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

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**HIGHLIGHTS**
- Polystyrene (PS) biodegrades in a mealworm strain from a U.S. source.
- Supplemental nutrition increases PS biodegradation rates.
- Optimal PS removal occurs at 25 °C using a bran feed that has 6–11% (w/w) PS.
- All PS foams degrade, with low-density foams degrading most rapidly.
- A 2nd generation of mealworms fed bran and PS has high rates of PS biodegradation.

**ABSTRACT**
Commercial production of polystyrene (PS) - a persistent plastic that is not biodegradable at appreciable rates in most environments - has led to its accumulation as a major contaminant of land, rivers, lakes, and oceans. Recently, however, an environment was identified in which PS is susceptible to rapid biodegradation: the larval gut of *Tenebrio molitor* Linnaeus (yellow mealworms). In this study, we evaluate PS degradation capabilities of a previously untested strain of *T. molitor* and assess its survival and PS biodegradation rates for a range of conditions (two simulated food wastes, three temperatures, seven PS waste types). For larvae fed PS alone, the %PS removed in the short (12–15 h) residence time of the mealworm gut gradually increased for 2–3 weeks then stabilized at values up to 65%. Thirty-two-day
Keywords:
Plastic wastes
Polystyrene
Biodegradation
Mealworms
Tenebrio molitor

1. Introduction

Plastic wastes are a major environmental concern of increasing global significance, with up to 6300 million metric tons of plastic waste generated to date (Geyer et al., 2017). Of these waste streams, one of the largest is that of polystyrene (PS), a commonly used plastic-eating capacity of insects and their ability to damage plastic wastes using a mealworm strain that is commercially available in the United States (strain CA). We identified that PS degradation rates are significantly enhanced by supplementing the diet with a conventional source of nutrition, and we establish that mealworms fed such a diet can reproduce and give birth to a second generation capable of PS degradation.

2. Methods

2.1. Mealworms and test materials

Mealworms (average weight of 75–85 mg/worm) were purchased from Petco Animal Supplies, Inc, a chain store in Mountain View, California, USA (named strain CA). The larvae were identified based on morphology and coloration as Tenebrio molitor Linnaeus. Prior to testing, they were fed bran, a common agricultural by-product, as their source of nutrition (Stevenson et al., 2012), for at least two days. Natural wheat bran was purchased from General Nutrition Corporation, Pittsburgh, PA.

The biodegradability of seven PS materials was evaluated in this study. Tests to assess the impacts of added nutrients, temperature, and waste PS properties on survival rates, PS degradation rates, and life cycle completion were performed with EPS foam plate from Insulfoam (Carlisle Construction Materials, Puyallup, WA). The
number-average molecular weight ($M_n$) of this material was 95,750 ± 1300, and the weight-average molecular weight ($M_w$) was 238,700 ± 2400 (n = 3, mean ± standard deviation). PS waste streams examined included six PS foam materials purchased from local vendors (coffee cups and packaging for electronics, meat, and frozen foods) then characterized for density and molecular weight (Table 1).

### 2.2. Characterization of PS degradation

To assess PS degradation, strain CA mealworms (n = 120, average weight 79.2 ± 14 mg per mealworm) were incubated in food grade polystyrene storage containers (volume of 475 mL; density of ~2 worms/cm³). These incubators were maintained at different temperatures and at a humidity of 70–80%.

To assess the effects of nutrition on PS degradation, two tests were performed. The first compared PS feedstock (1.8 g) alone to PS (1.8 g) plus added soy protein or bran (1.8 g every four days) at 20 °C. The cumulative ratio of soy protein or bran to PS (total B:PS ratio) was 8:1 g/g over a 32-day period. The second test compared PS degradation rates at three different temperatures (20 °C, 25 °C and 30 °C). PS (1.8 g) with bran added initially, and with different amounts of bran added every four days to give final ratios of bran to PS (total B:PS ratio) of 1:3:1, 2:7:1, 8:1, 16:1, and 24:1 over a 32-day period.

