTIR, ^{1}H NMR, and TGA (Peng et al., 2019; Brandon et al., 2018; Yang et al., 2019a).

FTIR was used to detect the functional group change of PS and

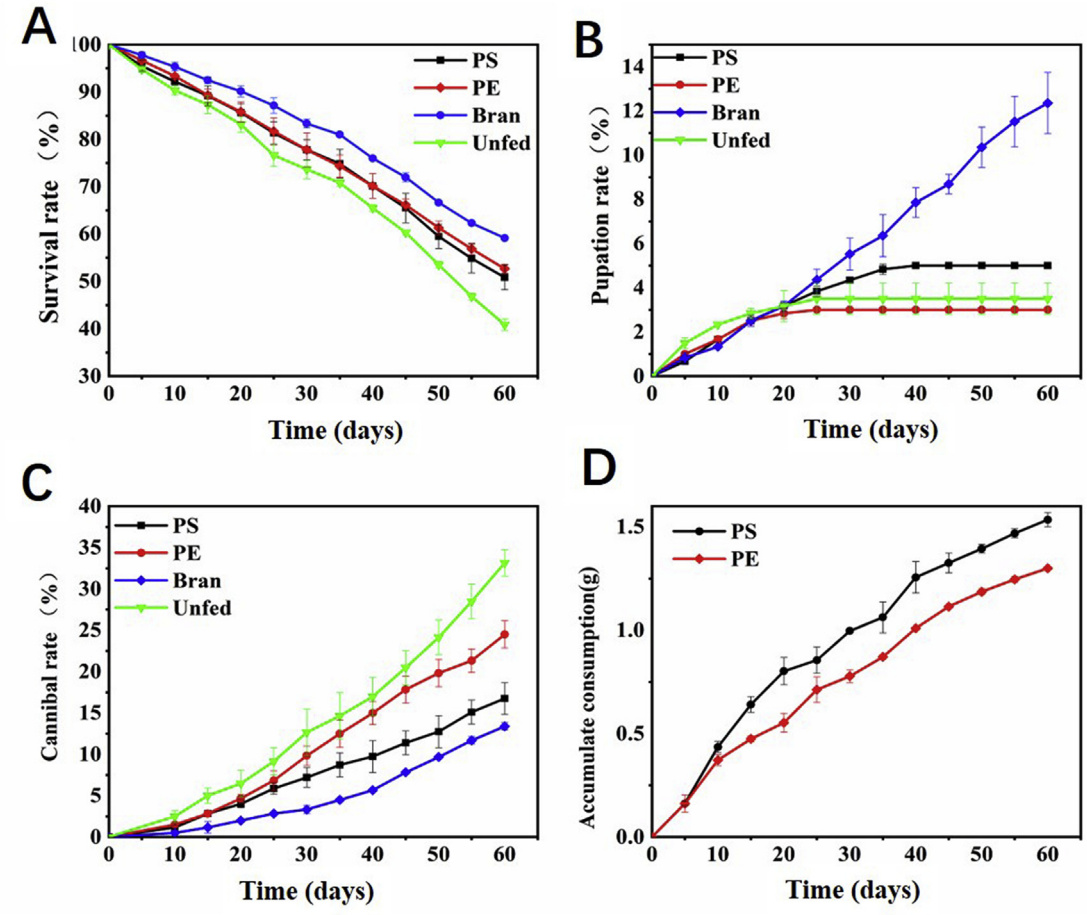

Fig. 1. Survival rates and plastic consumption by *Tenebrio molitor* larvae under different dietary conditions. **A**. Survival rates of the larvae fed with PS, PE-1, and bran versus unfed. **B**. Pupation rates under different dietary conditions. **C**. Comparison of cannibal rates. **D**. Comparison of accumulated consumption of PS and PE-1. PE = PE-1 LDPE foam; PS = EPS foam; B = bran; Test temperature was 25 °C.

LDPE ingested by *T. molitor* larvae at the end of the 32-day experiment (Fig. 3A). The intensities of the peaks at 625–970 cm^{-1} (ring-bending vibration) were strong in PS feedstock but much weaker in frass samples. Characteristic peaks known to represent the PS benzene ring (C=C stretch, 1550–1610 and 1800–2000 cm^{-1}) were dampened in frass samples, providing evidence of ring cleavage. Further evidence of biodegradation was observed in a decrease in intensities of peak characteristic for PS and the appearance of carbonyl groups (C=O stretch, 1700 cm^{-1}). The broadening of peaks at 2500–3500 cm^{-1} in all FTIR spectra of frass samples is associated with the hydrogen bond of hydroxyl groups and/or carboxylic acid groups, suggesting a shift from hydrophobic to more hydrophilic surface properties. FTIR spectra from the residual polymers from mealworms fed PS and PE revealed incorporation of oxygen as indicated by the appearance of peaks associated with C–O stretching (1000–1200 cm^{-1}) and alcohol groups (R–OH stretching, 3000–3500 cm^{-1}). These peaks were not observed in the control PE spectra (Fig. 3B).

