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RESEARCH ARTICLE

# Biodegradation of waste refrigerator polyurethane by mealworms

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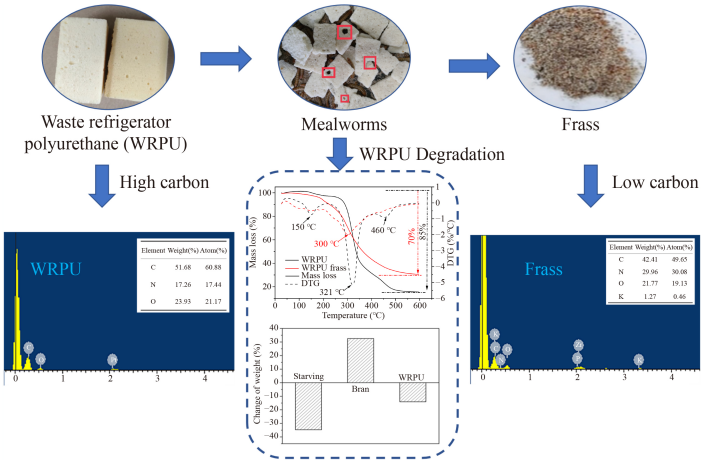
## HIGHLIGHTS

- Waste refrigerator polyurethane (WRPU) was ingested and biodegraded by mealworms.
- The carbon in WRPU-based frass was lower than that in WRPU.
- Urethane groups in WRPU were broken down after ingestion by mealworms.
- Thermal stability of WRPU-based frass were deteriorated compared to that of WRPU.
- Gut microbiomes of mealworms fed using WRPU were distinct from that fed using bran.

## GRAPHIC ABSTRACT

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## ABSTRACT

Refrigerator insulation replacement results in discarding a large amount of waste refrigerator polyurethane (WRPU). Insect larvae like mealworms have been used to biodegrade pristine plastics. However, knowledge about mealworms degrading WRPU is scarce. This study presents an in-depth investigation of the degradation of WRPU by mealworms using the micro-morphology, composition, and functional groups of WRPU and the egested frass characteristics. It was found that the WRPU debris in frass was scoured, implying that WRPU was ingested and degraded by mealworms. The carbon content of WRPU-based frass was lower than that of WRPU, indicating that mealworms utilized WRPU as a carbon source. The urethane groups in WRPU were broken, and benzene rings' C=C and C–H bonds in the isocyanate disappeared after being ingested by mealworms. Thermal gravimetric-differential thermal gravimetry analysis showed that the weight loss temperature of WRPU-based frass was 300 °C lower than that of WRPU, indicating that the thermal stability of WRPU deteriorated after being ingested. The carbon balance analysis confirmed that carbon in the ingested WRPU released as $CO_2$ increased from 18.84 % to 29.80 %, suggesting that WRPU was partially mineralized. The carbon in the mealworm biomass ingesting WRPU decreased. The possible reason is that WRPU does not supply sufficient nutrients for mealworm growth, and the impurities and odor present in WRPU affect the appetite of the mealworms. The microbial community analysis indicated that WRPU exerts a considerable effect on the gut microorganism of mealworms. These findings confirm that mealworms degrade WRPU.



## 1 Introduction

The renewal cycle of refrigerators is expected to shorten

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significantly over time (Kang et al., 2016) due to the continuous upgrading of associated technology. In China, the production of household refrigerators has increased by 87.5 %, from 48 million units in 2008 to 90 million units in 2020 (NBSC, 2009, 2021), resulting in a large number of discarded refrigerators. Rigid polyurethane (RPU) foam accounts for about 10 % of the total weight of the refrigerator, and approximately 93200 t of waste refrigerator polyurethane (WRPU) are generated every year in China alone (Terakado et al., 2014; Kang et al., 2016). Currently, most waste refrigerator polyurethane (WRPU) is disposed of in landfills worldwide. It occupies about $2.33 \times 10^6$–$3.11 \times 10^6$ $m^3$ of land yearly due to its low density (about 30–40 $kg/m^3$) and inadequate compaction (Tantisattayakul et al., 2018; Gong et al., 2022). The WRPU landfill degradation takes over 100 years because WRPU is a thermosetting polymer with giant-molecule and internally-cross-linked structure (Maitra and Shukla, 2014; Akindoyo et al., 2016; Park et al., 2018; Fesseha and Abebe, 2019; Beran et al., 2020; Liu et al., 2021).

Disposal WRPU approaches, besides landfill disposal, include incineration and physical, chemical, and biological methods (Yang et al., 2012; Badola et al., 2022). Unfortunately, these methods generate many undesirable gases such as $NO_x$, HCl, and $CHCl_3$, which cause secondary environmental pollution (Tantisattayakul et al., 2018; Charitopoulou et al., 2021). Recycling generally results in "down-cycling" to less valuable products (Liu et al., 2021) or degradation by-products that are difficult to separate and purify for industrial applications.

Compared to the above methods, WRPU biodegradation is a unique approach due to the environmentally friendly outcomes. Past researchers have shown that many organisms including bacteria and fungi, can degrade plastics (Peng et al., 2014; Magnin et al., 2020; Liu et al., 2021; Shilpa et al., 2022). *Pseudomonas putida* isolated from farm soils and activated sludge was found to have a strong ability to degrade polyurethane (PU) at the optimum temperature of 25 °C and pH of 8.4. The results showed the reduction of ester functional groups and the emergence of the amide group (Peng et al., 2014). Filamentous fungi are relatively more efficient in degrading PU than bacteria due to their outstanding enzymatic arsenal and abiotic features linked to filament formation (Magnin et al., 2019). A soil fungus (*Aspergillus flavus G10*), isolated and reported in past research, was found to biodegrade polyester PU as the sole carbon source (Khan et al., 2020). During the biodegradation process, methylene di-aniline formation was detected due to fungal hydrolysis of urethane bonds in the polymer backbone. Thermal analysis of polyester thermoplastic PUs also confirmed biological hydrolysis of the ester bonds (Magnin et al., 2020). However, microbial degradation of WRPU is often slow, needs stringent conditions for microbial cultivation and requires complex pretreatment (Brandon et al., 2018).