Controls were fed PS alone or bran alone at 20 °C, 25 °C and 30 °C. A total of 21 treatments was evaluated. During these tests, a nutritional supplement (soy protein or bran) was consumed within 2 days. Every four days, mealworms were counted, dead larvae removed, residual PS weighed, frass collected and weighed, and nutrients (soy protein or bran) added to the respective incubators. Survival rates (SR) were calculated as the percentage of live mealworms based on the initial number of live mealworms (120). All treatments were carried out in duplicate.

### 2.3. Biodegradation of different PS products

The primary PS material used for mealworm feed in this study was EPS foam, a typical construction industry waste, where it is used as insulation. The biodegradability of additional six PS wastes were evaluated in incubators maintained at 25 °C and 70–80% humidity. PS materials evaluated included an electronic packaging container made from EPS (density of 0.021 g/cm³); and an XPS coffee cup with a density of 0.042 g/cm³. These materials are typical of PS foams in trash wastes, and have $M_n$ ranging from 100,590 to 126,590 and $M_w$ from 262,210 to 334,300 (Table 1, Fig. 4A).

Each incubator was seeded with 120 mealworms plus 1.8 g of each PS product, cut into 2–3 cm irregular sized pieces. All tests were initiated by the addition of 3.6 g bran followed by additional 3.6 g bran every four days. A final B:PS ratio of 16:1 g bran per g PS was maintained over a 32-day period. All tests were carried out in duplicate.

### 2.4. Collection and characterization of frass and analytical methods

Procedures used to collect and analyze frass were similar to those previously reported (Yang et al., 2015a). Larvae were cleansed of residual PS powder with a stream of compressed air, transferred to a clean box for collection of frass for 12 h then returned to the original incubator. Frass was collected and stored at −80 °C. To analyze PS content, frass or control PS feedstock (50 mg) was transferred to a 30-mL glass vial, then extracted for 2 h with 10 mL tetrahydrofuran (THF) (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) at room temperature. The extract was filtered with a 0.22 μm PVDF sterile syringe filter (Thermo Fisher Scientific Inc., Dublin, Ireland), transferred to a clean 30-mL glass vial, and evaporated via rotary evaporation. The polymer residue remaining after evaporation was weighed to determine the THF extractable fraction, a measure of residual PS in the frass (Fig. 1F). The polymer residue was then re-suspended in THF to a final concentration of 5 mg PS extracted/mL, and the extract (1 mL) filtered through a 0.22 μm PVDF syringe filter into a glass vial. $M_n$, $M_w$, and molecular weight distribution (MWD) were determined by gel permeation chromatography (GPC). THF extract samples (100 μl) were injected into a GPC operating at a THF eluent flow rate of 1.0 mL/min and temperature of 40 °C (Viscotek GPCmax VE 2001 GPC Solvent/Sample Module, Viscotek Corporation, Houston, Texas, USA).

To characterize changes in the end groups of the gested polymer, liquid-state $^1$H nuclear magnetic resonance ($^1$HNMR) analysis was conducted at ambient temperature. Fresh frass samples (50 mg) were placed in 10-mL glass vials and extracted for 2 h with 2 mL chloroform-D (purity 99.8%, Cambridge Isotope Laboratories, Inc., Tewksbury, MA). Extracts were filtered through 0.22 μm PVDF

### Table 1

Characteristics of six polystyrene (PS) products tested before and after biodegradation by mealworms.