TGA was used to detect the thermal modification of PS and PE ingested by *T. molitor* larvae at the end of the 32-day experiment, showing patterns similar to those reported previously. PS foam had a 98.29% weight loss occurring at 360 °C–480 °C, with the maximum decomposition rate temperature of about 450 °C (Fig. 3C). PE foam had a 98.17% weight loss occurring at 420 °C–530 °C; the maximum decomposition rate was about 490 °C (Fig. 3D). There were three decomposition stages of the frass fed PS: in the first stage, 6.62% weight loss occurred at 175 °C–275 °C; in the second stage, 12.89% weight loss occurred at 275 °C–360 °C; in the third stage, 53.12% weight loss occurred at 360 °C–480 °C. The frass of larvae fed with PE only had three decomposition stages: in the first stage, 10.83% weight loss occurred at 175 °C–275 °C; in the second stage, 20.09% weight loss occurred at 275 °C–360 °C; in the three stages, 26.92% weight loss occurred at 360 °C–480 °C. In the normal diet group, the frass of bran fed larvae had three decomposition stages: in the first stage, 14.46% weight loss occurred at 175 °C–275 °C; in the second stage, 22.96% weight loss occurred at 275 °C–360 °C; 18.11% weight loss occurred at 360 °C–480 °C. Under the same heating program, PS fed larvae frass had more decomposition stages than PS foam. Similarly, PE fed larvae frass had more decomposition stages than PE foam, indicating that the frass of *T. molitor* larvae contained new components other than PS and PE foams. In addition, in the third stage, the weight loss rate of the frass was significantly lower than that of PS and PE foams, indicating that the PS and PE components in the frass of *T. molitor* larvae were significantly reduced after passage through the digestive system of *T. molitor* larvae. Decompositions below 100 °C may be classified as volatile organic compounds (intestinal secretions, carboxylic acid compounds from PS, PE biodegradable, etc.), while decomposition from 100 °C to 360 °C may be attributed to other organic wastes and biodegradable residues. In the stage of 360–480 °C, the mass loss rate of PS was 98.29%, and the loss rate of the frass from larvae fed with PS

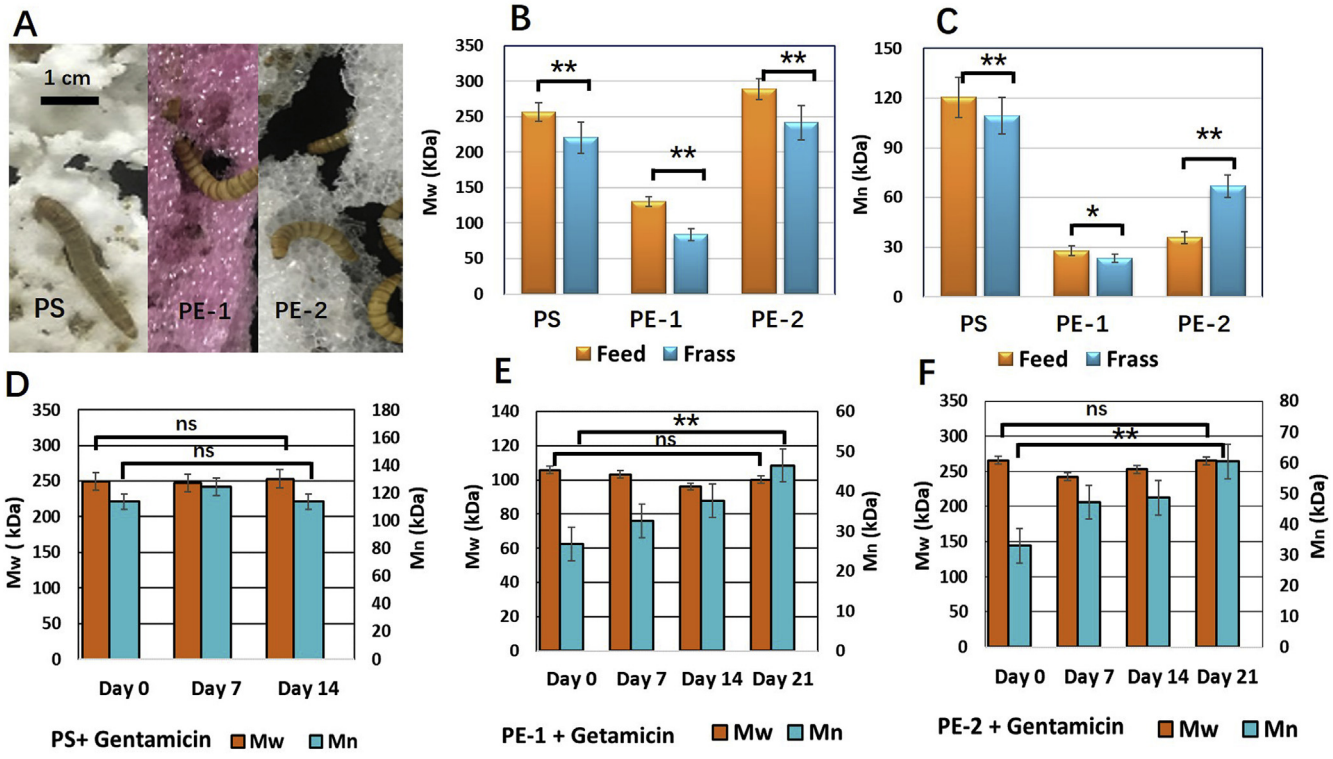

Fig. 2. Characterization of depolymerization and effect of the antibiotic gentamicin. **A**. *Tenebrio monitor* larvae fed with PS, PE-1 and PE-2 plastic foams. **B.** Changes in M_w of tested polymer foams. **C.** Changes in M_n of tested polymer foams. **D.** Effect of antibiotic gentamicin on depolymerization of PS. **E**. Effect of gentamicin on depolymerization of PE-1. **F.** Effect of gentamicin on depolymerization of PE-2. Samples on day 0 were feedstock PS, PE-1 and PE-2, and others were residual polymers extracted from frass of larvae fed with PS, PE-1 and PE-2 during antibiotic treatment on different days. All values represent mean ± SD, n = 2. Significance (Student's t tests) $p < 0.05$ indicated by *, $p < 0.01$ indicated by **, and no statistical significance indicated by ns.

Table 1
Consumption of PS and LDPE foams in *Tenebrio molitor* larvae.

