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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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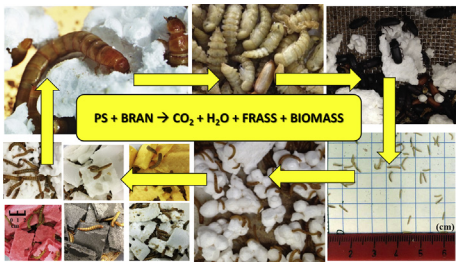
HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



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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

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survival rates were >85% versus 54% for unfed larvae. For mealworms fed ~10% w/w PS and ~90% bran, an agricultural byproduct, rates of PS degradation at 25 °C nearly doubled compared to mealworms fed PS alone. Polymer residues in the frass showed evidence of partial depolymerization and oxidation. All of the tested PS wastes degraded, with the less dense foams degrading most rapidly. Mealworms fed bran and PS completed all life cycle stages (larvae, pupae, beetles, egg), and the second generation had favorable PS degradation, opening the door for selective breeding.

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 polymer ([-CH (C_6H_5) CH_2-]$_n$) with an annual production rate exceeding 20 million tons per year (PlasticsEurope, 2016). PS wastes result from widespread commercial use of expanded PS (EPS), trade name Styrofoam, in building insulation and packing, and of extruded PS (XPS) in containers, such as coffee cups and food trays. In 2014, the global market for PS was valued at $32 billion with a projected 2020 market valued at $42 billion (Market Research Store, 2015). PS is a major pollutant of soils, rivers, lakes, and oceans (Zhou et al., 2011), and is among the major microplastics (<5 mm) accumulating in the ocean (Hidalgo-Ruz et al., 2012; Wu et al., 2017).

Since the early 1970s, many research groups have studied PS biodegradation in soils, seawater, landfill sediment, activated sludge, and compost. Some of these studies included use of ^{14}C-labeled PS (Guillet et al., 1974; Jones et al., 1974; Sielicki et al., 1978; Kaplan et al., 1979; Mor and Sivan, 2008; Råberg and Hafrén, 2008). The scientific consensus was that rapid PS degradation would require photolytic or thermolytic cleavage of -C-C- bonds prior to biodegradation (Guillet et al., 1974; Motta et al., 2009; Krueger et al., 2015; Geyer et al., 2017). Recently, however, rapid PS degradation was reported in the guts of mealworms (Yang et al., 2015a, b), indicating a biological mechanism that is functional in the absence of light or added heat.

Yellow mealworms, or mealworms for short, are the larvae of *Tenebrio molitor* Linnaeus, the second of four life stages: egg, larva, pupa, and beetle (Roberson, 2005; Löbl et al., 2008). *T. molitor* belongs to the family Tenebrionidae (common name “darkling beetle”), a cosmopolitan family with more than 20,000 beetle species. In its native temperate regions, *T. molitor* is viewed as a pest because it infects homes and stored grain facilities (Roberson, 2005), but in other environments, *T. molitor* is a resource. The larvae are mass produced as feed for birds, reptiles, amphibians, and fish using bran, an agricultural byproduct, as the primary feed. Frass generated by mealworms is sold as fertilizer. Researchers have concluded that mealworms are a more sustainable source of edible protein for humans than milk, chicken, pork, or beef (Oonincx and de Boer, 2012), and some have proposed their use as an astronaut food supply in bioregenerative life support systems (Li et al., 2013). Recent tests indicate that mealworms are a suitable alternative source of protein for broiler chickens (Bovera et al., 2015).