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Density (g/cm³)</th>
<th>$M_n$ (g/mol)</th>
<th>$M_n$ reduction (%)</th>
<th>$M_w$ (g/mol)</th>
<th>$M_w$ reduction (%)</th>
<th>SR, % PS consumption, g</th>
<th>PS consumption % specific PS consumption rate</th>
<th>Specific PS consumption rate a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Styrofoam white color</td>
<td>0.021</td>
<td>100590 ± 2680</td>
<td>13.7 ± 0.9</td>
<td>269370 ± 3580</td>
<td>16.5 ± 0.3</td>
<td>0.825 ± 0.034</td>
<td>87.5 ± 0.8</td>
<td>45.8 ± 1.9</td>
</tr>
<tr>
<td>Frass-1#</td>
<td>–</td>
<td>86830 ± 2740</td>
<td>–</td>
<td>22500 ± 3890</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2 White color</td>
<td>0.039</td>
<td>108720 ± 6130</td>
<td>18.2 ± 4.9</td>
<td>283870 ± 12670</td>
<td>13.5 ± 1.4</td>
<td>0.513 ± 0.018</td>
<td>90.0 ± 0.8</td>
<td>28.5 ± 1.2</td>
</tr>
<tr>
<td>Frass-2#</td>
<td>–</td>
<td>88780 ± 3110</td>
<td>–</td>
<td>245380 ± 7290</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3 Yellow color</td>
<td>0.036</td>
<td>101730 ± 6340</td>
<td>21.0 ± 4.1</td>
<td>262200 ± 6170</td>
<td>11.4 ± 2.6</td>
<td>0.596 ± 0.021</td>
<td>89.2 ± 1.7</td>
<td>33.1 ± 1.2</td>
</tr>
<tr>
<td>Frass-3#</td>
<td>–</td>
<td>80250 ± 4850</td>
<td>–</td>
<td>232080 ± 1450</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4 Red color</td>
<td>0.038</td>
<td>126590 ± 12370</td>
<td>22.5 ± 3.0</td>
<td>325850 ± 18740</td>
<td>24.2 ± 2.9</td>
<td>0.574 ± 0.021</td>
<td>88.3 ± 1.7</td>
<td>13.9 ± 1.1</td>
</tr>
<tr>
<td>Frass-4#</td>
<td>–</td>
<td>97860 ± 6100</td>
<td>–</td>
<td>285260 ± 13240</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5 Black color</td>
<td>0.036</td>
<td>105770 ± 2090</td>
<td>23.5 ± 0.3</td>
<td>269640 ± 550</td>
<td>10.6 ± 0.1</td>
<td>0.602 ± 0.014</td>
<td>91.7 ± 1.7</td>
<td>33.4 ± 0.8</td>
</tr>
<tr>
<td>Frass-5#</td>
<td>–</td>
<td>80950 ± 5790</td>
<td>–</td>
<td>241090 ± 750</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6 Coffee cup, white color</td>
<td>0.042</td>
<td>113560 ± 2870</td>
<td>20.6 ± 3.5</td>
<td>334300 ± 10510</td>
<td>9.0 ± 0.9</td>
<td>0.358 ± 0.010</td>
<td>86.7 ± 2.5</td>
<td>19.9 ± 0.5</td>
</tr>
<tr>
<td>Frass-6#</td>
<td>–</td>
<td>90402 ± 3600</td>
<td>–</td>
<td>304160 ± 12200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Key to products: product 1 is EPS: electronic package; products 2–5 are XPS for food packaging of meat, fruits and vegetables; and product 6 is XPS for a coffee cup. EPS = expanded polystyrene foam; XPS = extruded polystyrene foam.

a Specific PS consumption rate, mg 100 worms−1 day−1. SR = Survival rate (%). This test was conducted in duplicate with 120 mealworms per incubator over a 32-day period. $M_n$ = number-average molecular weight. $M_w$ = weight-average molecular weight. Based on t-tests (Table 54), all $M_n$ and $M_w$ values of frass fraction were significantly lower than those of the original PS feedstock (T-test, p < 0.05).
filters and transferred to a clean 10-mL glass vial. Approximately 1 mL of extract was transferred into 5 mm NMR sample tubes (Wilmad, LabGlass, Vineland, NJ, USA). $^1$H NMR spectra were measured on a 500-MHz NMR spectrometer (32 scans, delay time ($d1$) = 0.0 s). $^1$H spectra were reported in parts per million (ppm) and referenced to a peak for residual deuterated chloroform ($^1$H-7.26 ppm).

Fourier transform infrared spectroscopy (FTIR) (EQUINOX 55 FT-IR Spectrometer, Bruker Corporation, Ettlingen, Germany) was used to characterize major functional groups in the range 400–4000 cm$^{-1}$. Analyses were conducted in triplicate for each sample.

At the end of each test, the weight of PS consumed was computed as the difference in weight between the PS in the feed and the sum of uneaten residual PS and PS residue in the frass. The weight of PS in the frass was estimated by multiplying the THF-extracted fraction by the weight of frass. PS consumption (%) over different time periods was estimated as the weight of PS consumed for an indicated time period divided by the initial weight of PS x 100. For some experiments, the time period was variable, while for others, it was held constant at 32 days. Specific rates of PS consumption (weight PS consumed per 100 mealworms per day) were
computed as the weight of PS consumed (mg) per day divided by the number of live mealworms.