Diet	Weight mg/larva		Weight	SR,%	Specific consumption rate	M_w (kDa)		M_n (kDa)		M_z (kDa)	
	Initial	End	Change %		mg100 larvae^{-1}d^{-1}	Feed	Frass	Feed	Frass	Feed	Frass
Bran	76.2 ± 1.3	77.0 ± 1.3	1.8 ± 1.7	59.2 ± 0.2	–			–	–	–	–
EPS	75.8 ± 0.8	77.7 ± 1.2	2.5 ± 1.0	50.9 ± 2.6	10.0 ± 0.4 (60 d)	256.4 ± 2.5	220.7 ± 1.7	120.0 ± 2.5	109.5 ± 2.9	569.0 ± 2.8	474.6 ± 0.9
PE-1	75.1 ± 0.7	81.9 ± 1.7	8.8 ± 2.1	52.7 ± 0.9	8.2 ± 0.2 (60 d)	130.6 ± 5.5	84.0 ± 3.5	27.9 ± 0.4	23.3 ± 3.6	461.1 ± 1.0	188.7 ± 1.4
PE-2	81.0 ± 2.5	79 ± 1.9	3.4 ± 1.6	71.8 ± 2.1	4.8 ± 0.2 (40 d)	288.7 ± 9.8	241.5 ± 10.4	35.8 ± 0.9	66.8 ± 10.1	930.4 ± 3.9	576.6 ± 1.8
Unfed	75.6 ± 0.7	73.5 ± 1.4	3.0 ± 2.2	40.9 ± 1.2	–			–	–	–	–

Note: Duplicates were used with 300 larvae each in incubator. Tests were performed for 60 days at 25 °C. Initially, EPS or LDPE (3.000 g) foam was added in each incubator. Specific LDPE consumption was calculated on the basis of the mass of LDPE consumed over the test period of 60 days for EPS and FE-1, and 40 days for PE-2, respectively.

was 53.12%. The mass loss rate of PE alone was 98.17%, while the loss rate of the frass from larvae fed with PE was 26.92%. The three stage decomposition of the frass of PS or PE fed larvae were similar to those of bran-fed larvae frass, which indicated that the frass of PS and PE fed larvae had changed their composition to degraded products after passage through the intestinal tract.

The results indicated that both PS and PE were modified and oxidized after passage through *T. molitor larvae*, confirming the biodegradation as reported previously (Yang et al., 2018a, 2018b; Brandon et al., 2018).

3.4. Antibiotics suppression

Gentamicin suppression tests were performed to examine the role of gut bacteria in PS and PE depolymerization. The number of gut bacteria in *T. molitor* larvae receiving gentamicin treatment decreased sharply from 7.50×10^6 to 7.23×10^3 and 7.88×10^6 to 2.45×10^4 CFU per gut by almost three magnitudes after gentamicin treatment for 7, 14, or 21 days. Meanwhile, the controls without antibiotics remained unchanged (near 10^7 CFU per gut), implying that the gut bacteria were singnificantly suppressed by gentamicin sulfate. Because gentamicin is active against mostly Gram-negative bacteria and some Gram-posstive bacteria a limted population of bacteria still survived (Fig. 4A).

The effect of antibiotic gentamicin suppression was examined based on the impact on depolymerization. On days 7, 14, 21, the frass of the larvae fed on EPS, PE-1, and PE-2 was collected for GPC analysis. The M_w and M_n of the residual PS remained basically unchanged over a 14 day period as observed previously (Yang et al., 2015b; Yang et al., 2018b) (Fig. 2D). However, the changes in M_n and M_w of the residual PE showed different patterns. During the 21-day period, the M_n of the residual PE-1 increased from 26.8 to 32.6, then 37.6 and finally to 46.5 kDa, while the M_w decreased slightly from 105.9 to 103.3, then 100.6, and finally to 100.2 kDa (statistically insignificant, $p > 0.05$, Fig. 2E). The M_w of residual PE-2 was basically unchanged (265.6 to 265.2 kDa) but M_n increased