In recent years, insect larvae such as mealworms have gained increasing attention related to plastics' biodegradation (Yang et al., 2018; 2019a; 2019b; Sun et al., 2022). Studies have shown that plastics are rapidly degraded and mineralized in the insect larval gut, which is regarded as a naturally formed "microbial petri dish" (Peng et al., 2019; Yang and Wu, 2020). Yang et al. (2015a; 2015b) found that about 1.3 g of polystyrene (PS) was consumed within 30 d when fed to 500 mealworms. Frass carbon analysis showed that almost half of the carbon in ingested PS was converted to $CO_2$ in the mealworm gut (Yang et al., 2015a). Brandon et al. (2018) found that 120 mealworms could degrade 0.87 g polyethylene (PE) in 32 d. The degradation of polyvinyl chloride (PVC), polypropylene (PP), and polylactic acid (PLA) by mealworms has also been reported and proven using FT-IR, Liquid-state $^1H$ nuclear magnetic resonance ($^1H$ NMR), thermogravimetric analysis (TGA), and gel permeation chromatography (GPC) characterization. Microbial community analysis has shown that PVC, PP, and PLA can be biodegraded via gut microbe-dependent depolymerization with diversified microbiomes. The influence on the growth and development of mealworms of PLA-bran ratio in a mixture co-diet has also been explored in past research (Peng et al., 2020; 2021; Yang et al., 2021b).

Currently, PU biodegradation by mealworms is rarely reported. Guo et al. (2019) investigated the changes in the DNA methylation pattern of *Tenebrio molitor* (mealworm) mitochondria genome at different development stages using PU as the sole diet. Bulak et al. (2021) demonstrated the ability of *Tenebrio molitor* to biodegrade different plastic waste such as PS (styrofoam), PU1 (soft PU in kitchen sponge), PU2 (rigid PU in commercial thermal insulation foam), and PE. The mass loss rates were 46.5 %, 41.0 %, 53.2 %, and 69.7 %, respectively, and higher than most of the data reported in the research literature. However, the specific consumption rate for each plastic was low because of the appearance of pupae and cannibalistic behavior. Additionally, elements such as Br, Cl, and P in the fillers and flame retardants of the waste plastics also adversely influenced the consumption rates of mealworms. Studies based on scanning electron microscopy (SEM) analysis illustrated that plastics transform from smooth surfaces to corrugating and pitting after being ingested by mealworms (Bulak et al., 2021). This confirmed that the plastics could be biodegraded by the action of microorganisms and enzymes secreted by mealworms. However, the degradation mechanism of WRPU by mealworms is not yet fully understood.

Liu et al. (2021) reviewed the degradation of PU by microbes and enzymes. They noted that bacteria and fungi degrade polyester-based PU and low-molecular-

weight urethane-based PU into organic acids, organic alcohols, or diamines, by the cleavage of ester or urethane bonds according to the complexity of the PU structure. Generally, PU-degrading enzymes are mostly polyester hydrolases that can hydrolyze ester bonds in the soft segments of polyester-based PU. However, only a few microorganism species were reported to degrade polyether PU (Liu et al., 2021).

WRPU is a polyether PU and can contain metals, freon, or other pollutants, which could slow and complicate the degradation processes (Dement'ev et al., 1991; Zhao et al., 2011; Yazici et al., 2014; Magnin et al., 2020; Zhang et al., 2020b). A previous study undertaken by the research team which investigated the biodegradation of WRPU using *Galleria mellonella* (Zhu et al., 2021) found the consumption of WRPU by the larvae to be lower compared to the pristine RPU. It is hypothesized that freon, heavy metals, and other impurities present in WRPU reduce the larvae's appetite for ingesting WRPU as it adversely affects gut microbes (Kwadha et al., 2019). The mechanism attributed to the biodegradation of WRPU and pristine RPU by *G. mellonella* was also explored by Zhu et al. (2021). It was found that WRPU is partially biodegraded mainly due to the breakdown of the urethane bond in its hard segment, which is consistent with the finding literature on the biodegradation of PU (Peng et al., 2014; Magnin et al., 2020). Consequently, further investigations on whether mealworms have similar performance and biodegradation mechanisms concerning WRPU are important to be carried out.

In this study, the mealworms were used to gnaw WRPU. The WRPU biodegradation characteristics using mealworms were investigated by comparing the changes the feedstock and frass chemical compositions utilizing various techniques. The WRPU feedstock and egested frass were analyzed using X-ray fluorescence spectroscopy (XRF), Fourier transform infrared spectroscopy (FT-IR), thermal gravimetric-differential thermal gravimetry (TG-DTG), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS), and carbon mass balance.