2.5. Growth of a second generation of polystyrene-fed mealworms

A second generation of PS-fed mealworms was reared in an incubator at 28 °C and a relative humidity of 80%. Mealworms were fed excess PS supplemented with bran (5 g) every week until pupation. Pupae were separated and transferred on a moist paper towel to a clean incubator where they developed into beetles. The beetles were then transferred to an incubator divided into two floors by a stainless-steel sieve mat. Male and female beetles placed on the upper floor with bran and PS foam were able to mate. Females laid fertilized eggs that fell through the sieve mat to the lower floor where bran and PS foam was present to support hatching development. Juveniles on the lower floor were raised on PS-bran feed until the larvae were large enough (30–60 mg each) to be transferred into other incubators for growth and testing of PS degradation.

2.6. Statistical analyses

Pearson correlation and partial correlation test, redundancy analysis (RDA) and variation partitioning analysis (VPA) based on partial RDA were used to evaluate correlations between survival rate and PS consumption with temperature, B:PS ratios, and PS characteristics, using the “stats”, “gmgm”, and “vegan” packages in R (Team, 2017; Marchetti et al., 2015; Oksanen et al., 2017). Prior to correlation analyses, the R “scale” function was used to adjust for differences in variable units by standardizing data for each variable as Z values (the difference between the observed value and mean value divided by the standard deviation, such that the mean and standard deviation of Z values are 0 and 1, respectively).

3. Results and discussion

3.1. Effects of PS consumption on survival rates and PS biodegradation

Strain CA mealworms were able to chew and burrow into block EPS (Fig. 1A). At the end of the 32-day test at 25 °C, the SR of mealworms fed EPS alone was 86.7 ± 3.3%, significantly greater than that of unfed controls (54.2 ± 2.5%), and not significantly less than bran-fed mealworms (90.0 ± 0.8%) (Fig. 1B). Over the 32-day test period of the test, starved mealworms lost 2.6 ± 0.2% of their average weight; mealworms fed PS alone maintained a stable weight; and bran-fed mealworms experienced a 32.0 ± 1.5% weight gain.

From the initial 1.8 g PS feedstock, PS consumption progressively increased (Fig. 1C) resulting in a total consumption of 0.83 ± 0.04 g PS by the end of the test. The frass contained undegraded PS polymer particles (Fig. 1E), modified PS polymers, and other residues, such as undegraded exoskeletons. The percentage of undegraded PS residue in the frass (w/w, %) decreased from 66.2 ± 2.3% on day 4 to 35.2 ± 1.2% by day 24, stabilizing thereafter (Fig. 1D). At the end of the 32-day test, the PS content of the frass was 0.42 ± 0.002 mg/mg frass. The percentage of initial PS consumed was 23.5 ± 0.1%, an average specific consumption rate of 11.8 ± 0.1 mg PS/100 mealworms per day.

PS depolymerization and biodegradation were characterized by GPC analysis, FTIR, and liquid-state 1H NMR spectra. GPC analysis provides information on three key indicators of depolymerization and degradation of plastic materials: Mn, Mw, and MWD (Albertsson et al., 1998; Yang et al., 2015a). GPC analysis of the extracted PS residues (Fig. 1F) revealed a progressive shift of MWD from higher to lower molecular weights over time compared to PS feedstock (control) (Fig. 1G), with about 16 days required to reach relatively stable lower levels of Mn and Mw (Fig. 1H). Mn and Mw values for PS residues after 32 days were significantly lower than Mn and Mw for PS in the feed. Mn decreased from 95,800 ± 1300 to 77,000 ± 4000 (p = 0.0214); Mw decreased from 239,000 ± 2400 to 212,000 ± 4800 (p = 0.0206). The results (Fig. 1G and H) suggest that the PS degradation activity increased gradually, and stabilized after a 16- to 24-day adaptation period. Analysis of frass extracts by 1H NMR and FTIR confirmed modification of eggested PS associated with degradation and incorporation of oxygen as seen in the increase in signals associated with carbonyl groups (Fig. 3A) as well as shifting associated with hydroxyl groups (Fig. 3B, Table S5).