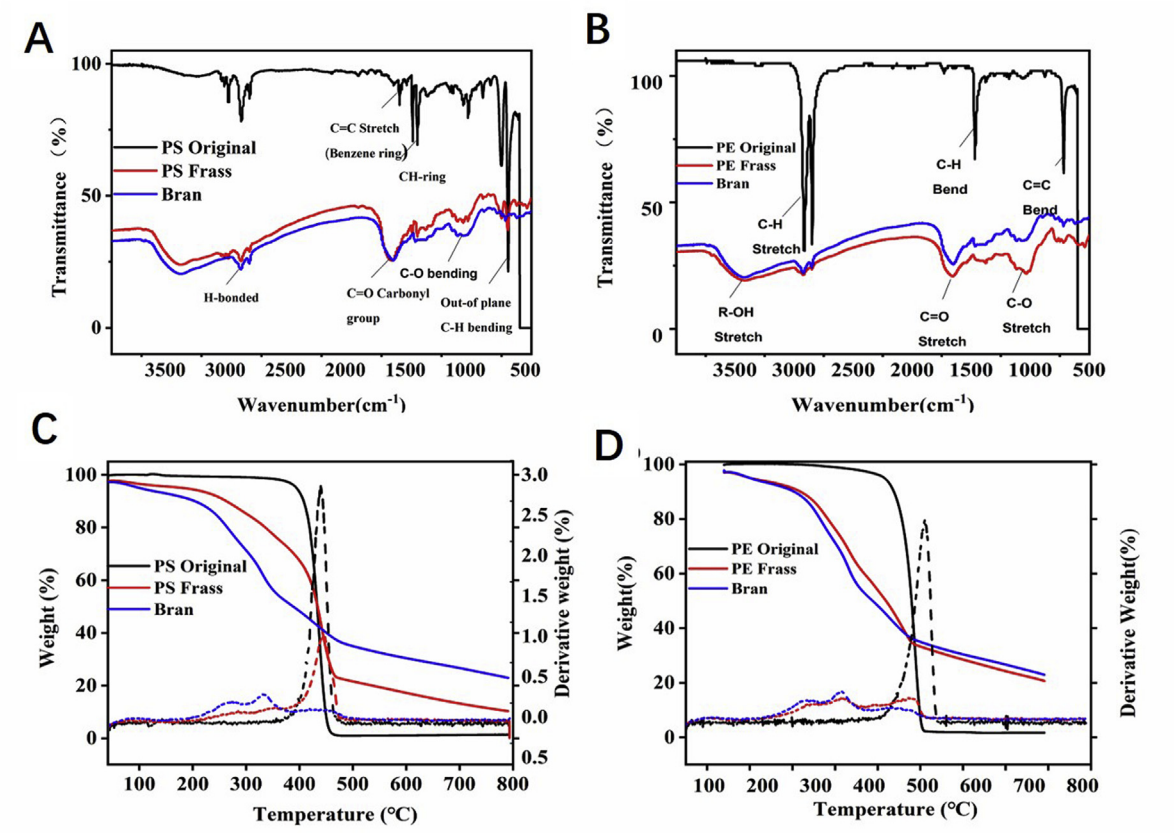

Fig. 3. Characterization of biodegradation and oxidation of plastic polymers by *T. molitor* larvae. **A.** Fourier transform infrared spectroscopy (FTIR) analysis of PS sample and frass of *T. molitor* larvae fed with PS diets. **B.** FTIR analysis of PE-1 sample and frass of the larvae fed with PE-1. **C.** Thermogravimetric analysis (TGA) of PS sample and frass of *T. molitor* larvae fed with PS and bran diet. **D.** TGA of PE-1 sample and frass of *T. molitor* larvae fed with PE-1 and bran. In this study, we tested LDPE using PE-1 sample.

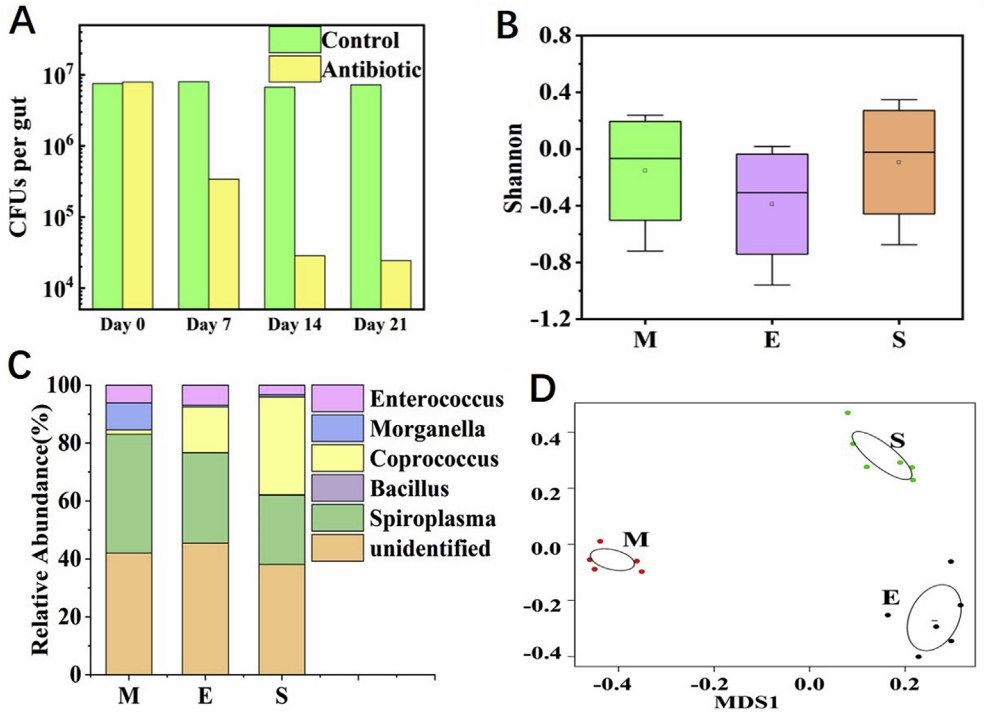

Fig. 4. Microbial community analysis of gut microbiome in different diets. **A**. Comparison of CFU in the intestine of *Tenebrio molitor* larvae before (control) and after antibiotic treatment. **B**. Shannon index for different treatment groups. **C.** Relative abundance of predominant genera in the gut of *T. molitor* larvae fed with different diets. **D.** Nonmetric multidimensional scaling based on weighted UniFrac. M = bran, E = LDPE, and S= PS. In this study, we tested LDPE using PE-1 sample.

progressively from 33.0 to 47.1, then 48.6, and finally to 60.4 kDa ($p < 0.005$, Fig. 2F). The results indicated that PE modification or depolymerization did not stop in the presence of gentamicin, *i.e.*, when most gut bacteria are inhibited, the *T. molitor* did not lose the ability to depolymerize PE. This pattern is different from the observations of this study and previously reported inhibition of PS depolymerization in *T. molitor* larvae via antibiotic gentamicin (Yang et al. 2015b, Yang et al., 2018a, Yang et al., 2018b). Therefore, we conclude that LDPE depolymerization and thus biodegradation in *T. molitor* larvae is likely less gut microbe dependent. Moreover, we further hypothesize that the depolymerization of LDPE may be partially contributed to the intestinal digestive system of *T. molitor* (*e.g.*, enzymatic, physical, and chemical reactions). However, the contribution of limited survived gut microbes should also be further examined. We also have hypothesis that PE-biodegradation could be accelerated by the synergistic driving forces from the larval digestive system and microbial activities. Further research is needed to prove feasibility.

