



Biodegradation of polyether-polyurethane foam in yellow mealworms (*Tenebrio molitor*) and effects on the gut microbiome

Jiawei Liu^a, Jingyuan Liu^a, Bin Xu^a, Anming Xu^a, Shixiang Cao^a, Ren Wei^b, Jie Zhou^{a, **}, Min Jiang^a, Weiliang Dong^{a,*}

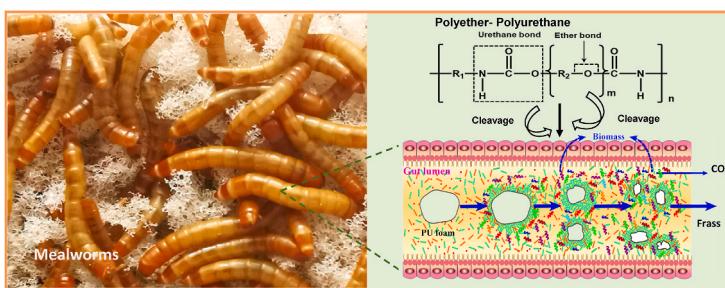
^a State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, 211800, PR China

^b Junior Research Group Plastic Biodegradation, Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, University of Greifswald, Greifswald, Germany

HIGHLIGHTS

- Yellow mealworms can grow on a diet of polyether-PU foam alone.
- FTIR, XPS, TGA revealed the cleavage of chemical bonds and the changes in thermal stability of polyether-PU foam found in feces.
- GPC confirmed a decreased molecular weight of polyether-PU foam found in feces as a result of depolymerization.
- The gut microbial community of yellow mealworms varied significantly in association with the polyether-PU diet.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Michael Bank

Keywords:

Waste plastics
Polyether-polyurethane
Biodegradation
Yellow mealworms
Tenebrio molitor
Gut microbiome

ABSTRACT

Polyurethane (PU) is one of the mass-produced recalcitrant plastics with a high environmental resistance but extremely low biodegradability. Therefore, improperly disposed PU waste adds significantly to plastic pollution, which must be addressed immediately. In recent years, there has been an increasing number of reports on plastic biodegradation in insect larvae, especially those that can feed on polyethylene and polystyrene. This study revealed that yellow mealworm (*Tenebrio molitor*) larvae can chew and ingest polyether-PU foams efficiently, resulting in a significant mass loss of nearly 67% after 35 days at a similar survival rate compared to when fed on bran. However, polyether-PU fragments were found in the frass of *T. molitor*, indicating that polyether-PU biodegradation and bioconversion in intestinal tracts were not complete. The scission of ether and urethane bonds in the polyether-PU can be evidenced by comparing polymer fragments recovered from frass with the pristine ones using Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. Gel permeation chromatography suggested the release of low-molecular-weight oligomers as a result of the biodegradation, which also resulted in poor thermal stability of the polyether-PU foam as determined by thermogravimetric analysis. High-throughput sequencing of the gut microbiome revealed significant changes in the microbial community populations due to the polyether-PU diet, for

* Corresponding author.

** Corresponding author.

E-mail addresses: jayzhou@njtech.edu.cn (J. Zhou), dwl@njtech.edu.cn (W. Dong).

example, an increase in the families Enterobacteriaceae and Streptococcaceae, suggesting that these microorganisms may contribute to the polyether-PU biodegradation.

1. Introduction

Polyurethane (PU) is a form of petroleum-based plastics that consists of repeating units linked by carbamate bonds. With a global production of 18 million tons in 2016, PU is one of the most extensively used synthetic polymers (Cornille et al., 2016). PU is available in thermoplastics, thermosets, coatings, adhesives, sealants and elastomers and is used in a wide variety of applications including building insulation, refrigerators and freezers, furniture and bedding, footwear, automotive, and textiles, as well as coatings and adhesives (Magnin et al., 2020). Due to their complicated chemical compositions and the use of various additives such as surfactants, catalysts, stabilizers, blowing agents, and flame retardants, commercial PU products cannot be efficiently recycled after their end-of-life cycles. As a result, PU waste that is improperly disposed of can accumulate in landfills and the natural environment (Liu et al., 2021; Skleničková et al., 2020). In the context of a circular plastic economy, effective disposal methods for PU waste remain a technological challenge (Wei et al., 2020).

Depending on the polyols employed in the synthesis, PU is classified as polyester-PU or polyether-PU. At the industrial level, polyether-PU is mostly used for thermal insulation (Cregut et al., 2013). To date, biodegradation of PU has primarily been described for polyester-PU, in which the ester bonds in the polyol soft segments can be hydrolyzed. This high biodegradability will enable the release of degradation products that may be mineralized by microorganisms (Akutsu et al., 1998; Russell et al., 2011; Mathur and Prasad, 2012; Shah et al., 2013a, 2013b; Alvarez-Barragan et al., 2016; Khan et al., 2017; Osman et al., 2017; Brunner et al., 2018; Liu et al., 2021). Biodegradation of polyether-PU, on the other hand, has been reported less frequently and is generally without substantial scientific proof. In the early 1990s, Jansen et al. isolated a *Staphylococcus epidermidis* strain that could modify the surface properties of a polyether PU polymer (Jansen et al., 1991). *Alternaria* sp. PURDK2 degraded a polyether-PU polymer, resulting in a 27.5% weight loss presumably by cleaving the urethane-bonds (Matsumiya et al., 2010). The highest so far reported polyether-PU degradation abilities have been found in *Cladosporium tenuissimum* A2.PP.5 and A3.I.1 and *Cladosporium pseudocladosporioides* T1.PL.1 that can result in up to 65% weight loss after 21 days of incubation (Alvarez-Barragan et al., 2016). Until recently, research on polyether-PU biodegradation has been in its infancy, and various challenges such as a lack of biodegrading microorganisms or enzymes, low biodegradation efficiency, and an unknown biodegradation mechanism must be addressed promptly.

Yellow mealworms (larvae of *Tenebrio molitor*), superworms (larvae of *Zophobas atratus*), wax moth (larvae of *Galleria mellonella*), snails (*Achatina fulica*), and honeybees have been identified as plastic-munching species in recent years (Riudavets et al., 2007; Yang et al., 2021; Song et al., 2020; Lou et al., 2020; Deng et al., 2021; Wang et al., 2022). They can ingest and consume plastic diets containing recalcitrant plastics such as polyethylene (PE), polystyrene (PS), and others. Mealworms are regarded as the most promising of these insect larvae due to their ability to digest a variety of plastic types. Yellow mealworms, the larvae of the darkling beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae), are used commercially as animal feed and are a potential sustainable alternative to food protein for human consumption (Borremans et al., 2020). Furthermore, yellow mealworms have been shown to be able to consume and degrade PS, LDPE, polypropylene (PP), polyvinyl chloride (PVC), and polylactic acid (PLA) polymers (Yang et al., 2015b, 2018b, 2021a, 2021b; Peng et al., 2020, 2021). The larvae have an inherent ability to mineralize various PS degradation products into CO₂ with the assistance of the gut microbiota (Yang et al., 2015b, 2018b, 2015b). Yang et al. (2021b) found that lower molecular weight LDPE