3.2. Effects of added nutrition and temperature on SR and PS degradation

Soy protein and bran are normal feed for mealworms and can be obtained from agricultural or food processing industries. When mealworms were fed soy protein or bran in the presence of PS, they first ate protein or bran then PS. As shown in Fig. 2A, all feed conditions resulted in higher SR values than the unfed control (60.8 ± 2.5%). SR values were similar for mealworms fed PS alone (87.5 ± 1.7%) and for mealworms fed PS plus soy protein (89.2 ± 0.8%) or bran (90.8 ± 1.7%). Added soy protein or bran significantly increased rates of PS degradation compared to PS alone (control). Average specific PS consumption rates (mg PS/100 mealworms per day) were 22.2 ± 1.8 for mealworms fed PS alone, 49.1 ± 4.1 for mealworms fed PS plus soy protein, and 44.1 ± 4.8 per day for mealworms fed PS plus bran (Fig. 2B). The 32-day PS consumption (%) was 39.1 ± 3.6% for PS alone, 76.8 ± 2.8% for PS plus soy protein, and 67.6 ± 4.3% PS plus bran. The weight gain of mealworms fed PS plus soy protein was 63.6 ± 1.2% greater than that of mealworms fed PS alone, and the weight gain of mealworms fed PS plus bran was 33.5 ± 1.5% greater than that of mealworms fed PS alone.

The combined effects of temperature and bran:PS ratios on SR values and PS consumption rates were evaluated over a 32-day period. Three temperatures (20°C, 25°C, 30°C) and seven B:PS ratios (all bran, 24:1, 16:1, 8:1, 2.7:1, 1.3:1, all PS) were evaluated. Fig. 2A summarizes SR values after 32 days, and Fig. 2B summarizes 32-day PS consumption rates (%). SRs were greater for fed mealworms compared to unfed controls, but it did not matter whether the feed was PS alone, bran alone, or PS plus bran. By contrast, the nature of the feed did affect the specific rates of PS consumption: feed supplemented with bran significantly increased specific rates of PS consumption compared to mealworms fed PS alone, and these rates were sensitive to both the B:PS ratios and temperature. Highest 32-day percentages of PS consumed were 84.0 ± 2.7% at 25°C for a B:PS ratio of 16:1; 78.5 ± 2.7% at 30°C for a B:PS ratio of 16:1; and 67.6 ± 4.3% at 20°C for a B:PS ratio of 8:1. Visibly less PS residue remained in incubators fed bran plus PS than in incubators fed PS alone. The B:PS ratio correlated positively with the 32-day PS consumption (%) (Pearson r = 0.75, p = 0.0005) and average specific PS consumption rate over 32 days (Pearson r = 0.70, p = 0.0011), indicating that higher B:PS ratios generally increase PS consumption, but most mealworms preferred bran to PS. This may explain somewhat lower values for the 32-day PS consumption percentages at the highest bran:PS ratios (16:1 and 24:1).

Temperature had a significant impact on SR values. For the same B:PS ratio, survival rates were significantly lower at 30°C than at 20°C and 25°C (Fig. 2C). At 20°C and 25°C, survival rates were similar regardless of feed ratio (Fig. 2C), but sensitive to temperature. A Pearson correlation test showed a significant correlation between survival ratio and temperature (partial correlation...
removing B:PS ratio influence, $r = 0.89, p < 0.0001$) as opposed to B:PS ratio ($r = 0.04, p = 0.88$). More larvae died at 30 °C than at 20 °C and 25 °C, resulting in lower survival rates.

Temperature also correlated with average specific PS consumption rates (Fig. 2D), where the highest specific degradation rates were observed at 25 °C. A Pearson correlation test showed a significant correlation between PS and temperature ($r = 0.57, p = 0.01$; with a partial correlation after accounting for the influence of the B:PS ratio, of $r = 0.81, p < 0.0001$), indicating that increased temperature increased specific rates of PS consumption.

3.3. Characterization of egested PS frass residues from bran-fed mealworms

GPC spectra were obtained for PS residues extracted from frass collected on day 32 after incubation under optimal condition for PS degradation (B:PS ratio of 8:1 at 20 °C; 16:1 at 25 °C; and B 16:1 at 30 °C). All samples exhibited similar changes in MWD, with shifts to lower molecular weights than those of the PS feed (Fig. 2E, F, and G).