## 2 Materials and methods

### 2.1 Materials

The mealworms (Fig. S1(a) in the Supporting Information) purchased from a flower and bird market in Shanghai, China, were about 20–25 mm long. All mealworms were starved for 72 h to empty their guts before the experiment. The WRPU (Fig. S1(b) in the Supporting Information) was obtained from a waste refrigerator in a recycling company in Shanghai, China. Initially, WRPU was soaked in 75 % alcohol (v/v) for 30 s, rinsed with sterile water 2–3 times to remove microorganisms and impurities on the surface, and dried under sterile conditions (Zhang et al., 2020a). All chemicals used were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

### 2.2 Mealworm gnawing of WRPU

Each mealworm group consisted of 100 individuals, fed with 2.0 g WRPU as the only carbon source, and cultured for 33 d in a petri dish with a diameter of 15 cm at 25 °C ± 1 °C, 80 % ± 2 % humidity, and 16:8 (light/dark) photoperiod (Yang et al., 2015a). The WRPU mass loss and larvae survival rate (SR) were measured every three days. The residues of dead larvae were immediately removed to reduce the impact of fratricide (Peng et al., 2020). Unfed larvae and larvae fed with bran were used as controls. The bran-fed larvae were fed with 3 g of bran every three days to ensure enough food for the mealworms (Yang et al., 2021c). All tests were carried out in triplicate (Yang et al., 2021b). ANOVA was undertaken using SPSS 17.0 (SPSS for Windows, USA) to investigate the statistical significance of the differences among the groups.

At the end of the experiment, the larvae were cleaned with compressed air and transferred to new containers for frass collection. The frass was collected within 24 h under starvation conditions to avoid the influence of any nutrients and then stored at −20 °C for further analysis.

### 2.3 Carbon mass balance of WRPU

The carbon mass balance was conducted according to the procedure described by Yang et al. (2015a). Sixty mealworms from each group were randomly collected and placed in an incubator, as shown in Fig. S2(a) in the Supporting Information. A total of 12 incubators were set up for 20 d. During the incubation, the ingested WRPU mass, dry weight of the mealworms and egested frass, and total $CO_2$ generation were determined every five days. A lifeless control was also used to ensure that no $CO_2$ was generated ($n$ = 3) during the process (Fig. S2(b) in the Supporting Information). The carbon content of the residual WRPU, dried mealworms, and frass samples was measured using an elemental analyzer (Vario EL, 2400 II, PerkinElmer, Llantrisant, CF728YW, UK). The carbon content in the form of $CO_2$ from the conversion of ingested WRPU and the mealworm growth were estimated according to Yang et al. (2015a). A detailed description of the method and calculation formula for determining the carbon mass balance is given in M1 of the Supporting Information.

### 2.4 Characterization of the WRPU and the egested frass

Micro-morphologies of WRPU, egested frass, and bran samples were determined using SEM-EDS equipment

(JSM-6700F, Royal Dutch Philips Electronics Ltd, Eindhoven, Netherlands) (Peng et al., 2019). XRF-1800 spectroscopy (Shimadzu Limited, Kyoto, Japan) was employed to investigate the changes in macro elements such as C, N, O, P, and S of the samples. FT-IR (NICOLET 6700 FT-IR Spectrometer, Thermo Fisher Scientific Corporation, MA, USA) was used to characterize the major functional groups of the WRPU, bran, and the egested frass. The spectra of all samples were obtained in the region of 400–4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ and a scanning step of 1 $cm^{-1}$ (Kim et al., 2020; Zhu et al., 2022). For XRF and FT-IR analyses, the sample preparation method was similar to that reported previously (Brandon et al., 2018; Zhu et al., 2022). The samples were dried, ground, and sieved using a 200 mesh. The thermal analysis was undertaken using TG-DTG equipment (STA 449C, NETZSCH, Selb, Germany), and 15 mg samples were put into a Pt-Rh crucible and heated at a rate of 10 °C/min from 30–800 °C under nitrogen atmosphere.

### 2.5 Gut microbial community analysis

At the end of the 33-d experiment, 20 mealworms were randomly collected from each group (WRPU, bran, and unfed) and washed with 75 % ethanol and sterile water three times to remove microorganisms and impurities on the larvae surface. The guts of the mealworms were accessed using sterile scissors, dissecting needles, tweezers, and other anatomical tools in a sterile environment (Zhang et al., 2020a). The DNA extraction and PCR amplification methods were similar to that reported previously (Peng et al., 2019; Yang et al., 2021a). Sequencing reads were filtered and taxonomically assigned using QIIME 2 (v2020.2). Finally, the free online Majorbio I-Sanger Cloud Platform (Shanghai, China) was used to run the microbial community composition, alpha diversity analysis, and principal coordinate analysis (PCoA). Specific details of DNA extraction, PCR amplification, and other methods are available in M2 in the Supporting Information.

## 3 Results and discussion

### 3.1 Behavior of mealworm gnawing of WRPU

Some cavities became evident in the WRPU at the end of the experiment, as illustrated in Fig. 1(a). About 0.6 g of WRPU were consumed in 33 d, and the consumption rate was 181.82 μg WRPU/(day larvae), indicating that mealworms can ingest the WRPU (Fig. 1(b)). The consumption rate was higher than that reported in the literature, which was 168.7 μg PU1 (soft PU)/(day larvae) and 158.9 μg PU2 (rigid PU)/(day larvae) on the Day 10 of the experiment (Bulak et al., 2021). This is attributed to the different properties of plastics and experimental conditions.

The SRs of the bran-fed mealworms was higher than that of the WRPU-fed mealworms (Fig. 1(b)). However, the SRs of WRPU-fed mealworms also reached 80 % after 33 d, suggesting that the degradation of WRPU provides energy and carbon for the mealworms, but does not enhance metabolic activities and growth (Peng et al., 2021). Past literature reported that the SRs of mealworms fed using different plastics were different, with 85.0 %, 86.7 %, 95.6 %, 88.7 %, 80.0 %, and 82.0 % for PS, expanded PS (EPS), PE, PP, PVC, and PLA, respectively (Yang et al., 2015a; 2018; 2021b; 2021c; Peng et al., 2020; 2021). These differences are attributed to the different physicochemical properties of plastics with varying nutrient compositions. For example, low SRs of mealworms fed with PVC could result from the PVC chloride release to the larval guts, negatively affecting the appetite of mealworms.