depolymerized preferentially during mealworm larval ingestion, whereas higher molecular weight LDPE depolymerization was less evident. They also discovered that LDPE depolymerization is less dependent on gut microbes (Yang et al., 2021b). Yang et al. (2021a) reported PP depolymerization to a limited extent during mealworm ingestion. PP biodegradation in the larvae is dependent on gut microbes, and *Kluyvera* sp. was identified as the dominant species in the gut microbiome of *T. molitor* larvae fed PP (Yang et al., 2021a). Peng et al. (2020) reported a decrease in Mw, Mn and Mz of PVC microplastic powders by 33.4%, 32.8%, and 36.4%, respectively, as a result of mealworm feeding, which also resulted in the limited mineralization of PVC to chloride (Peng et al., 2020). Peng et al. (2021) proposed a circular approach for PLA waste management involving the resource recovery of used PLA as the feedstock for insect biomass production, the management of mealworm feces waste as fertilizer, and the use of agricultural products for PLA production (Peng et al., 2021). *Tenebrio* larvae have a diverse gut microbiome that aids in the breakdown of recalcitrant plastics (Brandon et al., 2018; Wei et al., 2020; Lou et al., 2021; Zhu et al., 2021). In addition, yellow mealworms can secrete emulsifying substances into intestinal tracts to facilitate the plastic biodegradation (Brandon et al., 2021). In addition, numerous effective plastic-degrading bacteria and fungi have been isolated from larval guts. *Enterobacter* sp. and *Aspergillus flavus* have been isolated from the gut of greater wax moth, and they may be involved in PE degradation (Ren et al., 2019; Zhang et al., 2020). *Exiguobacterium* sp. strain YT2 isolated from yellow mealworm, *Pseudomonas* sp. isolated from superworm, and *Acinetobacter* isolated from red flour beetle may contribute to PS degradation (Kim et al., 2020; Wang et al., 2020; Yang et al., 2015b).

PU biodegradation in *Tenebrio* larvae has received little attention to yet. Guo et al. have described that *T. molitor* can survive solely on PU foam. However, their research focused on the alterations in the DNA methylation pattern of the mitochondrial genome of *T. molitor* at different development stages when PU foam was provided as the only diet (Guo et al., 2019). *T. molitor* was fed two types of PU 1 (kitchen sponge) and PU 2 (commercial thermal insulation foam) to assess biodegradability and the effects of heavy metals and other components in plastics on mealworm decomposition performance (Bieganowski, 2021). In addition, *G. mellonella* larvae have shown a strong preference for corresponding pristine plastics over two types of waste electrical and electronic equipment (WEEE) plastics, waste rigid polyurethane (WRPU) and waste polystyrene (WPS), and presented the following decreasing preference for pristine plastics when fed individually: RPUs > phenol-formaldehyde resin > PE > PP > PS ≈ PVC; this could be due to differences in physical properties and chemical structures of the plastics (Zhu et al., 2022). However, the type of PU foam used in their studies was unknown, and no analysis of the gut microbial community of the insect larvae was performed.

In this study, the ability of yellow mealworms (*T. molitor*) to degrade PU wastes was investigated using two types of flexible PU (polyether- and polyester-PU) foams as the sole diet, with a particular emphasis on the biodegradation of polyether PU, which has a high degradability for *T. molitor*. Additionally, the gut microbial community of *T. molitor* was examined. Frass from polyether-PU-eating mealworms was collected and characterized by a scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), gel permeation chromatography (GPC), X-ray photoelectron spectroscopy (XPS) and thermogravimetric analysis (TGA) to determine changes in the physical or chemical structure and compositions of the ingested polyether-PU foam after passage through the gut. High-throughput sequencing was performed to evaluate the change in the population of gut microbial community, which can be attributed to the consumption and degradation of

the ingested polyether-PU foam.

2. Materials and methods

2.1. Mealworms and test materials

Polyether-PU foams were purchased from Dagong sponge Co., Ltd. (Nantong, China). Toluene 2,4-diisocyanate (TDI) is the isocyanate used to synthesize polyether-PU foam, while the other components are unknown. These PU foams were cut into regular rectangular dimensions (Long × Width × High = 5 × 4 × 2 cm) prior to degradation experiments. Yellow mealworms (growth age of approximately 3–4 instars) used in this work were purchased from Qimo Pet Products Co., Ltd. (Xiamen, China), as well as the natural wheat bran. In order to extract the gut of the yellow mealworm, the insect dissection needle was purchased from Anning Education Equipment Co., Ltd. (Zhengzhou, China). Other chemical reagents (analytically reagents) used in this work were purchased from either Sinopharm Chemical Reagent Co., Ltd. (Xiamen, China) or Sigma-Aldrich (German).

2.2. Determination of *T. molitor* survival and characterization of polyether-PU foam degradation

Yellow mealworms used in this study were fed natural wheat bran, and then starved for five days before being fed the experimental diets: polyether-PU foam, bran and unfed (control diet). To assess the mealworm survival rate and plastic mass loss, a group of yellow mealworms (120 randomly selected as a group) were reared on polyether-PU foam (1.0 g) as sole diet in a food grade high density polypropylene container (Long × Width × High = 14 × 8 × 6 cm) and the spatial density of the mealworms was ~1 worms cm⁻². Bran-fed containers initially received 1 g of bran and 1 g of additional bran every 7 days. The unfed control containers provided no diet and the mealworms were kept in a state of starvation. These polypropylene containers were stored in incubators maintained at 25 °C and 70% humidity. Three experimental diet groups were prepared in triplicate ($n = 3$).

The survival rate of yellow mealworms was evaluated by counting the total number of mealworms and the dead mealworms every 7 days for 35 days. All the dead yellow mealworms, appearing black all over, were removed to prevent transmission of death. At the same time, frass produced by the yellow mealworm were also collected and stored in liquid nitrogen for further analysis. To assess the biodegradation effect of yellow mealworms, polyether-PU foam was also removed every 7 days and weighed after a simple surface cleaning. In addition, the changes of surface morphology of polyether-PU foam after being fed to yellow mealworms was characterized by SEM (Zeiss Sigma 300 vp, Carl Zeiss AG, German). Polyether-PU foams were thoroughly washed with sterilized distilled water and then dried under vacuum. They were then mounted, sputter-coated with gold, and examined by SEM with accelerating voltage from 3 to 7 KV. The control of polyether-PU foam (not eaten by mealworms) was also examined for comparison.

2.3. Collection and characterization of polyether-PU fragments from frass

As indicated in previous studies, plastic fragments that are not completely degraded can be recovered from the frass of yellow mealworms. In order to evaluate the biodegradability of polyether-PU foam by yellow mealworm, polyether-PU fragments were extracted from frass using an organic solvent. According to Yang et al. approximately 1.0 g of fresh frass was extracted with 150 mL of tetrahydrofuran (THF) as the solvent in a Soxhlet extractor at 90 °C for 12 h (Yang et al., 2015a). After concentration and solvent volatilization, solid frass extracts were obtained for further analysis. All the subsequent characterizations of frass extracts were carried out with pristine polyether-PU foam as the control.

The changes of functional groups in the PU polymer were investigated using FTIR spectrometer (Thermo Nicolet Corporation, Madison).

Triplicate FTIR analyses were performed to characterize functional groups in polyether-PU foam and frass samples over the range 500–4000 cm⁻¹ (Yang et al., 2021b). All treatments were carried out with three replicates.

The thermal characterization was performed using a TGA analyzer (TGA5500, USA) under nitrogen atmosphere. The instrument was calibrated using alumina. The thermogravimetric curves were obtained according to the following conditions: samples of approximately 5 mg, temperature range of 50–700 °C, heating rate of 10 °C/min and atmosphere of N₂ (dynamic) of 100 cm³/min (Zhu et al., 2021). All treatments were carried out with three replicates.

Surface chemical components were investigated using XPS (Thermo Scientific K-Alpha, USA). During the XPS spectra analysis, scanning was carried out over a broadband energy range (0–1200 eV) at an electron takeoff angle of 90° from the sample areas less than 1 mm in diameter (Yang et al., 2015b). All treatments were carried out with three replicates.

The molecular weights of PU polymers were performed using GPC (Agilent 1260, Waters, USA). The THF solution is mixed with the sample on a magnetic stirrer, heated gently (60 °C), then settled and filtered through a 0.22 μm PVDF filter. The THF extracted solution was concentrated to 5 mL in volume to achieve about 4 mg polymer per mL. 20 μL of the extract was injected into the GPC for analysis, with a flow rate of 0.8 mL/min (Lou et al., 2021). All treatments were carried out with three replicates.