3.5. Broad versus limited extent depolymerzation

Depolymerization is the essential step of biodegradation of plastic polymers. Ohtake et al. (1998) found that after buried under bioactive soil for more than 30 years, biodegraded LDPE showed decreased M_w and M_n *i.e.* broad deplymerization. Previous reports on PS and LDPE depolymerization by *Tenebrio* genus (Brandon et al., 2018; Peng et al., 2019; Yang et al., 2018a; Yang et al., 2018b; Yang et al., 2015a, b; Yang et al., 2020) and bacterial culture (Syranidou et al., 2019) have been that of broad depolymerization, *i.e.*, the decrease in both M_n and M_w of the plastic polymer. In this study, we found different depolymerization patterns for PE-2, *i.e.,* a decrease in M_w and increase in M_n. Recently, we found the limited extent depolymerization during biodegradation of EPS and LDPE in superworms (*Zophobas atratus* larvae) from Guangzhou, China (strain G) and Marion, USA (strain M) (Peng et al., 2020). Strain G performed broad depolymerization of EPS (M_w of 345 kDa and M_n 107.0 kDa) i.e. M_w was reduced by 39.0% and M_n by 45.3% but strain M performed limited extent depolymerization of EPS (M_w of 167.9 kDa and M_n 69.6 kDa) *i.e.,* M_w increased by 50.1% and M_n by 63.4%. Both strain G and strain M larvae fed with LDPE foam with respective M_w of 173.4 and 248.0 k Da, and M_n of 40.4 kDa and 36.3 kDa. M_w was increased by 63.8% and 8.8% in strain G and stain M; M_n was increased by 26.2% and 44.1% with strain G and stain M, respectively. In addition, a publication on biodegradation of polyurethane (PUR) by a landfill microbial culture reported that initially, the M_w of PUR increased from 208.5 to 229.4 kDa and then decreased to 169.9 kDa (Gaytán et al., 2019). Another report indicated PS degradation in *Galleria mellonlla* larvae (Lou et al., 2020) with an increase in M_n from 132.1 to 146.9 kDa as well as in M_w from 361.7 to 377.8 kDa. Recent study indicated that biodegradation of EPS by land snails *Achatina fulica* also occurred through limited extent depolymerization with increase in both M_w and M_n and the depolymerization was gut-microbe independent based oxytetracycline antibiotics suppresion test (Song et al., 2020). The limited extent depolymerization of PE-2 was likely related to the selective depolymerization of lower molecular polymers at higher rate than longer chain polymers although the longer chain polymers were degraded, which is supported by decrease in M_z values. In addition, the presence of crosslinking reactions during depolymerization/biodegradation could cause the increase in M_n. Further study is needed to understand this mechanism.

3.6. Gut microbial community response

In this study, we used second-generation high-throughput sequencing to investigate how intestinal microbial community structure responded to a diet shift to PS and LDPE (PE-1). We analyzed microbial diversities in the larvae fed on bran, PS and PE-1. The alpha diversity of microbial communities measured by the Simpson index did not differ significantly, but by measuring with the Shannon Index, the diversity was discovered to be different among the diets (Fig. 4B). The results showed great differences in the gut flora fed on the three diets. Abundances of *Coprococcus,* genus of anaerobic gut cocci, increased significantly in the portion of the larvae that were fed with PS. The abundances of another gut microbe *Morganella* decreased in both PE and PS fed larvae. Abundances of *Spiroplasma* was higher in the proportion of the normal diet bran fed larvae than those fed with PE and PS (Fig. 4C). However, no above microbes have been found degrading plastics (Ru et al., 2020). They likely associate with digestion of other food (like residue of the larvae) or PE or PS degraded intermediates. *Bacillus* sp. was found at low level in the larvae fed all diets. PE- and PS-degrading bacterial strain was isolated from Indian meal moth (Yang et al., 2014) and *T. molitor* (Suh and Lee, 2016). NMDS analysis found that the samples of the PS, PE, and bran fed larvae were significantly different from each other (Fig. 4D). Differential abundance analysis was used to assess whether particular OTUs were associated with different diets (Fig. 5). The observations are similar to the community shift reported in *T. molitor* fed PE, PS and bran (Brandon et al., 2018), *T. obscurus* fed PS vs bran (Peng et al., 2019), and *T. molitor* fed cellulose, PE and PS (Przemieniecki et al., 2020). Four OTUs were a minority of microbial communities and three were significantly associated with PE-fed microbiome ($p < 0.05$): *Dyella*, *Lysobacter*, and *Leptothrix* were significantly associated with the microbiome fed with PS. This is different from the two specific OTU *Citrobacte*r sp. and *Kosakonia* sp. were strongly associated with both PE and PS degradation in *T. molitor* (Brandon et al., 2018), and *Lactococcus* and *Elizabethkingia* found in *T. molitor* fed PE and PS (Przemieniecki et al., 2020). However, no strain of above genera with PE or PS-degrading capacity has been reported in updated review papers (Jacquin et al., 2019; Ru et al., 2020). *Dyella* sp. increased in the larvae fed with PS (Fig. 5 B). *Dyella ginsengisoli* strain *LA-4* was isolated from the activated sludge, which can utilize biphenyl as the sole source of carbon and energy, which degrades over 95 mg/L of biphenyl in 36 h (Li et al., 2009). *Dyella* sp. may involve in degradation of PS intermediates in *T. molitor*. *Bacillus* sp. increased in PS fed larvae (Fig. 5 A). The differential abundance analysis showed the significant difference between PE and PS fed larvae. Since PE and PS has significantly different molecular structure and metabolic pathways from bran (Ru et al., 2020), it is likely that the depolymerization and biodegradation of these two polymers involve different microbes, enzymes, and metabolic pathways, resulting in different depolymerizing patterns and that distinct roles of microbes that we observed in this study. The diversied microbiomes in *T. molitor* larvae could be related to their diets e.g., bran which contains lignins (Yang et al., 2019a,2019b). *T. molitor* larvae can degraded lignin, cellulose and hemicellulose in wheat straw, rice straw and corn straw. The microbes related to degradation of lignocellulosic materials could be a source of plastic degraders. Further research is needed to address on this hypothesis. In this study, the results of community structure analysis indicated the significant community shift due to change of diet from bran to PS or PE. The community structures associated with bran, PS and PE-degradation are extremely diversified and significantly different from previously reported in literatures (Brandon et al., 2018; Yang et al., 2018b; Przemieniecki et al., 2020). This analysis did provide more detailed information of individual functional bacterial species associated with depolymerization/biodegradation of PS and PE but could not identify the functional bacterial genus or species. Research on characterization of enzymes for depolymerization and