### 3.2 Analysis of WRPU and egested frass

#### 3.2.1 SEM and XRF analyses

Surface morphologies of the WRPU feedstock and egested frass were investigated using the SEM. The RPU

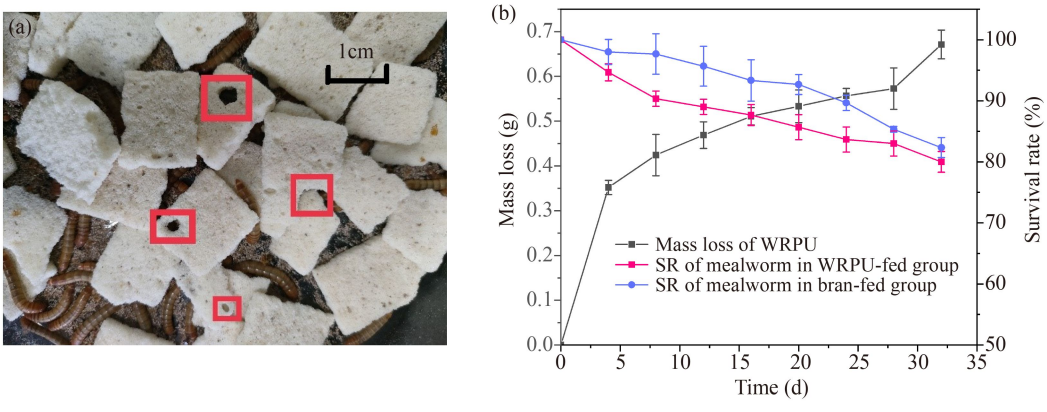


**Fig. 1** (a) An image of mealworms gnawing at WRPU with some damage points marked with a red frame; (b) cumulative consumption of WRPU and survival rate of mealworms fed with bran and WRPU (mean ± SD; $n$=3).

from refrigerator thermal insulation material often represents foam plastic with a honeycomb-like structure. Fig. 2(a) shows that the honeycomb of the WRPU is incomplete and very different from the bran (Fig. 2(b)). This result implies that the structure of WRPU feedstock was damaged due to environmental aging and wearing (Liu et al., 2020; Li et al., 2021). SEM of WRPU was different from that of PU2 (Bulak et al., 2021), as WRPU has different structural composition. As illustrated in Fig. 2(c), the WRPU in the frass was severely scoured. However, the morphology of undigested plastic debris can still be discerned vaguely in the WRPU-based frass compared with the bran-based frass (Fig. 2(d)). These observations indicate that the physical structure of the WRPU was destroyed by mealworms, possibly resulting from the gnawing by mealworms and biodegradation by intestinal microorganisms (Brandon et al., 2018; Lou et al., 2020; Bulak et al., 2021). In addition, the EDS analysis showed that C, N, O, and H were the main elements of WRPU (Oprea, 2010), and carbon content in the WRPU-based frass was about 42.41 wt.%, lower than the WRPU feedstock (51.68 wt.%) (Figs. 2(e) and 2(f)). These results evidence the biodegradation and mineralization of carbon in WRPU by mealworms.

XRF analysis was used further to analyze the overall elements of the WRPU feedstock and egested frass. The contents of macro-elements such as C, N, P, and S in the WRPU feedstock differ from those in the egested frass, as shown in Fig. 3 and Table S1 in the Supporting Information. The carbon content in the WRPU-based frass was about 42.72 % and lower than that of WRPU (55.33 %), corresponding to the results from the SEM-