Since the 1950's, many researchers have investigated the plastic-eating capacity of insects and their ability to damage packaging materials. Beetles and larvae that exhibited this behavior were identified in the Tenebrionidae family, the Anobiidae family and the Dermestidae family (Gerhardt and Lindgren, 1954; Cline, 1978; Newton, 1988). But in these studies, no effort was made to investigate the fate of ingested plastics. Thereafter, occasional reports on the internet and in social media described the plastic-eating behavior of mealworms and superworms (larvae of *Zophobas morio*, also in the Tenebrionidae family). Consumption of EPS foams by mealworms was first reported by students competing in middle and high school science fairs. In 2003, Chong-Guan Chen reported mealworms eating XPS food containers (Sina, 2003); in 2009, I-Ching Tseng reported EPS consumption by mealworms and isolation of two PS-degrading bacterial strains from the mealworm gut (Burkart, 2009). These early studies appear to have escaped academic attention as they did not result in peer-reviewed publications or archiving of bacterial isolates.

The situation changed in 2015, when Yang and coworkers demonstrated that a mealworm strain from Beijing, China, can survive on EPS alone for one month (Yang et al., 2015a). Almost half of the ingested EPS carbon was converted to CO_2 in the mealworm gut. Analysis of egested frass from mealworms fed EPS and antibiotics to inhibit microbial activity confirmed gut microbe−dependent cleavage of long-chain PS molecules and formation of depolymerized metabolites (Yang et al., 2015a, b). Limitations of the Yang study included its focus on a single strain of mealworm (strain Beijing) and on a single type of EPS, and no effort was made to assess factors influencing PS biodegradation rates or impacts of PS on the life cycle of *T. molitor*. Additional research is needed to understand and exploit biodegradation mechanisms that could yield solutions to the enormous problem of plastic wastes in the environment.

In this report, we assess the biodegradation potential of seven PS wastes using a mealworm strain that is commercially available in the United States (strain CA). We find 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 byproduct, as their source of nutrition (Stevenson et al., 2012), for at least two days. Natural wheat bran was purchased from Exotic Nutrition, Newport News, VA. Soy protein 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 venders (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 ± 1.4 mg per mealworm) were incubated in food grade polyethylene storage containers (volume of 475 mL; density of ~2 worms/cm^2). 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 six additional 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/cm^3); XPS food packaging containers (densities ranging from 0.036 to 0.039 g/cm^3); and an XPS coffee cup with a density of 0.042 g/cm^3. 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 egested polymer, liquid-state ^{1}H nuclear magnetic resonance (^{1}H NMR) 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.

Product	Description	Density (g/cm^3)	M_n	M_n reduction (%)	M_w	M_w reduction (%)	PS consumption, g	SR, %	PS consumption %	Specific PS consumption rate[a]
1	Styrofoam white color	0.021	100590 ± 2680	13.7 ± 0.9%	269370 ± 3580	16.5 ± 0.3%	0.825 ± 0.034	87.5 ± 0.8	45.8 ± 1.9	23.5 ± 0.1
	Frass-1#	–	86830 ± 2740		225000 ± 3890					
2	White color	0.039	108720 ± 6130	18.2 ± 4.9%	283870 ± 12670	13.5 ± 1.4%	0.513 ± 0.018	90.0 ± 0.8	28.5 ± 1.0	14.4 ± 0.0
	Frass-2#	–	88780 ± 3110		245380 ± 7290					
3	Yellow color	0.036	101730 ± 6340	21.0 ± 4.1%	262200 ± 6170	11.4 ± 2.6%	0.596 ± 0.021	89.2 ± 1.7	33.1 ± 1.2	17.0 ± 0.0
	Frass-3#	–	80290 ± 4850		232080 ± 1450					
4	Red color	0.038	126590 ± 12370	22.5 ± 3.0%	325850 ± 18740	12.4 ± 2.9%	0.574 ± 0.021	88.3 ± 1.7	31.9 ± 1.1	16.2 ± 0.1
	Frass-4#	–	97860 ± 6100		285260 ± 13240					
5	Black color	0.036	105770 ± 7090	23.5 ± 0.3%	269640 ± 550	10.6 ± 0.1%	0.602 ± 0.014	91.7 ± 1.7	33.4 ± 0.8	16.7 ± 0.1
	Frass-5#	–	80950 ± 5790		241090 ± 750					
6	Coffee cup, white color	0.042	113860 ± 2870	20.6 ± 3.5%	334300 ± 10510	9.0 ± 0.9%	0.358 ± 0.010	86.7 ± 2.5	19.9 ± 0.5	10.2 ± 0.1
	Frass-6#	–	90420 ± 3600		304160 ± 12200					

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 S4), all M_n and M_w values of frass fraction were significantly lower than those of the original PS feedstock (T-test, $p < 0.05$).