For the PS-alone diet, $M_n$ decreased by $15.1 \pm 2.0\%$ at 20 °C,
14.6 ± 2.3% at 25°C, and 15.9 ± 2.8% at 30°C; M_w decreased by 11.9 ± 1.7 at 20°C, 11.6 ± 0.4% at 25°C, and 12.7 ± 0.3% at 30°C. Co-feeding PS with bran resulted in a slight decrease in M_w and M_n, but the differences were not statistically significant. M_n decreased by 16.7 ± 2.5% at 20°C, 18.6 ± 1.3% at 25°C, and 17.6 ± 2.4% at 30°C; M_w decreased by 14.1 ± 1.8% at 20°C, 13.1 ± 1.5% at 25°C, and 15.5 ± 4.0% at 30°C (Fig. 2E–F).

To determine how PS polymers were modified, frass extracts were analyzed by FTIR (Albertsson et al., 1998; Shang et al., 2003; Stevenson et al., 2012; Yang et al., 2014; Al-Kadhemy et al., 2016; Mecozzi et al., 2016; Sekhar et al., 2016) and liquid-state 1H NMR spectra. Comparison of FTIR spectra for the feed PS and PS in egested frass (Fig. 3A) revealed bond changes and the incorporation of oxygen previously associated with plastic degradation via aging, irradiation, and biotransformation (Mecozzi et al., 2016; Al-Kadhemy et al., 2016; Sekhar et al., 2016). The intensities of the peaks at 625-970 cm⁻¹ (ring-bending vibration) were strong in PS feedstock but much weaker in frass samples. Characteristic peaks known to represent the PS benzene ring (C=\(C\) stretch, 1550–1610 and 1800–2000 cm⁻¹) were dampened in frass samples, providing evidence of ring cleavage. Further evidence of degradation was the observed decrease in intensities of peaks characteristic for PS (Shang et al., 2003; Sekhar et al., 2016) and the appearance of carbonyl groups (C=O stretch, 1700 cm⁻¹) (Yang et al., 2014). The broadening of peaks at 2500-3500 cm⁻¹ in all FTIR spectra of frass samples is associated with the hydrogen bond of hydroxyl groups and/or carboxylic acid groups, suggesting a shift from hydrophobic to more hydrophilic surface properties. Overall changes in FTIR spectra in frass samples collected at 20°C, 25°C and 30°C were similar (Fig. 3A), but PS oxidation was most extensive for frass from mealworms co-fed bran at 20°C and 25°C.

Comparison of 1H NMR spectra for PS to the spectra of frass extracts revealed new peaks in the frass from mealworms fed PS only and PS plus bran (Fig. 3B, Table S5). These peaks were detected in regions of chemical shift associated with \(-\text{CH}=\text{CH}_2\), carbonyl (H\_2C=O), and hydroxyl (-OH) groups. Their presence in PS residues of frass, but not in the control PS, is evidence of transformations and modifications to the PS within the mealworm gut.

Fig. 3. FTIR spectra and 1H NMR spectra of Control and frass samples for mealworms fed PS, bran plus PS, and bran alone at 20°C, 25°C and 30°C. Samples were obtained on day 32. A. FTIR spectra. B. 1H NMR spectra. Results were obtained for a final B:PS ratio of 8:1 g/g at 20°C (a); with final B:PS ratio of 16:1 g/g at 25°C (b); and with final B:PS 16:1 g/g at 30°C.
3.4. Biodegradation of different PS waste materials

Mealworms consumed not only EPS insulation material describe above but also all six PS wastes tested (Fig. 4B). The SR values for mealworms fed different PS wastes ranged from 86 to 91% at the end of the 32-day test period (Fig. 4C). The 32-day PS consumption (%) and average specific consumption rates depended upon the density of the materials tested (Fig. 4D): the highest PS consumption of 45.8 ± 1.9% was obtained with the EPS material with the lowest density of any product tested (product 1), and lowest PS consumption of 19.9 ± 0.5% was obtained for an XPS coffee cup material with the highest density of any material tested (product 6). Specific consumption rates had the same pattern (Table 1).