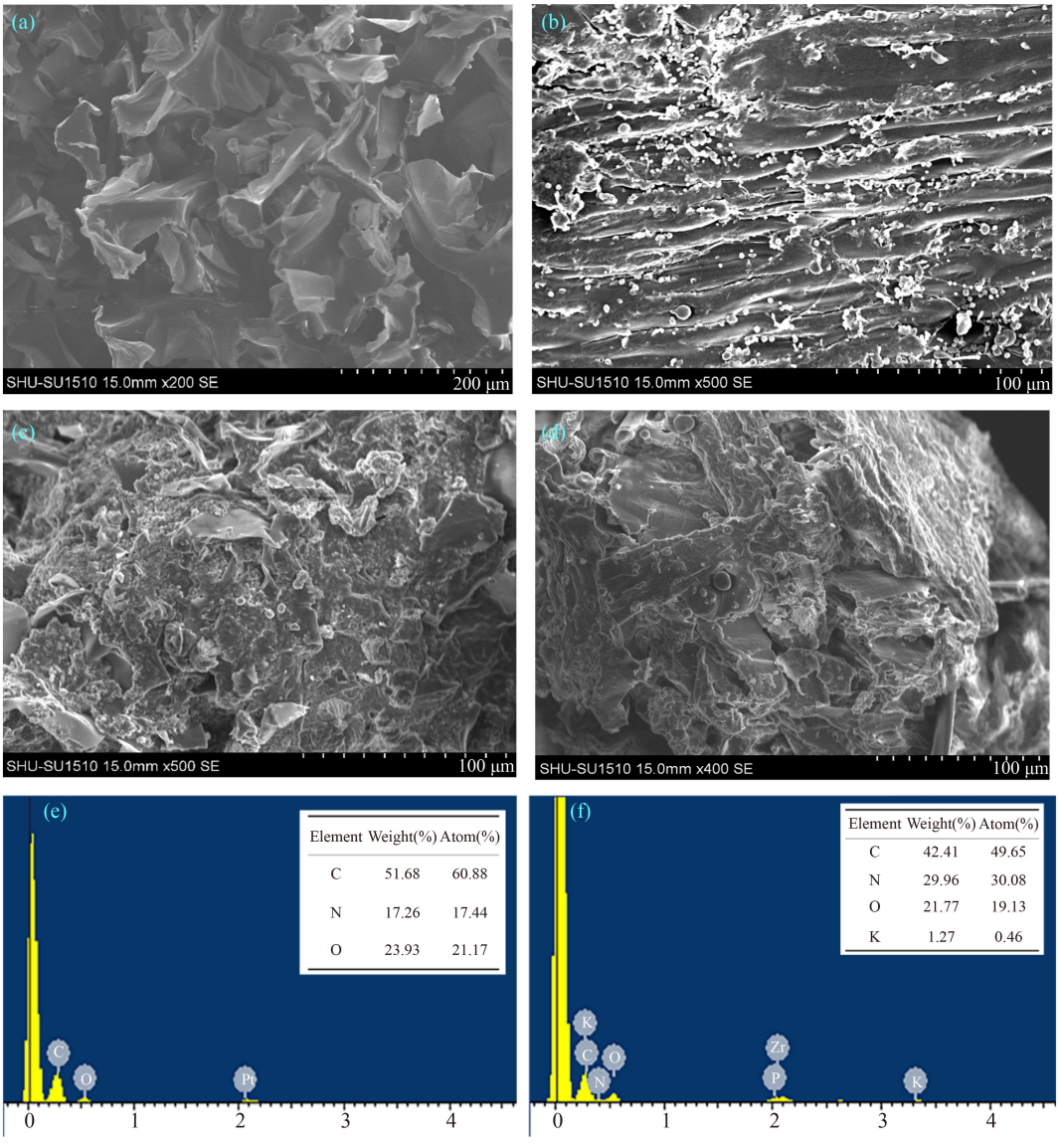


**Fig. 2** SEM-EDS of the WRPU, bran, and their egested frass: (a) SEM of WRPU feedstock; (b) SEM of bran feedstock; (c) SEM of WRPU-based frass; (d) SEM of bran-based frass; (e) EDS of WRPU feedstock; (f) EDS of WRPU-based frass.

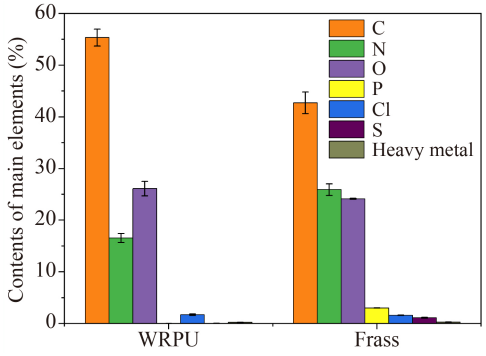


**Fig. 3** Elemental analysis of the WRPU and the egested frass.

EDS (Fig. 2). The presence of Cl in WRPU could be due to the adsorption of freon in the foaming agent (Zhao et al., 2011; Yazici et al., 2014). The Cl in the egested frass could originate from the mealworms as their bodies also contain Cl (Nowak et al., 2016) and the residual WRPU in the frass. The heavy metals are impurities in the WRPU (Dement'ev et al., 1991). Compared with the WRPU feedstock, higher contents of N, P, and S in the WRPU-based frass are ascribed to the metabolism of mealworms using their own biomass, which merits further investigations.

### 3.2.2 FT-IR analysis

The changes in surface functional groups of the WRPU before and after being ingested by mealworms were investigated using FT-IR spectroscopy. As shown in Fig. 4, the FT-IR spectra of WRPU-based frass are very different from the bran-based frass. This result indicated that the mealworms ingest the WRPU, thus causing changes in the WRPU-based frass. WRPU, as a thermosetting polymer material, is mainly synthesized by the reaction between polyols and polyisocyanate (Maitra and Shukla, 2014; Akindoyo et al., 2016; Magnin et al., 2019; Beran et al., 2020), and has a characteristic unit of urethane in its structure (Oprea, 2010; Magnin et al., 2020).

Compared to the WRPU feedstock, the main changes in the spectra of the egested frass were in three regions: 3000–3500, 1500–1750, and 500–900 $cm^{-1}$. The absorption peak of N–H and –OH disappeared at 3293 $cm^{-1}$ and a wider peak occurred at 3282 $cm^{-1}$, implying the possible transformation from hydrophobicity to hydrophilicity (Oprea et al., 2018). The free carbonyl group at 1712 $cm^{-1}$ disappeared, and the hydrogen-bonded carbonyl group at 1639 $cm^{-1}$ was generated. These results indicate the formation of intermolecular or intramolecular hydrogen bonds between N–H and C=O due to the breakdown of the urethane bond. It is hypothesized that the isocyanates, polyether polyols, and other degradation by-products are generated during these conversions (Pellizzi et al., 2014). The disappearance of C=C and C–H peaks from the benzene rings in the isocyanate at 765, 817 and 1600 $cm^{-1}$ suggested that the isocyanate in the WRPU is mineralized to $CO_2$ by the mealworms (Khan et al., 2020; Bulak et al., 2021). The results indicated that all the changes in the WRPU occur in the urethane groups, which is the hard segment of the WRPU and relatively easy to degrade (Oprea, 2010). However, the ether bond of the soft segment of the WRPU at 1070 $cm^{-1}$ had a slight variation after being ingested, as this segment was resistant to biochemical degradation (Khan et al., 2017; Li et al., 2019). These outcomes correspond to the results reported in past research on WRPU biodegradation using *G. mellonella* and microorganisms (Zhu et al., 2021).