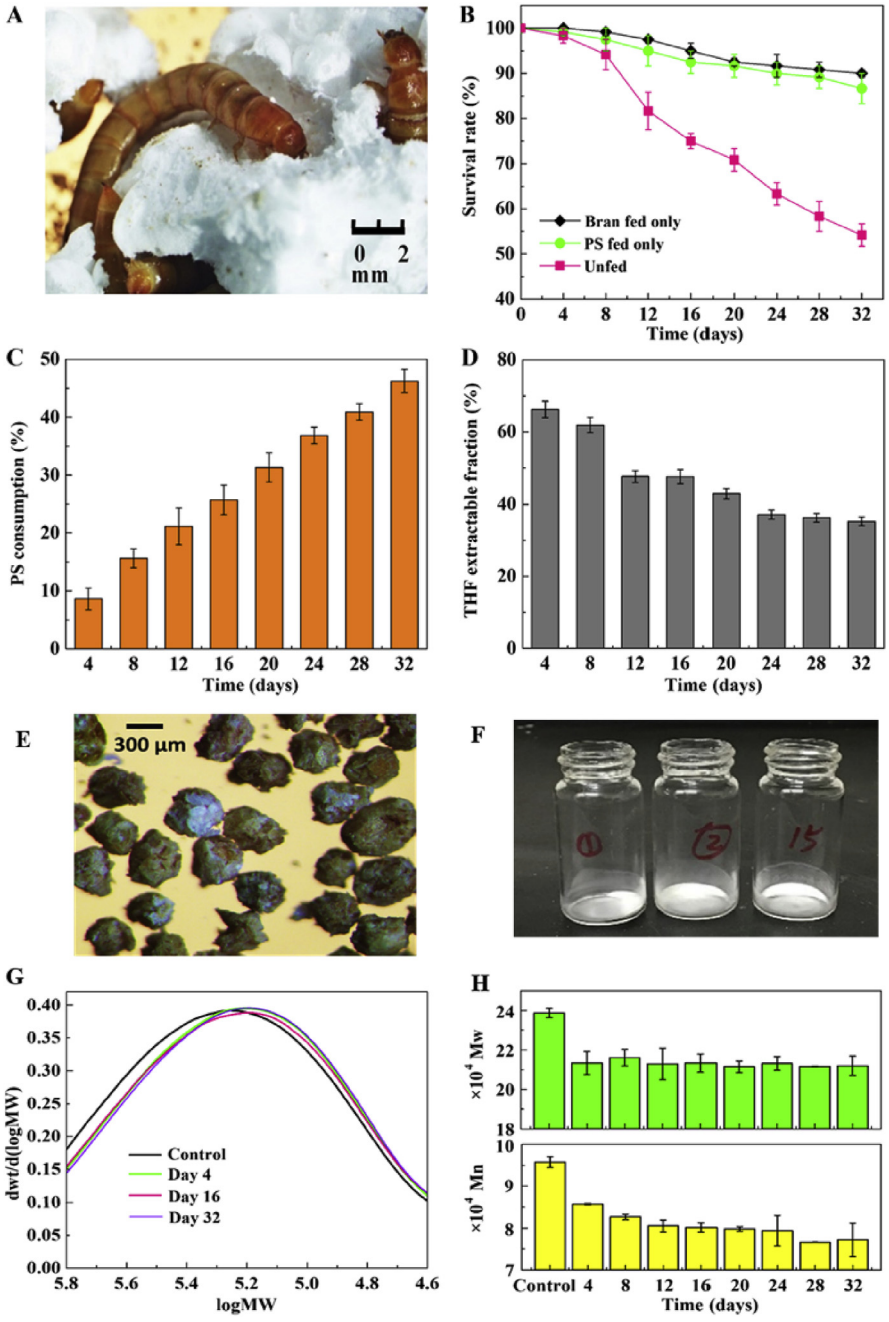

Fig. 1. *T. molitor* strain CA mealworms fed PS alone. **A**. The larvae chew, eat, and tunnel into PS. **B.** Survival rates for unfed mealworms compared to those fed PS alone and bran alone. **C**. PS consumption (%) over time. **D** Progreasssive decrease in the THF-extractable fraction. **E.** Frass (black) with embedded white polymer residuals. **F.** Residual polymer extracted from frass. **G**. Molecular weight distribution (MWD) of THF extracts of the feed PS and frass (days 4, 16, 32), showing shifts to lower molecular weights in the frass. **H.** Changes in M_w and M_n versus feed PS (control) over a 32-day period. This test was conducted in duplicate with 120 mealworms in each incubator over a 32-day period. Analyses were performed in triplicate, and the data are reported as the mean ± one standard deviation.

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 hatchling 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", "ggm", 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 undigested PS polymer particles (Fig. 1E), modified PS polymers, and other residues, such as undigested exoskeletons. The percentage of undigested 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 23.6 ± 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 ^{1}H NMR spectra. GPC analysis provides information on three key indicators of depolymerization and degradation of plastic materials: M_n, M_w 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 M_w and M_n (Fig. 1H). M_n and M_w values for PS residues in the frass after 32 days were significantly lower than M_n and M_w for PS in the feed. M_n decreased from 95,800 ± 1300 to 77,000 ± 4000 ($p = 0.0214$); M_w 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 ^{1}H NMR and FTIR confirmed modification of egested 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 6.3 ± 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 $r = 0.70$, $p = 0.001$), 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 or 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