GPC analysis of the PS residuals extracted from frass samples revealed significant depolymerization following PS ingestion (Table 1 and Fig. 5A). The MWD of frass residues shifted toward lower molecular weights compared to the PS feedstock. Moreover, lower density samples, such as product 1 (density 0.021 g/cm³), shifted more than higher density samples, such as product 6 (0.042 g/cm³) (Fig. 5A). A t-test established that shifts in Mw and Mn were significant (p < 0.05, Table 1).

PS consumption was more affected by foam material density than molecular weight. Density is related to product hardness and likely affects the extent to which a given material can be chewed and ingested by the mealworms. A Pearson correlation test indicated that both PS consumption rate and specific PS consumption rate had strong negative correlations with density (r = −0.94, p = 0.006, for PS consumption rate; r = −0.94, p = 0.005, for specific PS consumption rate), and there was no significant relationship between PS consumption rate and feedstock Mn or Mw (p > 0.15). A Redundancy Analysis (RDA) indicated that PS density, Mn, and Mw explain 99.8% of the variation (p = 0.01) of the 32-day PS consumption (%) and specific PS consumption rate (Fig. 4D). A variation partitioning analysis (VPA) based on partial RDA revealed that PS density alone can explain 53.5% (p = 0.003), while interaction of PS density and Mw (21.8%, p = 0.004) and interaction of all three factors (density, Mn, Mw) (16.8%, p = 0.01) also contributed.

3.5. PS waste degradation by a second generation of mealworms

Mealworms fed PS plus bran completed their life cycle, developing into pupae (Fig. 6A) then beetles in 2 weeks at 28 °C (Fig. 6B). A new generation of mealworms was then reared for three months with PS and bran (Fig. 6C); this generation appeared to have a higher affinity for PS materials (Fig. 6D). Survival rates and PS degradation patterns for the second generation fed PS and bran were similar to those of the first generation (Fig. 6E). In one test at 25 °C, 120, second generation juvenile mealworms weighing ~30 mg per mealworm had a specific PS consumption rate of 16.9 ± 1.9 mg PS/100 mealworms per day or 5.6 ± 0.6 mg PS/1000 mg mealworms per day on a weight basis. These values fall within the range of values measured for the mature first generation PS-degrading mealworms that weighed 75–85 mg per mealworm. GPC analyses of frass THF extracts confirmed a shift in MWD, with slightly lower Mw and Mn values than those of the first generation (Fig. 6F). The second generation also was able to consume the other six PS products tested. These results indicate that the capacity to consume and degrade PS can be maintained, and perhaps enhanced, through selective breeding. The second generation juveniles mealworms eventually grew to be mature larvae (weighing 90 mg or higher, like the first generation), then developed into pupae and beetles.
3.6. Generality of PS biodegradation and nutrition effects

This work establishes that PS degradation capacity is not limited to a specific strain of *T. molitor* or to a specific type of PS. Survival rates for PS-fed *T. molitor* strain CA were consistent with Yang 2015 report for strain Beijing showing that mealworms fed PS alone can survive and maintain their biomass by eating and digesting PS (Yang et al., 2015a). Counts of dead mealworms and observations of exoskeleton residuals indicated that unfed mealworms and mealworms fed PS alone scavenge nutrients needed for survival by consuming shed exoskeleton fragments and dead mealworms (prior to their removal). Mealworms fed PS alone developed into pupae which then converted into beetles, but the nutrition made available by scavenging of shed exoskeletons and dead insects was evidently insufficient to support reproduction and development of a secondary generation.

Addition of nutrition is important for biodegradation of PS and likely other plastics. Supplementing the mealworms with a source of nutrition, such as soy protein or bran, enabled faster rates of PS degradation — in the case of bran, nearly double the rates observed without nutrient addition. Given this fact and the detection of partially oxidized products in the frass, it can be hypothesized that PS degradation involves an initial oxygenase-mediated attack, and a diet with added nutrition may facilitate such an attack, by providing nutrients or trace metals needed for enzyme production or serving as a source of reducing equivalents. Also important is the fact that provision of added nutrition enables reproduction and mating and could therefore enable selective breeding. The generality of PS biodegradation by mealworms will be further examined using mealworms from different geographic locations.