2.4. Microbial community analysis

At the end of the 35 d experiment, the gut tissues of the mealworm (30 in each group) were randomly collected for microbial community analysis. The mealworms fed with bran were used as controls. Each diet groups were prepared in three replicates.

Mealworm samples were collected, preserved in 100% ethanol at -80 °C, and shipped to the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) for gut microbial community analysis. DNA extraction from externally decontaminated mealworms was performed using the DNeasy Blood and Tissue kit (Qiagen) before library construction. The hypervariable region V3–V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA).

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw FASTQ files were de-multiplexed using an in-house perl script, and then quality-filtered by fastp version 0.19.6 and merged by FLASH version 1.2.7.

The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA gene database using confidence threshold of 0.7. The metagenomic function was predicted by PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) based on OTU representative sequences. PICRUSt2 is a software containing a series of tools as follows: HMMER was used to align OTU representative sequences with reference sequences. EPA-NG and Gappa were used to put OTU representative sequences into a reference tree. The castor was used to normalize the 16S gene copies. Finally, alpha diversity, microbial community composition, differential abundance analysis and principal coordinate analysis (PCoA) were run on the online platform of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.5. Statistical analysis

Statistical analyses were performed in IBM SPSS Statistics (version 25). To evaluate the differences in survival and microbial diversity,

ANOVA were performed, followed by pairwise comparisons using Student's *t*-test with Tukey's correction to assess differences between diets and species. All p-values are adjusted p-values and all error values are average \pm standard deviation.

3. Results and discussion

3.1. Polyether-PU foam consumption and its effects on survival rate of mealworms

The mealworms began chewing polyether-PU foam as soon as them were fed, as indicated by their digging into the foam structure (Fig. 1A). The eating activity of the mealworms appeared to be high, causing hollows in the polyether-PU foam blocks (Fig. 1A). Especially, on the 7th day, visible pores emerged in the foam as a result of gnawing by yellow mealworms. On the 35th day, the foam was evidently devoured, and a large gap appeared in the center (Fig. 1B). In addition, SEM was used to characterize the surface morphological changes of the polyether-PU foam exposed to mealworms in comparison to pristine polyether-PU foam. The net structure of the untreated polyether-PU foam is intact, and the fiber surface is smooth (Fig. 2A), while the net structure of the exposed polyether-PU foam was fractured, and the fiber surface became loose with numerous fractures (Fig. 2B).

Fig. 2C shows the variation in survival rates of the larvae from nine treatment groups over 35 days at 25 °C. Two feeding treatments resulted in higher survival rates than the unfed control ($62.5 \pm 2.1\%$), indicating that the yellow mealworms could benefit from these two diets for proper growth. The survival rate of the mealworms fed polyether-PU was $86.3\% \pm 2.3\%$, which was not significantly different from that of the bran fed controls ($88.7\% \pm 1.9\%$) (Fig. 2C). The survival percentage of

mealworms fed polyether-PU was slightly lower than the bran fed controls, indicating that polyether-PU foam feeding had no negative impact on the survival capabilities of mealworms. Similar findings have been reported in research on biodegradation of PS, PE and PP, suggesting that feeding plastic or bran has minimal influence on survival rates and the mealworms could utilize plastics for regular metabolic activities (Brandon et al., 2018; Lou et al., 2021; Yang et al., 2021). Furthermore, the cannibalism among large-sized mature larvae under nutrition-limited condition could not be ruled out. Yang et al. find that *Zophobas atratus* larvae fed only PP had a lower survival rate ($12.1 \pm 4.1\%$) but a high cannibal rate ($45.1 \pm 4.4\%$) over 35 days, compared to *Tenebrio molitor* with a lower cannibal rate ($11.3 \pm 0.8\%$) (Yang et al., 2021).

Consumption of polyether-PU increased throughout the experiment period (Fig. 2D), with mealworms from Xiamen causing a total mass loss of polyether-PU by $67.0 \pm 0.8\%$ of the initial mass (1.0 g) within 35 days (Fig. 2D). It can also be seen that the mealworms had to adapt to polyether-PU feeding at first, as indicated by a slower consumption rate within first 7 days. After adaptation, the consumption rate of PU by mealworms increased and then slowed down later in the experiment, implying that the upper limit of PU consumption was approaching. We used the median numbers of active mealworms (about 108) throughout the course of 28 days to calculate the average consumption of polyether-PU foam per worm. The average polyether-PU foam consumption rate was approximately 0.18 mg/d per worm, which was slightly lower than those demonstrated in previous studies with PE (0.22 mg/d per mealworm) (California, USA), PP (1.0 mg/d per mealworm) (Henan, China) or PS (4.27 mg/d per mealworm) (Beijing, China) (Brandon et al., 2018; Yang et al., 2021a, 2021b).

Polyester- and polyether-PU are the two forms of PU depending on

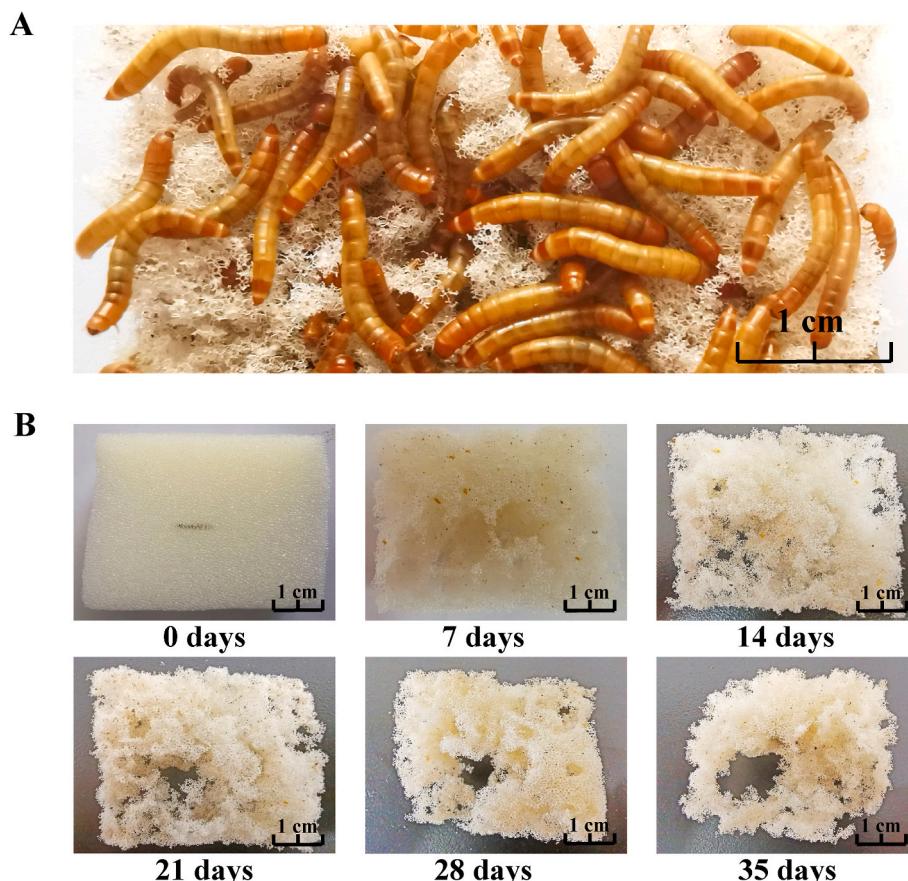


Fig. 1. Polyether-PU foam-eating behavior of mealworms (*T. molitor*). A: *T. molitor* chew and eat the polyether-PU foam block. B: The physical form of polyether-PU foam changes during 35 days under the *T. molitor* eating.