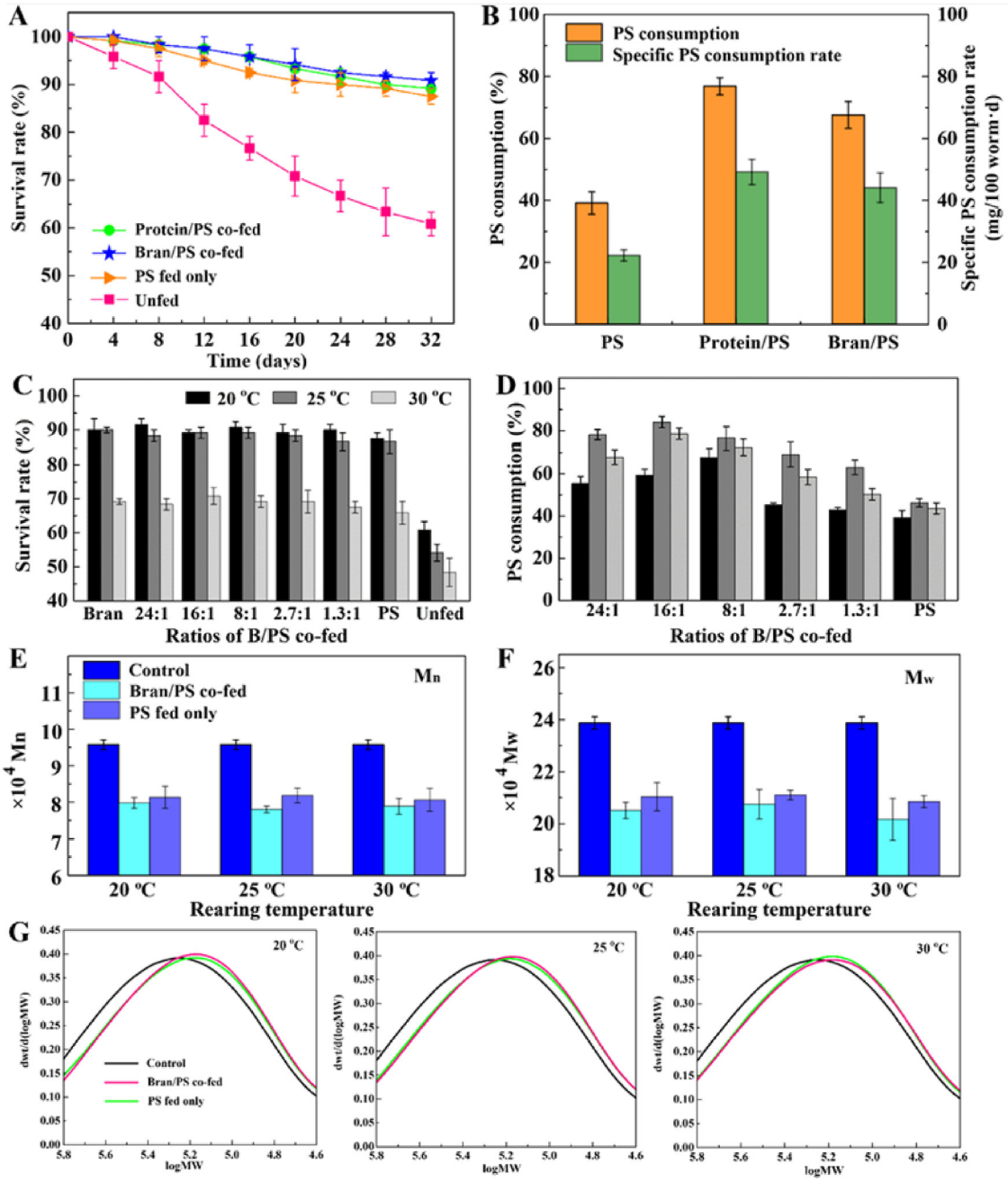

Fig. 2. Effects of nutrient supplements and impacts of temperature on PS degradation by *T. molitor* strain CA. **A**. Comparison of survival rates for different mealworms. **B**. PS consumption (%) and specific PS consumption rates for unfed mealworms compared to mealworms fed soy protein plus PS or bran plus PS over 32-day period. **C.** Survival rates for mealworms fed various ratios of bran versus PS at 20 °C, 25 °C and 30 °C over 32 days. **D.** PS consumption (%) for mealworms fed PS alone and various ratios of bran versus PS at 20 °C, 25 °C and 30 °C over 32 days (mean ± standard deviation); **E-F.** Comparison of M_n and M_w of PS feedstock (control) and THF extracts of frass samples fed PS plus bran. **G.** Shifts in MWD of THF extracts of frass from mealworms fed PS alone and PS plus bran versus PS feedstock (Control). **E-G**. Results were obtained for a final B to PS ratio of 8:1 g/g at 20 °C (a); with final B to PS ratio of 16:1 g/g at 25 °C (b); and with final B to PS ratio of 16:1 g/g at 30 °C for 32 days.

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 ± 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 ^{1}H 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 ^{1}H 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 –CH=CH-, 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.