3.7. Effects of temperature

The effects of temperature on survival rates and PS degradation rates are best explained by the known constraints of temperature on mealworm physiology, with a reported optimal range of 25–28 °C and by their inability to tolerate temperatures greater than 30 °C (Roberson, 2005). A t-test indicates that the SRs of

![Fig. 5. Mealworm-mediated depolymerization of the six PS waste materials. A. Comparison of shifts in $M_w$, $M_n$, and MWD of control (feed PS) and THF extract of frass residues after 32 days of incubation. B. PS consumption and specific PS consumption rates correlated negatively to PS foam density. C. Redundancy Analysis (RDA) of the specific rate of PS consumption and PS characteristics, including density, $M_w$, and $M_n$, and a Variation Partitioning Analysis (VPA) based on partial RDA. P1–PS represent products 1–6. The percentages represent proportions of variation explained by certain axes or factors. *, $p < 0.05$, **, $p < 0.01$, based on ANOVA of RDA or partial RDA. The product numbers (#) and test conditions are described in Fig. 4.](image-url)
mealworms at 20 °C is not significantly greater than those at 25 °C (p > 0.05). However, mealworms grown at a lower temperatures appeared to have somewhat higher SR values. For the PS-fed mealworms, SR values were higher at 20 °C and 25 °C than at 30 °C, but rates of PS degradation were also higher at 25 and 30 °C than at 20 °C (Fig. 2D and G). It is possible that lower metabolic activity at lower temperatures reduces death rates (increasing SR), and higher metabolic activity at higher temperatures increases death rates (decreasing SR). Because metabolic activity correlated with PS consumption, higher PS consumption rates are observed at higher temperatures, with lower rates at lower temperatures.

3.8. Characterization of plastic degradation in insect larvae

The fact that insect pests, especially darkling beetles (family Tenebrionidae) and their larvae as well as Indian meal moth (Plodia interpunctella) and honey comb wax worm (Galleria mellonella) can chew, eat, and penetrate various plastic packing materials has been well known since the 1950s (Gerhardt and Lindgren, 1954; Cline, 1978; Newton, 1988; Yang et al., 2014; Bombelli et al., 2017). What was not known was the fate of the materials consumed. It is now increasingly clear that different plastics can be degraded within the gut of a range of different insect larvae, including larvae of the Indian moth (Yang et al., 2014) and mealworms (Yang et al., 2015a). Undoubtedly, there are more. Research is clearly needed to understand whether gut microflora also play a role in the biodegradation of plastics by other plastic-eating insect larvae and factors influencing degradation rates. In the case of the mealworm, PS degradation is dependent upon their gut microflora: mealworms fed the antibiotic gentamicin lost the ability to degrade PS (Yang et al., 2015b). Many insects are known to consume plastics, but no effort has yet been made to systematically assess the fate of ingested plastics (Gerhardt and Lindgren, 1954; Cline, 1978; Newton, 1988). The methods used in previous studies (Yang et al., 2015a,b) and in this work should be of value for such an assessment, providing four independent lines of evidence: (1) mass balances for the plastic plus insect in which the weight of plastic degraded equals the weight of plastic ingested minus the weight of plastic recovered as residues in frass plus the change in weight of the insect; (2) changes in the fraction of residual plastic extracted by THF in frass egested from insects fed plastic only; (3) comparison of GPC analyses of feed plastic and extracted plastic residues to assess changes in molecular weight (Mw and Mn) and in the molecular weight distribution (MWD) due to depolymerization; and (4) characterization of frass residues using a suite of analytical tools (e.g., FTIR, 1H NMR, 13C NMR and TG-FTIR) to identify functional groups in plastic extracted from the frass due to depolymerization and oxidation.

4. Conclusion

The fact that two mealworm strains are now known to degrade PS wastes suggests that this capability is likely widely distributed among T. molitor species. Feeding a source of nutrition increases the rate of PS degradation and enables breeding of a second generation with favorable properties for PS biodegradation, potentially enabling selective breeding PS-degrading organisms. The capacity for PS biodegradation is not strain specific and extends to a wide range of PS wastes, with faster biodegradation rates observed for less dense products. Temperature affects growth and PS degradation in a manner consistent with the known temperature constraints on mealworm physiology.
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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2017.10.117.

References