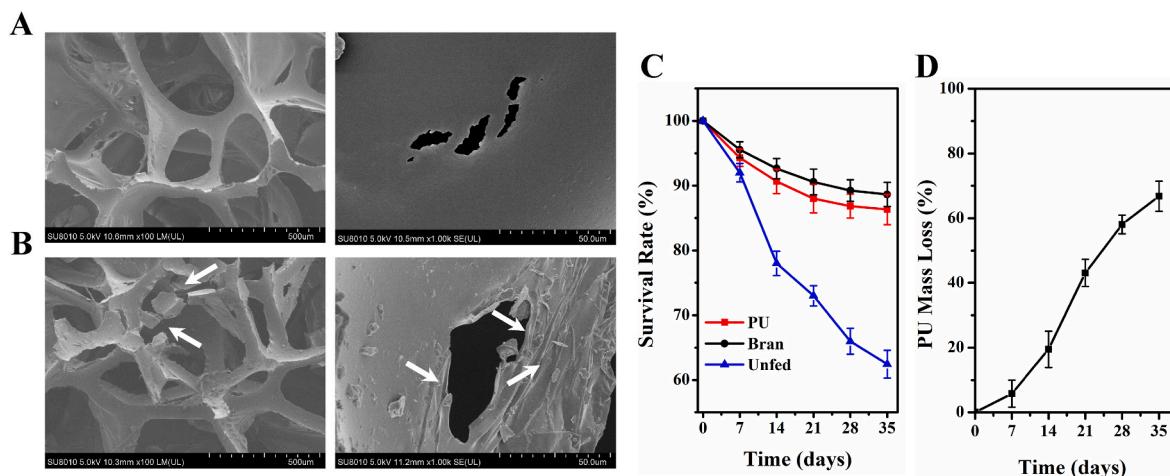


Fig. 2. SEM observations of the physical surface topography of the control of polyether-PU foam (not eaten by mealworms) (A) versus the polyether-PU foam eaten by *T. molitor* after 35 days (B); the *T. molitor* survival rates caused by the conventional diet (bran)-fed, polyether-PU foam-fed, and unfed mealworm populations (C); polyether-PU foam mass loss by *T. molitor* over 35 days (D) [mean \pm standard deviation (SD), n = 3 groups, 120 worms as a group].

the different types of polyols employed. Thus, we fed *T. molitor* with both forms of PU foam. In comparison to the readily consumed polyether-PU foam, polyester-PU foam decomposed to a very limited level when fed to mealworms, as evidenced by weak nibbling traces on

the polymer surface and no significant mass loss (Fig. S1). This phenomenon may be related to foam density, as polyether-PU foam (1.17 g/cm³) is less dense than polyester-PU foam (1.27 g/cm³), making polyether-PU foam easier for mealworms to chew and digest. Yang et al.

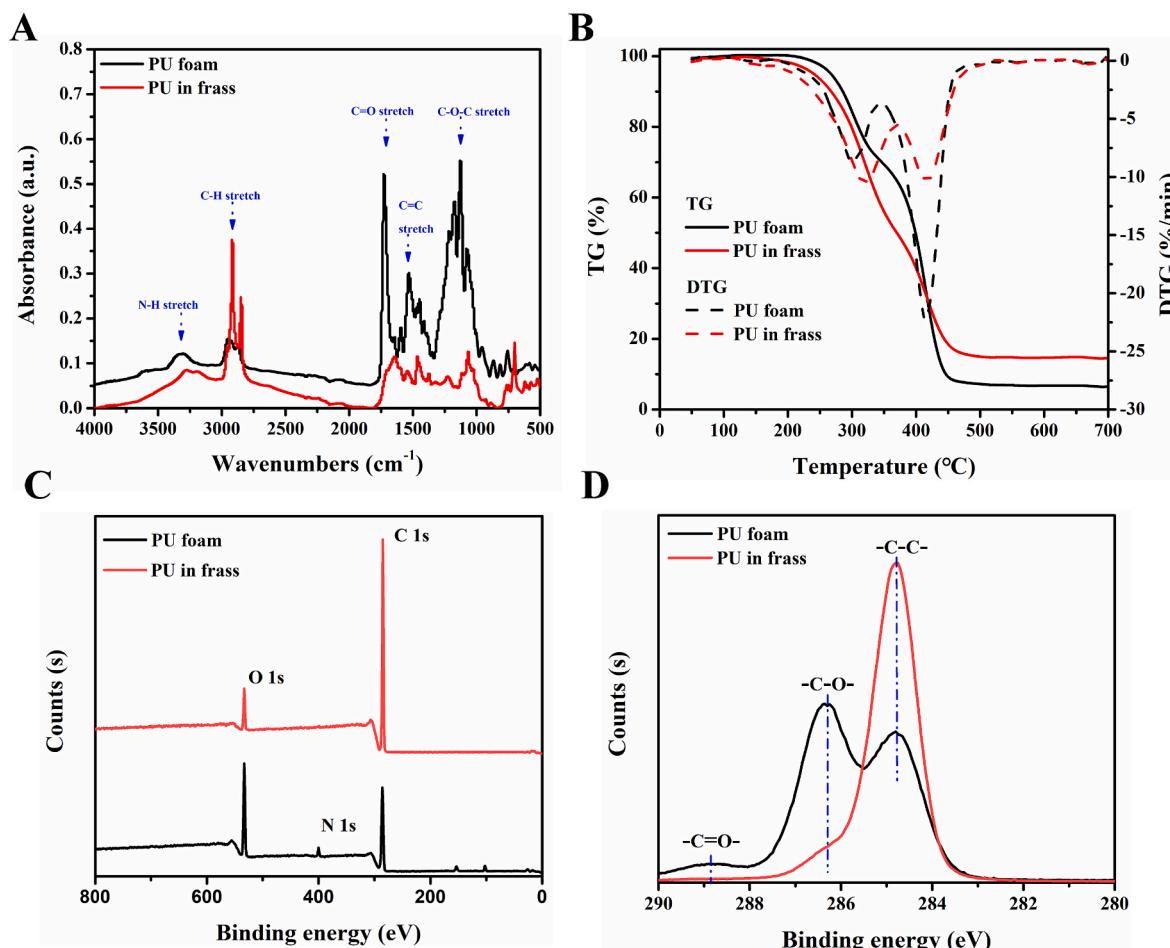


Fig. 3. Changes in the chemical structure and composition of polyether-PU foam after passage through the mealworm gut as frass. A: FTIR spectra of control polyether-PU foam and frass-residual polyether-PU foam. B: TG/DTG curves of the control polyether-PU foam and frass-residual polyether-PU foam (TG curves are solid lines, and DTG curves are dashed lines). C: XPS scanning of the control polyether-PU foam and frass-residual polyether-PU foam. D: C 1s spectra of the control polyether-PU foam and frass-residual polyether-PU foam.

also confirmed that when yellow mealworms (*Tenebrio molitor* Linnaeus) were used to degrade PS foam, the less dense foam was degraded faster (Yang et al., 2018a).