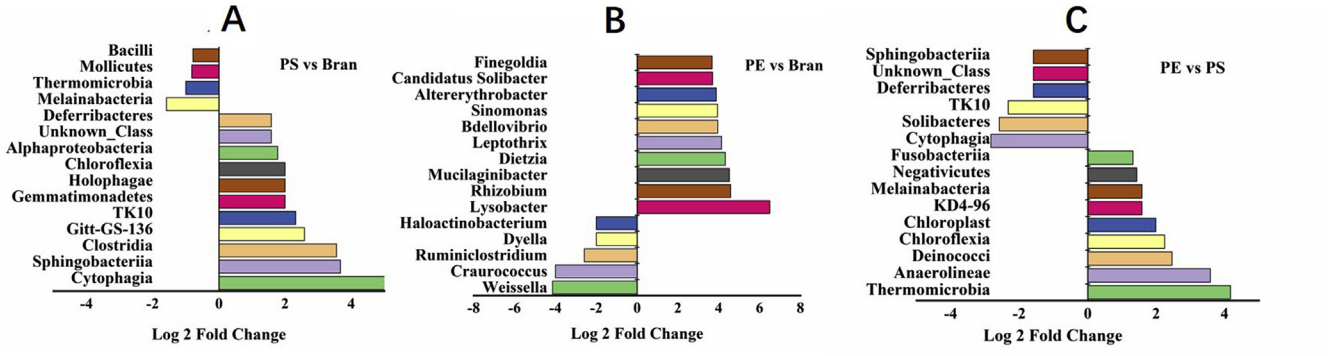

Fig. 5. Differential abundance analysis of gut microorganisms between experimental diets. Direction of fold change (log 2) indicates which diet each OTU is more strongly associated (labeled below X-axis). A. *T. molitor* larvae fed with PS versus bran. B. The larvae fed with PE-1 versus bran. C. The larvae fed with PE-1 versus PS. In this study, we tested LDPE using PE-1 sample.

related genes could help in finding functional genus or species. More investigation is also needed to explore mechanisms of different depolymerization patterns in relation to gut microbiome.

4. Conclusions

This study characterized biodegradation of low-density polyethylene (LDPE) foams from two sources and expanded polystyrene foam by *T. molitor* larvae obtained in Beijing, China. Both LDPE and PS polymers were depolymerized and then biodegraded. The PS biodegradation showed a similar ubiquitous broad depolymerization pattern, *i.e.,* a decrease in both M_w and M_n simultaneously, as reported previously. Biodegradation of LDPE foams showed two different depolymerization patterns. For material with average lower molecular weights (PE-1), both M_w and M_n were decreased, while for the material with a higher average molecular weight (PE-2), M_w decreased but M_n increased, indicating limited extent depolymerization. The M_z values of all polymers were decreased, indicating decrease in the highest molecular weight portion due to biodegradation. Biodegradation and oxidation of PS and LDPE were confirmed by formation of oxygen containing functional groups using FTIR analyses and polymer modification using TGA. PS depolymerization was completely inhibited during gentamicin antibiotic depression of gut microbes, confirming the microbe dependence of PS degradation reported previously with *T. monitor* larvae. However, limited extent LDPE depolymerization was still observed during gentamicin depression, indicating the presence of less gut microbe dependence of LDPE depolymerization in *T. molitor* larvae. Microbial community analysis indicated that the gut microbiome had a significantly different response to the feeding of different diets bran, PS, and LDPE, likely associated with development of corresponding microbes for respective substrates.