### 3.2.3 TG-DTG analysis

Thermal analysis results of the WRPU feedstock and frass are presented in Fig. 5. The analyzed WRPU showed three endothermic valleys at 150 °C, 321 °C and 460 °C for the WRPU. The weight loss of the WRPU at 150 °C was about 1.34 %, mainly resulting from the evaporation of the surface absorbed water and the

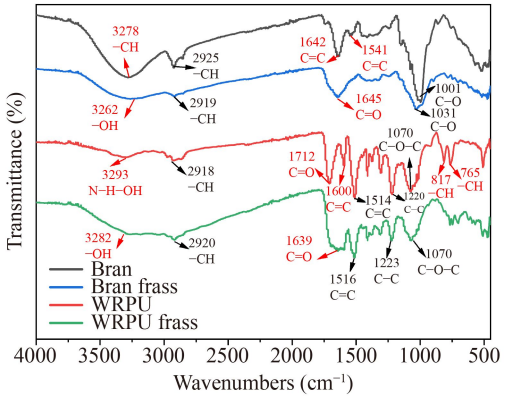


**Fig. 4** FT-IR spectra of the WRPU feedstock and frass, and bran feedstock and frass.

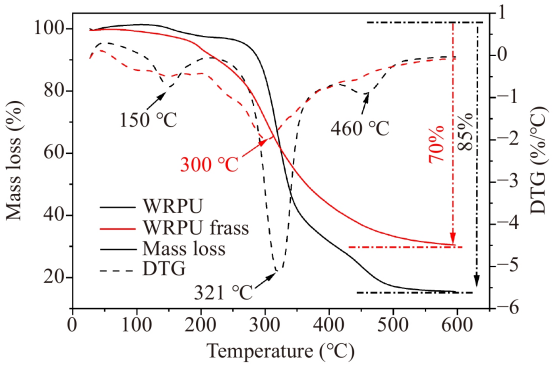


**Fig. 5** TG-DTG analysis of the WRPU feedstock and the egested frass.

decomposition of substances with poor thermal stability (Zhang et al., 2015). The WRPU weight loss at 321 °C was about 25.42 %, ascribed to the decomposition of the hard segment urethane groups. The WRPU weight loss at 460 °C was about 78.51 % due to the decomposition of the soft segment ether groups (Yuan et al., 2018). However, only one apparent endothermic valley at 300 °C was found in the egested frass. The measured weight loss was about 29.77 %, indicating that mealworms incompletely degraded WRPU. The temperature at the weight loss peak for the WRPU-based frass (300 °C) was lower than that for WRPU feedstock (321 °C). Compared with the WRPU feedstock, these results indicate that the thermal stability of the frass deteriorates, which is consistent with the previous findings based on *G. mellonella* (Zhu et al., 2021). According to the results of the FT-IR spectra (Fig. 4), the likely reason is that the microbes in the mealworm intestine attack the hard segment urethane bonds of the WRPU, then break the crosslink bond of the chain, and generate isocyanate and polyols. Isocyanates are unstable and easily transform into by-products with lower thermal stability such as biuret and urethane (Panda et al., 2018).

Meanwhile, the weight loss of the WRPU-based frass after 323 °C was lower than that of WRPU. This result implies that the mass percentage of the soft segment in the WRPU-based frass increased due to mealworm degradation of the hard segment in the WRPU feedstock (Magnin et al., 2020). The soft segment is mainly composed of polyols. The hydroxyl groups in polyols tend to form intermolecular or intramolecular hydrogen bonds, resulting in their high thermal stability. Therefore, more soft segments are found in the WRPU-based frass, compared with the WRPU feedstock. The total thermal weight loss of the WRPU-based frass (70 %) was lower than that of the WRPU feedstock (85 %), indicating that the WRPU-based frass contained fewer decomposable organic compounds than the WRPU feedstock. The above results showed that mealworm ingestion causes a thermal stability deterioration and organic content decrease of the WRPU.

### 3.3 Carbon balance analysis of the WRPU and the egested frass

Carbon mass balance was carried out by determining the carbon content change of the WRPU, mealworm biomass, and egested frass during the incubation process, to further investigate the biodegradation and mineralization of WRPU ingested by mealworms. The carbon content of WRPU decreases, and the conversion to $CO_2$ increases from 18.84 % at 5 d to 29.80 % at 20 d of incubation time, as shown in Table 1 and Fig. 6. These results indicate that WRPU was partially mineralized.

**Table 1** Carbon balance analysis during the ingestion of WRPU by mealworms (mean±SD; $n$=3)

| Incubation time (d) | Item | Initial carbon (mg) | Final carbon (mg) | Varying weight (mg) | Reduction rate of biomass (%) | Conversion rate (%) |
|---|---|---|---|---|---|---|
| 5 | WRPU | 822.5±12.61 | 736.3±22.22 | −86.2 | | |
| | Biomass | 535.8±6.57 | 523.3±32.34 | −12.5 | −2.3 | |
| | $CO_2$ | 0 | 18.6±3.48 | 18.6 | | 18.84 |
| | Frass | 0 | 62.3±1.11 | 62.3 | | 63.12 |
| | Total recovery | | | | | 81.96 |
| 10 | WRPU | 725.4±18.49 | 617.1±20.74 | −108.3 | | |
| | Biomass | 597.2±39.25 | 577.3±41.20 | −19.9 | −3.3 | |
| | $CO_2$ | 0 | 32.6±3.36 | 32.6 | | 25.43 |
| | Frass | 0 | 85.2±5.09 | 85.2 | | 66.46 |
| | Total recovery | | | | | 91.89 |
| 15 | WRPU | 855.6±23.13 | 677.8±14.18 | −177.8 | | |
| | Biomass | 624.5±12.78 | 598.6±71.15 | −25.9 | −4.1 | |
| | $CO_2$ | 0 | 56.1±5.31 | 56.1 | | 27.54 |
| | Frass | 0 | 133.2±7.18 | 133.2 | | 65.39 |
| | Total recovery | | | | | 92.93 |
| 20 | WRPU | 657.9±5.02 | 403.5±23.56 | −254.4 | | |
| | Biomass | 575.6±5.79 | 528.9±54.65 | −46.7 | −8.1 | |
| | $CO_2$ | 0 | 89.7±16.94 | 89.7 | | 29.80 |
| | Frass | 0 | 194.8±21.37 | 194.8 | | 64.70 |
| | Total recovery | | | | | 94.5 |