3.2. Evidence for depolymerization and biodegradation of polyether-PU foam

Residual polymer extracted from the frass of mealworms fed on polyether-PU foam was used to assess biodegradation and depolymerization. FTIR was used to analyze modified functional groups in the residual polymer (Fig. 3A). The pristine polyether-PU foam showed peaks assigned to —C—O—C— stretching (1217, 1174, 1126, 1077 cm $^{-1}$), —C=O— stretching (1726 cm $^{-1}$), N—H stretching, (3309 cm $^{-1}$), —C=C— stretching in benzene rings (1531 cm $^{-1}$) and —C—H— stretching (2950 cm $^{-1}$). By contrast, the peak of —C—O—C— stretching (1125 cm $^{-1}$) disappeared in the frass polyether-PU foam, indicating the scission of ether bonds in the soft segment of polyether-PU foam. Besides, the peak of —C=O— stretching (1726 cm $^{-1}$), which partly represents the urethane bond, vanished in the polyether-PU foam in frass, implying the hydrolysis of urethane bond in PU foam (Zhang et al., 2012). The peak of —C=C— stretching on benzene rings (1531 cm $^{-1}$) was similarly not observed in the frass polyether-PU (Zhang et al., 2012), suggesting that the hard segment was also cracked. However, nothing is known about the biodegradation mechanism of ether bonds in polyether PU foam. The biodegradation of (non-foamed) polyether PU has been suggested to be a result of oxidative reaction with initial cleavages of ether bonds in soft (polyol) segments (Christenson et al., 2006). On the other hand, it is still unknown whether or which enzymes may play a role in the oxidation of ether bonds in polyether PU foam (Alvarez-Barragan et al., 2016). Because ether bonds in polyether PU foam were assumed to be resistant to microbial attack, biodegradation of polyether PU foam was thought to occur with urethane and urea bonds instead via enzymatic hydrolysis (Matsumiya et al., 2010). Nevertheless, enzymatic degradation of urethane and urea bonds in the PU foam have been so far scarcely reported. Therefore, identifying potential enzymatic activities involved in the degradation of ether bonds or urethane bonds in PU foam, whether from insect larvae or gut microbiota, will be of great interest for future research.

TGA is a method of thermal analysis in which the mass of a sample is measured over time as the temperature changes. It is employed to study the thermal stability and composition of materials including various plastics. The TGA spectra for the control polyether-PU foam and polyether-PU foam in frass were shown in Fig. 3B as a function of temperature at a heating rate of 10 °C/min. The decomposition of urethane bond began between 150 and 220 °C, depending on the type of substituents on the isocyanate and polyol side (Lee et al., 2002). Although urethane bond decomposition dominated the early stage, the polyol component may contribute to weight loss at higher temperatures. The decomposition of PU foam samples proceeded in three stages (Fig. 3B). The first stage (100–150 °C) was mainly due to the release of volatile components, whereas the second stage (150–345 °C) was caused by the degradation of the urethane segments (Zhang et al., 2012). The weight loss in the third stage (>345 °C) could be attributed mainly to the decomposition of ester linkages and fatty acid chains which are more resistant to thermal decomposition than the urethane segments (Gómez et al., 2014). The maximum decomposition rates of the polyether-PU foam control sample occur at 154, 302, and 418 °C, respectively; the maximum decomposition rates of the frass sample occur at 153, 320, and 419 °C, respectively. Thermal parameters, such as the temperature at 5% weight loss (T_{5%}), 20% weight loss (T_{20%}) and 50% weight loss (T_{50%}) are generally used to evaluate the thermal properties of the materials (Yuan et al., 2018). As shown in Fig. 3B, T_{5%}, T_{20%} and T_{50%} of control are 267, 310 and 399 °C, while these of the corresponding frass sample are 240, 299 and 370 °C, respectively. The lower temperatures of T_{5%}, T_{20%} and T_{50%} determined with the frass sample indicate its inferior thermal stability compared with the control pristine polyether-PU foam

(Yuan et al., 2018; Zhu et al., 2021). Reduced thermal stability was thought to be the result of a decreased average molecular weight after degradation (Tsintzou et al., 2012; Yue et al., 2021). Total thermal weight loss ratios of the polyether-PU foam control and the frass are 93.10% and 85.30%, respectively, showing a decrease in the total weight loss rate after the polyether-PU ingestion, indicating the depletion of polyether-PU content in the frass (Yang et al., 2015a).

XPS was used to characterize the elemental content and chemical composition of the sample surface. Fig. 3C shows the XPS scanning spectra (0–800 eV) of the polyether-PU foam in frass in comparison with the polyether-PU foam control. Surface carbon (284.8 eV), oxygen (532.6 eV) and a trace amount of nitrogen (399.9 eV) were detected in the pristine polyether-PU foam. The proportion of carbon at the polymer surface increased from 70.7% to 91.1% after excreted from the mealworms, while the equivalent values for oxygen decreased from 25.2% to 7.9% and for nitrogen decreased from 4.1% to 1.0%. The loss of oxygen may be caused by the volatized oxidation products formed during the polyether-PU foam degradation (Yang et al., 2001) (Yang et al., 2001). The change in percentage of elemental composition indicates that chemical structure of the polymer was modified as a result of mealworm ingestion. A comparison of the XPS spectra of C 1s on the polyether-PU foam in frass versus the polyether-PU foam control (Fig. 3D) demonstrated that the peak-fitting result of C 1s for the polyether-PU foam control showed three peaks at 288.8 eV, 286.4 eV and 284.8 eV, which were assigned to a —C=O— , —C—O— and —C—C— group, respectively. The urethane linkages are represented by C-C (284.8 eV), C—O (286.4 eV) and C=O (288.8 eV) (Moghim et al., 2017). With the samples of the polyether-PU foam in frass, only the peak of —C—C— group and the peak of —C—O— group with limited intensity were visible whereas the peak of —C=O— disappeared. This implies the hydrolysis of urethane bond of polyether-PU foam in frass, and the results were consistent with the FTIR analysis. Previous PU degradation studies characterized by XPS have also shown chain scissions of CO—NH as a result of considerable oxidation following exposure to strong ultraviolet light (Liu et al., 2010, 2019).

GPC is commonly used to determine the molecular mass of polymer samples. We were unable to determine the molecular weight of the pristine polyether-PU foam by GPC due to its insolubility in any GPC-applicable solvent under room temperature (26 °C) or high temperature (150 °C). Because the thermoset PU foam materials we used are chemically cross-linked, they are insoluble in any of the common solvents used for GPC analysis such as dimethyl sulfoxide, 1,2,4-trichlorobenzene, dichloromethane, *N,N*-dimethylformamide, tetrahydrofuran (at 26 or 150 °C), hexafluoroisopropanol, dimethylacetamide, and chloroform (at 26 °C). Therefore, GPC can only be used to determine the molecular weights of larvae fragmented PU samples removed from the later cultivation stages, when organo-soluble PU oligomers are formed (Skleníčková et al., 2020; Zhu et al., 2021). Therefore, GPC was more widely used to analyze the biodegradation of vinyl polymers of which the initial molecular weight before treatment can be easily estimated, such as PE and PS (Yang et al., 2015a; Brandon et al., 2018; Song et al., 2020; Yang et al., 2020; Lou et al., 2021; Yang et al., 2021; Wang et al., 2022). The GPC results of the frass-residual polyether-PU foam over 35 days were analyzed (Fig. S2; Table S2), the molecules with number-average molecular weight (M_n) ranging from 14,000–18,000 g/mol, 2000–2700 g/mol, and less than 1000 g/mol were detected. Three groups of frass samples revealed distinct patterns of peak intensities of molecules in these three molecular mass ranges. Since the pristine PU foam is a highly cross-linked ultra-macromolecule, which is not soluble in any GPC solvent, it is reasonable to assume that the depolymerization did not occur uniformly at different regions in the PU ultra-macromolecule with different local polymer microstructures. Although a well-balanced average specific molecular weight value with PU samples in frass after 35 days of degradation was not get, oligomers with M_n of less than 1000 are abundant in all samples but not in the pristine PU foam. The GPC analysis with the polyether-PU foam in frass

revealed detectable M_n and the weight-average molecular weight (M_w) values, implying that the biodegradation occurred in the gut of mealworms, thereby releasing soluble oligomers from PU foam during the degradation process.