Credit author statement

Li Yang: Investigation, Writing - original draft; Jie Gao: Investigation, Supervision; Ying Liu: Investigation; Guoqiang Zhuang: Conceptualization, Writing-Review; Xiawei Peng: Investigation, Supervision; Xuliang Zhuang: Conceptualization, Funding acquisition, Resources, Writing-Review; Wei-Min Wu: Conceptualization, Methodology, Investigation, Supervision, Writing-Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Albertsson, A.C., 1978. Biodegradation of synthetic polymers. II. A limited microbial conversion of ^{14}C in polyethylene to $^{14}CO_2$ by some soil fungi. J. Appl. Polym. Sci. 22 (12), 3419–3433.
Albertsson, A.C., 1988. The three stages in degradation of polymers–polyethylene as a model substance. Appl. Polym. Sci. 35 (5), 1289–1302.
Albertsson, A.C., Erlandsson, B., Hakkarainen, M., Karlsson, S., 1998. Molecular weight changes and polymeric matrix changes correlated with the formation of degradation products in biodegraded polyethylene. J. Environ. Polym. Degrad. 6 (4), 187–195.
Amass, W., Amass, A., Tighe, B., 1988. A review of biodegradable polymers: uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. Polym. Bar Int. 47 (2), 89–144.
Andrady, A.L., 1994. Assessment of environmental biodegradation of synthetic polymers. J. Macromol. Sci. Polym. Rev. 34 (1), 25–76.
Andrady, A.L., 2003. Common plastics materials. In: Andrady, A.L. (Ed.), Plastics and environment.77-121. John Willey & Sons, Inc. Hoboken, New Jersey.
Brandon, A.M., Gao, S.H., Tian, R., Ning, D., Yang, S.S., Zhou, J., Wu, W.M., Criddle, C.S., 2018. Biodegradation of polyethylene and plastic mixtures in mealworms (larvae of *Tenebrio molitor*) and effects on the gut microbiome. Environ. Sci. Technol. 52 (11), 6526–6533.
Brandon, A.M., Abbadi, S.H., Ibekwe, U.A., Cho, Y.M., Wu, W.M., Criddle, C.S., 2020. Fate of hexabromocyclododecane (HBCD), a common flame retardant, in polystyrene-degrading mealworms: elevated HBCD levels in egested polymer but no bioaccumulation. Environ. Sci. Technol. 54 (1), 364–371.
Fan, P.X., Liu, P., Song, P.X., Chen, X.Y., Ma, X., 2017. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig mode. Sci. Rep.7 3431–3439.
Gautam, R., Bassi, A.S., Yanful, E.K., 2007. A review of biodegradation of synthetic plastic and foams. Appl. Biochem. Biotechnol.141(1), 85–108.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci Adv 3 (7), e1700782.
Guillet, J.E., Regulski, T.W., McAneney, T.B., 1974. Biodegradability of photodegraded