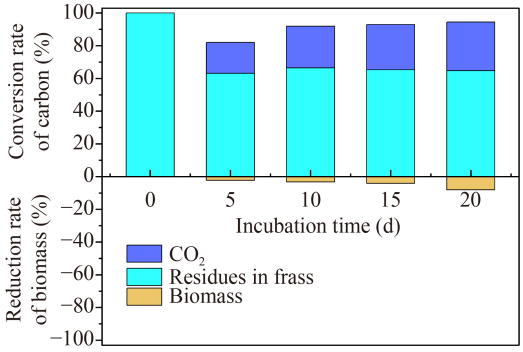


**Fig. 6** Conversion of carbon from the ingested WRPU and biomass to residues in frass and $CO_2$ at 0, 5, 10, 15 and 20 incubation times and reduction rate of carbon in biomass.

Meanwhile, the carbon content of the egested frass showed little change and mealworm biomass tended to decrease with the incubation time. The likely reason is that WRPU does not supply all the required nutrients for mealworm growth, such as protein, phosphorus, vitamins, and minerals. The mealworms need to consume their own biomass to maintain life activities. As shown in Fig. 7, the mealworm biomass for the bran-fed group increased by 32.6 % (dry weight), but the unfed and WRPU-fed groups decreased by 34.7 % and 14 %, respectively, after 30 d.

Similar results were obtained by Bulak et al. (2021). Their results showed that the mass of mealworms in the PS, PU1, PU2, and PE groups decreased by 18.37 %, 28.28 %, 26.26 %, and 24.71%, respectively, after 58 dof feeding waste plastics. The mass of mealworm larvae fed using microplastics such as polyvinyl chloride (PVC) and low density PE (LDPE) was also reported to decrease after incubation for one month (Wu et al., 2019). However, Yang et al. (2015a) reported a 0.2 % increase in the PS-fed mealworm biomass carbon after 16 d. Yang et al. (2021a) also found that the mass of mealworm larvae fed with EPS, PE1 (LDPE foam containing pink color additives), and PE2 (colorless LDFE film without additives) increased by 2.5 % ± 1.0 %, and 8.8 % ± 2.1 %, respectively, after 60 d, and 3.4 % ± 1.6 % after

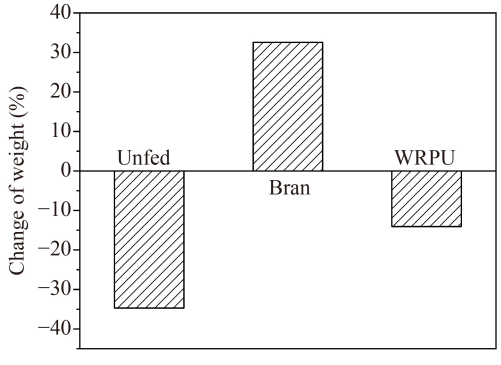


**Fig. 7** Change in the mealworms weight for the unfed, bran-fed and WRPU-fed conditions after 30 d.

40 d. These results are attributed to the release of odor-causing volatiles such as chlorine (Zhao et al., 2011; Yazici et al., 2014) and heavy metals (Fig. 3) from the waste plastics. These components would adversely affect the gut microbes and reduce the larvae's appetite (Kwadha et al., 2019; Demets et al., 2020). In turn, this would lead to a decrease in the ingestion of waste plastics by mealworms (Caravelli et al., 2004; Bulak et al., 2021; Zhu et al., 2022).

### 3.4 Response of the microbial community in the mealworms' gut

Plastics degradation is closely related to the intestinal microbiota. The microbial communities of the mealworms fed with WRPU, bran, and unfed were analyzed using IlluminaMiseq to better elucidate the WRPU biodegradation mechanism. As shown in Fig. 8, the gut microbiome of the unfed mealworms is different from that fed with bran and WRPU. Alpha diversity of the microbial community, as measured by the Shannon index, was not significantly different among the groups (Fig. 8(a)). However, WRPU-fed and bran-fed mealworms formed distinct clusters based on results from PCoA at the ASV level (Fig. 8(b)), suggesting that the composition of the gut microbial community in bran-fed mealworms is different from that of WRPU-fed mealworms.

Gut microorganisms of the mealworms in the three groups mainly consisted of 23 families. As shown in Fig. 8(c), the five dominant families are *Enterobacteriaceae*, *unclassified_o_Enterobacterales*, *Spiroplasmataceae*, *Streptococcaceae* and *Enterococcaceae*. However, the dominant families' relative abundance was different in the groups. The relative abundance of *Spiropasmataceae* was higher in the bran-fed group than in the unfed and WRPU-fed groups. The results were in accordance with the results reported by Brandon et al. (2018) for bran-fed mealworms at 32 d. Yang et al. (2021a)also found that the relative abundance of *Spiroplasma* in the gut microbiome of bran-fed mealworms was higher than larvae fed with PE and PS. Accordingly, it can be hypothesized that *Spiroplasmataceae* plays a more significant role in the biodegradation of bran in the gut of mealworms.