3.3. Effects of polyether-PU foam consumption on the gut microbial communities

A total of 721,724 sequences with an average length of 428 bps were obtained from the high throughput sequencing of the DNA extracted from the gut microbiome. Alpha diversity is an analysis of the diversity of species within a single sample, including Simpson, Ace, Chao1 and Shannon indexes, among other things (Table S1). Simpson indices represent the evenness of microbial communities, whereas other indices represent the richness of microbial communities. There was no significant difference between the two groups in terms of ACE and Chao1 index. A higher community biodiversity can be concluded on the basis of a higher Shannon or a lower Simpson index (Lou et al., 2021). Rarefaction curves with a Simpson index at the OUT level were generated for all samples at 0.97. The rarefaction curves of all samples achieved a plateau at about 2000 reads, implying that the amplicons were enough for sequencing (Fig. S3). Based on the Shannon and Simpson indices, the gut microbiome of PU-fed group showed higher species richness and evenness than the Bran-fed group. That may suggest that the digestion of polyether-PU foam in mealworms requires larger gut flora than the digestion of bran. In addition, a principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity index revealed clusters associated with different diets, with clear clusters for bran-fed and PU-fed mealworms (Fig. S4). The analysis of PCoA revealed that the gut microbiomes between different diets were notably different from each other and also from the triplicate samples in the same diet.

Relative abundance analysis revealed that the gut microbiome in the mealworms contained 5 main families (Fig. 4A). When fed with bran, the dominant families included Spiroplasmataceae and Enterococcaceae which are common insect gut-associated bacteria widely found in the *T. molitor* gut microbiome. When fed with polyether-PU foam, the relative abundances of Streptococcaceae and Enterobacteriaceae increased at the expense of decreasing Spiroplasmataceae and Enterococcaceae populations. The ingestion of polyether-PU foam resulted in an increased relative abundance of Streptococcaceae from 0.55% to 28.07%. The relative abundance of Enterobacteriaceae increased from 0.93% to 16.19% whereas the relative abundance of some other families decreased. Specifically, Spiroplasmataceae decreased from 50.99% to 23.43%, Enterococcaceae decreased from 23.46% to 12.85% (Fig. 4A).

The phenomenon observed here that the community shift is mainly associated with the distribution of Enterobacteriaceae after polyether-PU foam feeding was consistent with previous studies on PS or PE biodegradation. PE-degrading bacteria have been found in this family (Yang et al., 2014). In addition, two bacterial strains (*Aeromonas* sp. and *Klebsiella pneumoniae*) isolated from the guts of mealworms and superworms that are capable of growing on PS are members of the family Enterobacteriaceae (Tang et al., 2017). More recently, a similar increase in Enterobacteriaceae was also observed in guts of various species (e.g., yellow or dark mealworms and snails) when they were exposed to PS or PE feeding (Brandon et al., 2018; Yang et al., 2018b; Peng, 2019; Song et al., 2020; Lou et al., 2021). These findings are consistent with this study, suggesting that Enterobacteriaceae can also contribute to polyether-PU degradation.

Differential abundance analysis was used to assess whether particular OTUs were associated with different diets (Fig. 4B). This analysis revealed six OTUs that were strongly associated with polyether-PU-fed microbiome ($p < 0.05$): unclassified Enterobacteriaceae, unclassified Cryomorphaceae, *Collinsella* sp., *Staphylococcus* sp., *Adlercreutzia* sp. and *Enterobacter* sp.. *Enterobacter* sp. isolated from the gut of the larvae of Indian mealmoth with the capability of LDPE-degradation (Yang et al., 2014). The shift of the *Enterobacter* sp. to a higher relative abundance was observed in PP-fed, suggesting that these genera were significantly related to PP degradation in *Z. atratus* larvae (Yang et al., 2021). *Staphylococcus epidermidis* strain KH was found able to grow on polyether-PU foam, resulting in a decrease in elementary nitrogen in the polyether-PU foam surfaces and indicating that PU surface has undergone some degradation (Jansen et al., 1991). However, other OTUs that were strongly associated with polyether-PU-fed microbiome that whether with PU, PE or PS-degrading capacity has not been reported in updated papers. We speculated, therefore, that these genera could play a role in the polyether-PU foam degradation which should be comprehensively investigated in further research. Since the structure of PU was significantly different from PE and PS thus should be metabolized differently, it is likely that the depolymerization and biodegradation of these polymers involve different microbes, enzymes, and metabolic pathways, resulting in different depolymerizing patterns and the distinct roles of microbes observed in this study.

In addition, changes in the gut microbial community may be affected by trace amounts of chemical vesicants, flame retardants or other additives present in most commercially available polyether-PU foam products. Therefore, whether these trace chemical additives may affect the microbial community and their influence on individual genus or species necessitate further research.

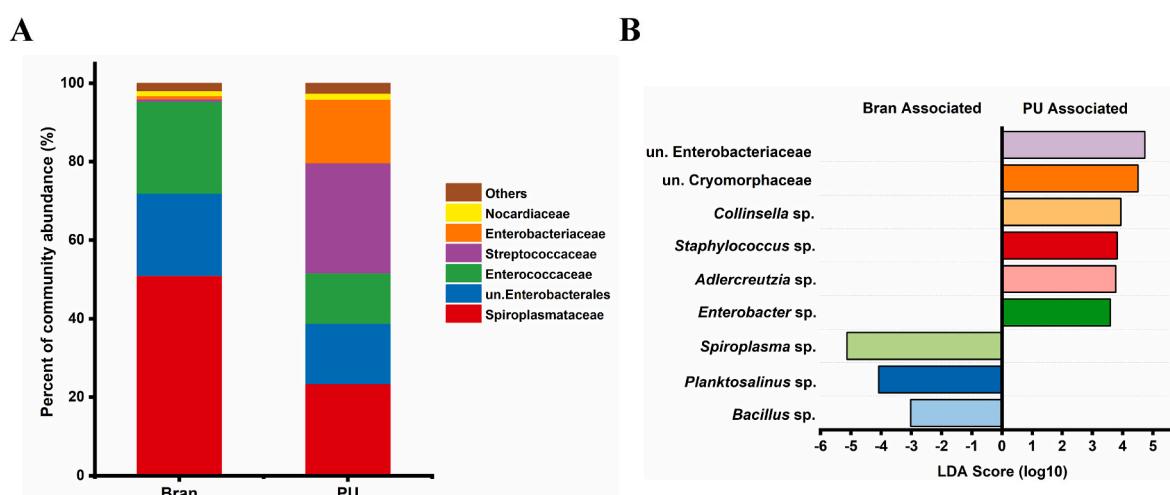


Fig. 4. Changes of gut microbial community of *T. molitor* between two experimental diets (bran fed versus polyether-PU foam fed). A: Relative microorganism abundances at family level. B: Differential abundance Lefse analysis of gut microorganisms at genus level.

4. Conclusions

This is the first study to demonstrate that polyether-PU foam depolymerized after being digested by yellow mealworms (*T. molitor*), as evidenced by weight loss determination, SEM, FTIR, TGA and XPS analyses. After feeding on polyether-PU foam, changes in the structure and populations of the gut microbial community revealed key families or genera closely related to polyether-PU foam degradation. More research is required to understand the synergistic actions of gut microorganisms and larvae digestive enzymes in the degradation of polyether-PU in order to elucidate the degradation mechanism. This could contribute to the development of an alternative plastic disposal strategy for mixed plastics containing PU using mealworms.

Author contributions statement

Jiawei Liu: Conceptualization, Formal analysis, Writing – original draft. Jingyuan Liu: Methodology, Formal analysis. Bin Xu: Methodology, Investigation. Anming Xu: Data curation, Investigation. Shixiang Cao: Investigation. Ren Wei: Conceptualization, Methodology, Formal analysis, Writing – review & editing. Jie Zhou: Conceptualization, Methodology, Supervision, Writing – review & editing. Min Jiang: Writing – review & editing, Project administration, Funding acquisition, Supervision. Weiliang Dong: Conceptualization, Methodology, Investigation, Validation, Supervision, Funding acquisition, Project administration, 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.