polymers. II. Tracer studies of biooxidation of Ecolyte PS polystyrene. Environ. Sci. Technol. 8, 923–925.
Hale, L., Crowley, D., 2015. DNA extraction methodology for biochar-amended sand and clay. Biol. Fertil. Soils 51 (6), 733–738.
Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-Pay, M., Barbe, V., Meistertzheim, A.-L., Ghiglione, J.-F., 2019. Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the "plastisphere. Front. Microbiol. 10, 865.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347 (6223), 768–771.
Jones, P.H., Prasad, D., Heskins, M., Morgan, M.H., Guillet, J.E., 1974. Biodegradability of photodegraded polymers. I. Development of experimental procedures. Environ. Sci.Technol.8 919–923.
Kaplan, D.L., Hartenstein, R., Sutter, J., 1979. Biodegradation of polystyrene, poly(-metnyl methacrylate), and phenol formaldehyde. Appl. Environ. Microbiol. 38, 551–553.
Krueger, M.C., Harms, H., Schlosser, D., 2015. Prospects for microbiological solutions to environmental pollution with plastics. Appl. Microbiol. Biotechnol. 99, 8857–8874.
Kyrikou, I., Briassoulis, D., 2007. Biodegradation of agricultural plastic films: a critical review. J. Polym. Environ. 15 (2), 125–150.
Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2017. Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*. Environ. Pollut 220, 523–531.
Lee, B., Pometto, A.L., Fratzke, A., Bailey, T.B., 1991. Biodegradation of degradable plastic polyethylene by phanerochaete and streptomyces species. Appl. Environ. Microbiol. 57 (3), 678–685.
Li, A., Qu, Y., Zhou, J., Gou, M., 2009. Isolation and characteristics of a novel biphenyl-degrading bacterial strain, *Dyella ginsengisoli* LA-4. J. Environ. Sci. 21 (2), 211–217.
Li, L.I., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: a review of sources, occurrence and effects. Sci. Total Environ. 566–567, 333–349.
Lu, S., Qiu, R., Hu, J., Li, X., Chen, Y., Zhang, X., Cao, C., Shi, H., Xie, B., Wu, W.-M., He, D., 2020. Prevalence of microplastics in animal-based traditional medicinal materials: widespread pollution in terrestrial environments. Environ. Pollut. 709, 136214.
Lv, X., Dong, Q., Zuo, Z., Liu, Y., Huang, X., Wu, W.-M., 2019. Microplastics in a municipal wastewater treatment plant: fate, dynamic distribution, removal efficiencies, and control strategies. J. Clean. Prod. 225, 579–586.
Mor, R., Sivan, A., 2008. Biofilm formation and partial biodegradation of polystyrene by the actinomycete *Rhodococcus ruber*. Biodegradation 19, 851–858.
Motta, O., Proto, A., Carlo, F.D., Caro, F.D., Santoro, E., Brunetti, L., Capunzo, M., 2009. Utilization of chemically oxidized polystyrene as co-substrate by filamentous fungi. Int. J. Hyg Environ. Health 212, 61–66.
Nakamiya, K., Sakasita, G., Ooi, T., Kinoshita, S., 1997. Enzymatic degradation of polystyrene by hydroquinone peroxidase of *Azotobacter beijerinckii* HM121. J. Ferment. Bioeng. 84, 480–482.
Ohtake, Y., Kobayashi, T., Asabe, H., Murakami, N., Ono, K., 1998. Oxidative degradation and molecular weight change of LDPE buried under bioactive soil for 32–37 years. Appl. Polym. Sci. 70 (9), 1643–1648.
Peng, B.Y., Su, Y., Chen, Z., Chen, J., Zhou, X.F., Benbow, M.E., Criddle, C.S., Wu, W.M., Zhang, Y.L., 2019. Biodegradation of polystyrene by dark (*Tenebrio obscurus*) and yellow (*Tenebrio molitor*) mealworms (Coleoptera: Tenebrionidae). Environ. Sci. Technol. 53, 5256–5265.
Peng, B.Y., Li, Y., Fan, R., Chen, Z., Chen, J., Brandon, A.M., Craig, S., Zhang, Y.L., Wu, W.M., 2020. Biodegradation of low-density polyethylene and polystyrene in superworms, larvae of *Zophobas Atratus* (Coleoptera: Tenebrionidae): broad and limited extent depolymerization. Environ. Pollut. 266 (115206) https://doi.org/10.1016/j.envpol.2020.115206.
PlasticsEurope, 2019. Plastics-the facts 2019. https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts 2019_14102019.pdf. (Accessed 20 March 2020).
Przemieniecki, S.W., Kosewska, A., Ciesielski, S., Kosewska, O., 2020. Changes in the gut microbiome and enzymatic profile of *Tenebrio monitor* larvae biodegrading cellulose, polyethylene and polystyrene waste. Environ. Pollut. 256, 113265.
Ru, J., Huo, Y., Yang, Y., 2020. Microbial degradation and valorization of plastic wastes. Front. Microbiol. 11, 442.
Shah, A.A., Hasan, F., Hameed, A., Ahmed, S., 2008. Biological degradation of plastics: a comprehensive review. Biotechnol. Adv 26 (3), 246–265.
Song, Y., Cao, C., Qiu, R., Hu, J., Liu, M., Lu, S., Shi, H., Raley-Susman, K.M., He, D., 2019. Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (*Achatina fulica*) after soil exposure. Environ. Pollut. 250, 447–455.
Song, Y, Qiu, R, Hu, J.N, Li, X, Zhang, X.T, Chen, Y.X, Wu, W.M, He, D.F, 2020. Biodegradation and disintegration of expanded polystyrene by land snails *Achatina fulica*. Science of the Total Environment 746, 141289. https://doi.org/10.1016/j.scitotenv.2020.141289.
Suh, D._E., Lee, D.-Young, 2016. Polyethylene terephthalate, polyvinyl chloride, polystyrene, polypropylene, polyethylene bio-degrading microbes and irs use for plastic recycling preprocess. J. Int. STEAM 1, 1–5.
Sivan, A., 2011. New perspectives in plastic biodegradation. Curr. Opin. Biotechnol. 22, 422–426.
Syranidou, E., Karkanorachaki, K., Amorotii, F., Avgeropoulos, A., Kolvenbach, B., Zhou, N.-Y., Fava, F., Corvini, F.X., Kalogerakis, N., 2019. Biodegradation of mixture of plastic films by tailored marine consortia. J. Hazard Mater. 375, 33–42.
Tian, L., Kolvenbach, B., Corvini, N., Wang, S., Tavanaie, N., Wang, L., Ma, Y., Scheu, S., Corvini, F.X., Rong, J., 2017. Mineralisation of ^{14}C-labelled polystyrene plastics by *Penicillium variabile* after ozonation pre-treatment. N. Biotech. 38, 101–105.
Wang, L., Wu, W.-M., Bolan, N.S., Tsang, D.C.W., Li, Y., Qin, M., Hou, D., 2021. Environmental fate, toxicity and risk management strategies of nanoplastics in the environment: current status and future perspectives. J. Hazard Mater. 401 (123415) https://doi.org/10.1016/j.jhazmat.2020.123415.
Wu, W.M., Yang, J., Criddle, C.S., 2017. Microplastics pollution and reduction strategies. Front. Environ. Sci. Eng. 11 (1), 6.
Yang, J., Yang, Y., Wu, W.M., Zhao, J., Jiang, L., 2014. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environ. Sci. Technol. 48, 13776–13784.
Yang, S.S., Brandon, A.M., Flanagan, J.C., Yang, J., Ning, D., Cai, S.Y., Fan, H.Q., Wang, Z.Y., Ren, J., Benbow, E., Ren, N.Q., Waymouth, R.M., Zhou, J.Z., Criddle, C.S., Wu, W.M., 2018a. 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, 979–989.
Yang, S.-S., Wu, W.-M., Brandon, A.M., Fan, H.-Q., Receveur, J.P., Li, Y., Wang, Z.-Y., Fan, R., McClellan, R.L., Gao, S.-H., Li, P., Cai, W.-W., Ding, L.-Y., Yang, J., Zheng, M., Ren, J., Zhang, Y.-L., Gao, J., Xing, D.F., Ren, N.Q., Waymouth, R.M., Zhou, J.Z., Taoi, H.-C., Picard, C.J., Benbow, M.E., CS, Criddle, 2018b. Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). Chemosphere 212, 262–271.
Yang, S.-S., Chen, Y.-H., Kang, J.-H., Xie, T.-R., He, L., Xing, D.-F., Ren, N.-Q., Ho, S.-H., Wu, W.-M., 2019a. Generation of high-efficient biochar for dye adsorption using frass of yellow mealworms (larvae of *Tenebrio molitor* Linnaeus) fed with wheat straw for insect biomass production. J. Clean. Prod. 227, 33–47.
Yang, S.S., Chen, Y.D., Zhang, Y., Zhou, H.M., Ji, X.Y., He, L., Xing, S.F., Ren, N.Q., Ho, S.H., Wu, W.M., 2019b. A novel clean production approach to utilize crop waste residues as co-diet for mealworm (*Tenebrio molitor*) biomass production with biochar as byproduct for heavy metal removal. Environ. Pollut 252, 1142–1153.
Yang, Y., Yang, J., Wu, W.M., Zhao, J., Song, Y.L., Gao, L.C., Yang, R.F., Jiang, L., 2015a. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 1. chemical and physical characterization and isotopic tests. Environ. Sci. Technol. 49, 12080–12086.
Yang, Y., Yang, J., Wu, W.M., Zhao, J., Song, J.L., Gao, L.C., Yang, R.F., Jiang, L., 2015b. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 2. role of gut microorganisms. Environ. Sci. Technol. 49, 12087–12093.
Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30, 614–620.