Additionally, the number of bacterial species in the bran group was higher than that in the unfed and WRPU-fed groups due to the abundant nutrients in the gut of bran-fed mealworms. *Enterobacteriaceae* and *Enterococcaceae* in the WRPU-fed group showed relatively higher abundance (31.66 % and 21.51 %, respectively) than to the bran-fed and unfed groups. Luo et al. (2021) also reported on the dominance of *Enterococcus* in the PU-fed superworm gut (Luo et al., 2021). Kay et al. (1991) buried polyester PUR fragments in the soil for 28 d and isolated *Enterobacter Agglomerans B7* from the soil with

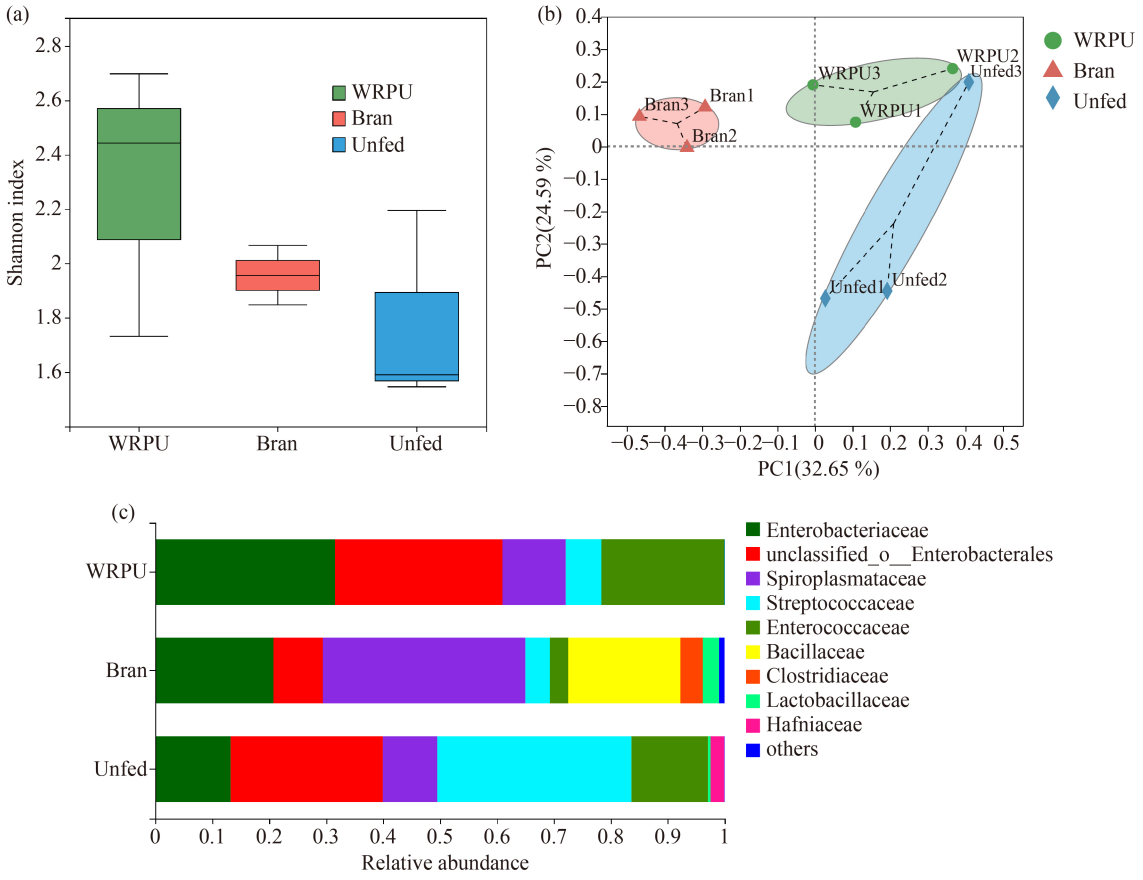


**Fig. 8** Microbial community composition of gut microbiome in WRPU-fed, bran-fed and unfed mealworms: (a) Shannon index; (b) principal coordinate analysis (PCoA); (c) relative abundance of predominant microbe at the family levels.

the capability of degrading PUR. This finding suggested that *Enterobacteriaceae* is closely linked to WRPU biodegradation in the mealworm gut. However, the role of *Enterococcaceae* in the biodegradation of WRPU needs further investigations to confirm this finding conclusively. Overall, the gut microbial composition of mealworms was different in the WRPU-fed and bran-fed groups, implying that the diet exerts a considerable influence on the gut microorganisms in mealworms.

## 4 Conclusions

The experimental data of this study showed that mealworms consumed approximately 0.6 g of WRPU after 33 d of incubation. It was found that the WRPU in the frass was severely scoured and partly mineralized based on SEM-EDS and XRF analyses. The FT-IR analysis results proved the cleavage of the urethane and disappearance of isocyanate groups in the hard segment of WRPU. The ether bond in the soft segment did not show a significant change. Meanwhile, based on TG-DTG analysis, it was found that the weight loss temperature of the egested frass was lower than that of WRPU, implying that the thermal stability of WRPU decreases. During the WRPU ingestion process, carbon released as $CO_2$ increased from 18.84 % at 5 d to 29.80 % at 20 d. These results indicated that the WRPU is partially mineralized and degraded by mealworms.

However, carbon in the mealworm biomass decreased with time, possibly due to inadequate nutrition from WRPU. The analyses of the microbial community of mealworms fed with WRPU, bran, and unfed showed differences in the gut microbial composition, implying that WRPU exerts a considerable effect on the gut microorganism of mealworms. Further investigation of the biodegradation characteristics of WRPU by the gut microorganisms, and the effect of different plastics on the gut microorganisms of the larvae is recommended.

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