Acknowledgements

This work was supported by the National Key R&D Program of China (2021YFC2103600), the National Natural Science Foundation of China (31961133017, 21978129), Natural Science Foundation of Jiangsu Province of China for Excellent Young Scholars (BK20211591). The authors gratefully acknowledge the financial support provided by the MIX-UP (“MIXed plastics biodegradation and UPCycling using microbial communities”) research project based on NSFC and EU H2020 collaboration. In Europe, MIX-UP has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 870294.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2022.135263>.

Abbreviations

FTIR	fourier transform infrared spectroscopy
GPC	gel permeation chromatography
OTU	operational taxonomic unit
PU	Polyurethane
PE	Polyethylene
PS	Polystyrene
PP	Polypropylene
PVC	Polyvinyl chloride
PCR	Polymerase chain reaction
PCoA	Principal coordinate analysis
SEM	Scanning electron microscope
TGA	Thermogravimetric analysis
TDI	Toluene 2,4-diisocyanate

THF	tetrahydrofuran
XPS	X-ray photoelectron spectroscopy

References

- Akutsu, Y., Nakajima-Kambe, T., Nomura, N., Nakahara, T., 1998. Purification and properties of a polyester polyurethane-degrading enzyme from *Comamonas acidovorans* TB-35. *Appl. Environ. Microbiol.* 64, 62–67.
- Alvarez-Barragan, J., Dominguez-Malfavon, L., Vargas-Suarez, M., Gonzalez-Hernandez, R., Aguilar-Osorio, G., Loza-Taveras, H., 2016. Biodegradative activities of selected environmental fungi on a polyester polyurethane varnish and polyether polyurethane foams. *Appl. Environ. Microbiol.* 82, 5225–5235.
- 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, 6526–6533.
- Brandon, A.M., Garcia, A.M., Khlystov, N.A., Wu, W.M., Criddle, C.S., 2021. Enhanced bioavailability and microbial biodegradation of polystyrene in an enrichment derived from the gut microbiome of *Tenebrio molitor* (mealworm larvae). *Environ. Sci. Technol.* 55 (3), 2027–2036.
- Brunner, I., Fischer, M., Ruthi, J., Stierli, B., Frey, B., 2018. Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics. *PLoS One* 13, e0202047.
- Bieganowski, A., 2021. Biodegradation of different types of plastics by *Tenebrio molitor* insect. *Polymers* 13, 3508.
- Borreman, A., Smets, R., van Campenhout, L., 2020. Fermentation versus meat preservatives to extend the shelf life of mealworm (*Tenebrio molitor*) paste for feed and food applications. *Front. Microbiol.* 11, 1510.
- Christenson, E.M., Patel, S., Anderson, J.M., Hiltner, A., 2006. Enzymatic degradation of poly(ether urethane) and poly(carbonate urethane) by cholesterol esterase. *Biomaterials* 27, 3920–3926.
- Cornille, A., Auvergne, R., Figovsky, O., Boutevin, B., Caillol, S., 2016. A perspective approach to sustainable routes for non-Isocyanate polyurethanes. *Eur. Polym. J.* 87, 535–552.
- Cregut, M., Bedas, M., Durand, M.J., Thouand, G., 2013. New insights into polyurethane biodegradation and realistic prospects for the development of a sustainable waste recycling process. *Biotechnol. Adv.* 31, 1634–1647.
- Deng, Y., Jiang, X., Zhao, H., Yang, S., Gao, J., Wu, Y., Diao, Q., Hou, C., 2021. Microplastic polystyrene ingestion promotes the susceptibility of honeybee to viral infection. *Environ. Sci. Technol.* 55, 11680–11692.
- Guo, B., Yin, J., Hao, W., Jiao, M., 2019. Polyurethane foam induces epigenetic modification of mitochondrial DNA during different metamorphic stages of *Tenebrio molitor*. *Ecotoxicol. Environ. Saf.* 183, 109461.
- Gómez, E.F., Luo, X., Li, C., Michel, F.C., Li, Y., 2014. Biodegradability of crude glycerol-based polyurethane foams during composting, anaerobic digestion and soil incubation. *Polym. Degrad. Stabil.* 102, 195–203.
- Jansen, B., Schumacher-Perdreade, F., Peters, G., Pulverer, G., 1991. Evidence for degradation of synthetic polyurethanes by *Staphylococcus epidermidis*. *Zentralbl. Bakteriol.* 276 (1), 36–45.
- Khan, S., Nadir, S., Shah, Z.U., Shah, A.A., Karunaratna, S.C., Xu, J., Khan, A., Munir, S., Hasan, F., 2017. Biodegradation of polyester polyurethane by *Aspergillus tubingensis*. *Environ. Pollut.* 225, 469–480.
- Kim, H.R., Lee, H.M., Yu, H.C., Jeon, E., Lee, S., Li, J., Kim, D.H., 2020. Biodegradation of polystyrene by *Pseudomonas* sp. isolated from the gut of superworms (larvae of *Zophobas atratus*). *Environ. Sci. Technol.* 54 (11), 6987–6996.
- Lee, S.H., Teramoto, Y., Shiraishi, N., 2002. Biodegradable polyurethane foam from liquefied waste paper and its thermal stability, biodegradability, and genotoxicity. *J. Appl. Polym. Sci.* 83, 1482–1489.
- Liu, F., Hao, Y., Wang, Z., Shi, H., Han, E., Ke, W., 2010. Flaking and degradation of polyurethane coatings after 2 years of outdoor exposure in Lhasa. *Chin. Sci. Bull.* 55, 650–655.
- Liu, J., He, J., Xue, R., Xu, B., Qian, X., Xin, F., Blank, L.M., Zhou, J., Wei, R., Dong, W., Jiang, M., 2021. Biodegradation and up-cycling of polyurethanes: progress, challenges, and prospects. *Biotechnol. Adv.* 48, 107730.
- Liu, J., Li, Z., Zhang, L., Hou, J., Lu, Z., Zhang, P., Wang, B., Jin, N., 2019. Degradation behavior and mechanism of polyurethane coating for aerospace application under atmospheric conditions in South China Sea. *Prog. Org. Coating* 136, 105310.
- Lou, Y., Ekaterina, P., Yang, S.S., Lu, B., Liu, B., Ren, N., Corvini, P.F.X., Xing, D., 2020. 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. *Environ. Sci. Technol.* 54 (5), 2821–2831.
- Lou, Y., Li, Y., Lu, B., Liu, Q., Yang, S.S., Liu, B., Ren, N., Wu, W.M., Xing, D., 2021. Response of the yellow mealworm (*Tenebrio molitor*) gut microbiome to diet shifts during polystyrene and polyethylene biodegradation. *J. Hazard Mater.* 416, 126222.
- Magnin, A., Pollet, E., Phalip, V., Averous, L., 2020. Evaluation of biological degradation of polyurethanes. *Biotechnol. Adv.* 39, 107457.
- Mathur, G., Prasad, R., 2012. Degradation of polyurethane by *Aspergillus flavus* (ITCC 6051) isolated from soil. *Appl. Biochem. Biotechnol.* 167, 1595–1602.
- Matsumiya, Y., Murata, N., Tanabe, E., Kubota, K., Kubo, M., 2010. Isolation and characterization of an ether-type polyurethane-degrading microorganism and analysis of degradation mechanism by *Alternaria* sp. *J. Appl. Microbiol.* 108, 1946–1953.
- Moghim, T.B., Abel, M.L., Watts, J.F., 2017. A novel approach to the assessment of aerospace coatings degradation: the HyperTest. *Prog. Org. Coating* 104, 223–231.

- Osman, M., Satti, S.M., Luqman, A., Hasan, F., Shah, Z., Shah, A.A., 2017. Degradation of polyester polyurethane by *Aspergillus* sp. Strain S45 isolated from soil. *J. Polym. Environ.* 26, 301–310.
- Peng, B.Y., 2019. Biodegradation of polystyrene by dark (*Tenebrio obscurus*) and yellow (*Tenebrio molitor*) mealworms (Coleoptera: Tenebrionidae). *Environ. Sci. Technol.* 53 (9), 5256–5265.
- Peng, B.Y., Chen, Z., Chen, J., Yu, H., Zhang, Y., 2020. Biodegradation of polyvinyl chloride (PVC) in *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae. *Environ. Int.* 145, 106106.
- Peng, B.Y., Chen, Z., Chen, J., Yu, H., Zhou, X., Criddle, C., Wu, W.M., Zhang, Y., 2021. Biodegradation of polylactic acid by yellow mealworms (larvae of *Tenebrio molitor* via resource recovery: a sustainable approach for waste management. *J. Hazard Mater.* 416, 125803.
- Riudavets, J., Salas, I., Pons, M., 2007. Damage characteristics produced by insect pests in packaging film. *J. Stored Prod. Res.* 43, 564–570.
- Russell, J.R., Huang, J., Anand, P., Kucera, K., Sandoval, A.G., Dantzler, K.W., Hickman, D., Jee, J., Kimovec, F.M., Koppestein, D., Marks, D.H., Mittermiller, P.A., Nunez, S.J., Santiago, M., Townes, M.A., Vishnevetsky, M., Williams, N.E., Vargas, M.P., Boulanger, L.A., Bascom-Slack, C., Strobel, S.A., 2011. Biodegradation of polyester polyurethane by endophytic fungi. *Appl. Environ. Microbiol.* 77, 6076–6084.
- Ren, L., Men, L., Zhang, Z., Guan, F., Tian, J., Wang, B., Wang, J., Zhang, Y., Zhang, W., 2019. Biodegradation of polyethylene by *Enterobacter* sp. D1 from the guts of wax moth *Galleria mellonella*. *Int. J. Environ. Res. Publ. Health* 16 (11), 1941.
- Shah, Z., Hasan, F., Krumholz, L., Aktas, D.F., Shah, A.A., 2013a. Degradation of polyester polyurethane by newly isolated *Pseudomonas aeruginosa* strain MZA-85 and analysis of degradation products by GC-MS. *Int. Biodeterior. Biodegrad.* 77, 114–122.
- Shah, Z., Krumholz, L., Aktas, D.F., Hasan, F., Khattak, M., Shah, A.A., 2013b. Degradation of polyester polyurethane by a newly isolated soil bacterium, *Bacillus subtilis* strain MZA-75. *Biodegradation* 24, 865–877.
- Sklenicková, K., Abbrent, S., Halecký, M., Kočí, V., Benes, H., 2020. Biodegradability and ecotoxicity of polyurethane foams: a review. *Crit. Rev. Environ. Sci. Technol.* 1, 1–46.
- Song, Y., Qiu, R., Hu, J., Li, X., Zhang, X., Chen, Y., Wu, W.M., He, D., 2020. Biodegradation and disintegration of expanded polystyrene by land snails *Achatina fulica*. *Sci. Total Environ.* 746, 141289.
- Tang, Z.L., Kuo, T.A., Liu, H.H., 2017. The study of the microbes degraded polystyrene. *Adv. Technol. Innovation.* 2, 13–17.
- Tsintzou, G.P., Antonakou, E.V., Achilias, D.S., 2012. Environmentally friendly chemical recycling of poly(bisphenol-A carbonate) through phase transfer-catalysed alkaline hydrolysis under microwave irradiation. *J. Hazard Mater.* 241–242, 137–145.
- Wei, R., Tiso, T., Bertling, J., O'Connor, K., Blank, L.M., Bornscheuer, U.T., 2020. Possibilities and limitations of biotechnological plastic degradation and recycling. *Nat. Catal.* 3, 867–871.
- Wang, Z., Xin, X., Shi, X., Zhang, Y., 2020. A polystyrene-degrading *Acinetobacter* bacterium isolated from the larvae of *Tribolium castaneum*. *Sci. Total Environ.* 726, 138564.
- Wang, S., Shi, W., Huang, Z., Zhou, N., Xie, Y., Tang, Y., Hu, F., Liu, G., Zheng, H., 2022. Complete digestion/biodegradation of polystyrene microplastics by greater wax moth (*Galleria mellonella*) larvae: direct *in vivo* evidence, gut microbiota independence, and potential metabolic pathways. *J. Hazard Mater.* 423, 127213.
- 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., Andrew 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., 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., Ning, D., Phillips, D.H., Peng, B.Y., Wang, H., Cai, S.Y., Li, P., Cai, W.W., Ding, L.Y., Yang, J., Zheng, M., Ren, J., Zhang, Y.L., Gao, J., Xing, D., Ren, N.Q., Waymouth, R.M., Zhou, J., Tao, H.C., Picard, C.J., Benbow, M.E., Criddle, C.S., 2018b. Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of *Tenebrio molitor Linnaeus* (Coleoptera: Tenebrionidae). *Chemosphere* 212, 262–271.
- Yang, S.S., Ding, M.Q., He, L., Zhang, C.H., Li, Q.X., Xing, D.F., Cao, G.L., Zhao, L., Ding, J., Ren, N.Q., Wu, W.M., 2021a. Biodegradation of polypropylene by yellow mealworms (*Tenebrio molitor*) and superworms (*Zophobas atratus*) via gut-microbe-dependent depolymerization. *Sci. Total Environ.* 756, 144087.
- Yang, L., Gao, J., Liu, Y., Zhuang, G., Peng, X., Wu, W., Zhuang, X., 2021b. 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. *Chemosphere* 262, 127818.
- Yang, Y., Yang, J., Wu, W.M., Zhao, J., Song, Y., Gao, L., Yang, R., 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, Y., Gao, L., Yang, R., Jiang, L., 2015b. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 2. role of gut microorganisms. *Environ. Sci. Technol.* 49, 12087–12093.
- Yue, W., Yin, C.F., Sun, L., Zhang, J., Xu, Y., Zhou, N.Y., 2021. Biodegradation of bisphenol-A polycarbonate plastic by *Pseudoxanthomonas* sp. strain NyZ600. *J. Hazard Mater.* 416, 125775.
- Yang, X.F., Vang, C., Talmor, D.E., 2001. Weathering degradation of a polyurethane coating. *Polym. Degrad. Stabil.* 74 (2), 341–351.
- Yuan, Y., Ma, C., Shi, Y., Song, L., Hu, Y., Hu, W., 2018. Highly-efficient reinforcement and flame retardancy of rigid polyurethane foam with phosphorus-containing additive and nitrogen-containing compound. *Mater. Chem. Phys.* 211, 42–53.
- Zhang, H., Pang, H., Zhang, L., Chen, X., Liao, B., 2012. Biodegradability of polyurethane foam from liquefied wood based polyols. *J. Polym. Environ.* 21, 329–334.
- Zhang, J., Gao, D., Li, Q., Zhao, Y., Li, L., Lin, H., Bi, Q., Zhao, Y., 2020. Biodegradation of polyethylene microplastic particles by the fungus *Aspergillus flavus* from the guts of wax moth *Galleria mellonella*. *Sci. Total Environ.* 704, 135931.
- Zhu, P., Pan, X., Li, X., Liu, X., Liu, Q., Zhou, J., Dai, X., Qian, G., 2021. Biodegradation of plastics from waste electrical and electronic equipment by greater wax moth larvae (*Galleria mellonella*). *J. Clean. Prod.* 310 (5), 127346.
- Zhu, P., Shen, Y., Li, X., Liu, X., Qian, G., Zhou, J., 2022. Feeding preference of insect larvae to waste electrical and electronic equipment plastics. *Sci. Total Environ.* 807, 151037.