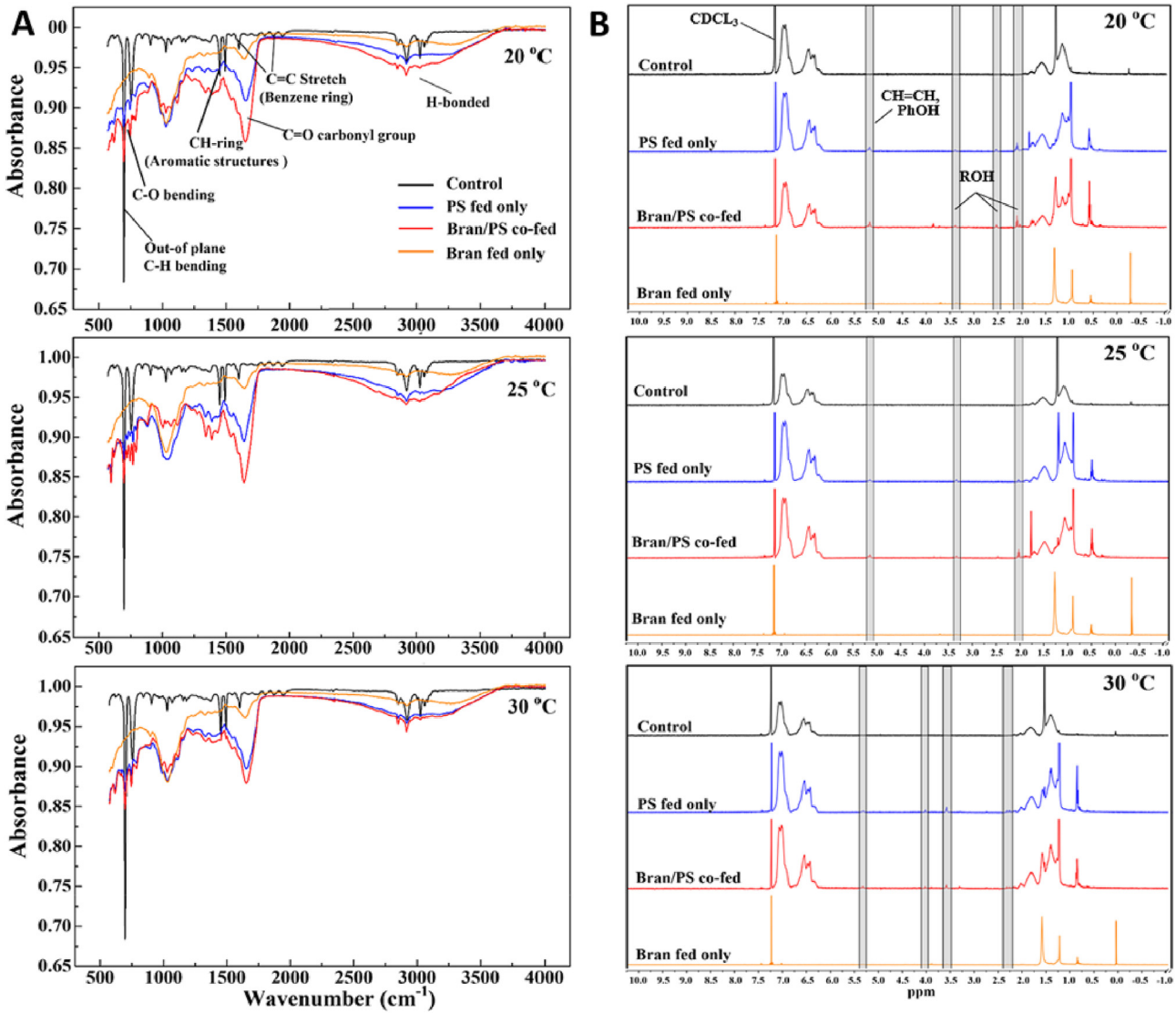

Fig. 3. FTIR spectra and ^{1}H NMR spectra of Control and frass samples for mealworms fed PS, bran plus PS, and bran alone at 20 °C, 25 °C and 30 °C. Samples were obtained on day 32. **A.** FTIR spectra. **B.** ^{1}H NMR spectra. Results were obtained for a final B:PS ratio of 8:1 g/g at 20 °C (a); with 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/cm^3), shifted more than higher density samples, such as product 6 (0.042 g/cm^3) (Fig. 5A). A *t*-test established that shifts in M_w and M_n 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 M_n or M_w ($p > 0.15$). A Redundancy Analysis (RDA) indicated that PS density, M_n, and M_w 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 M_w (21.8%, $p = 0.004$) and interaction of all three factors (density, M_n, M_w) (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 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 M_w and M_n 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.

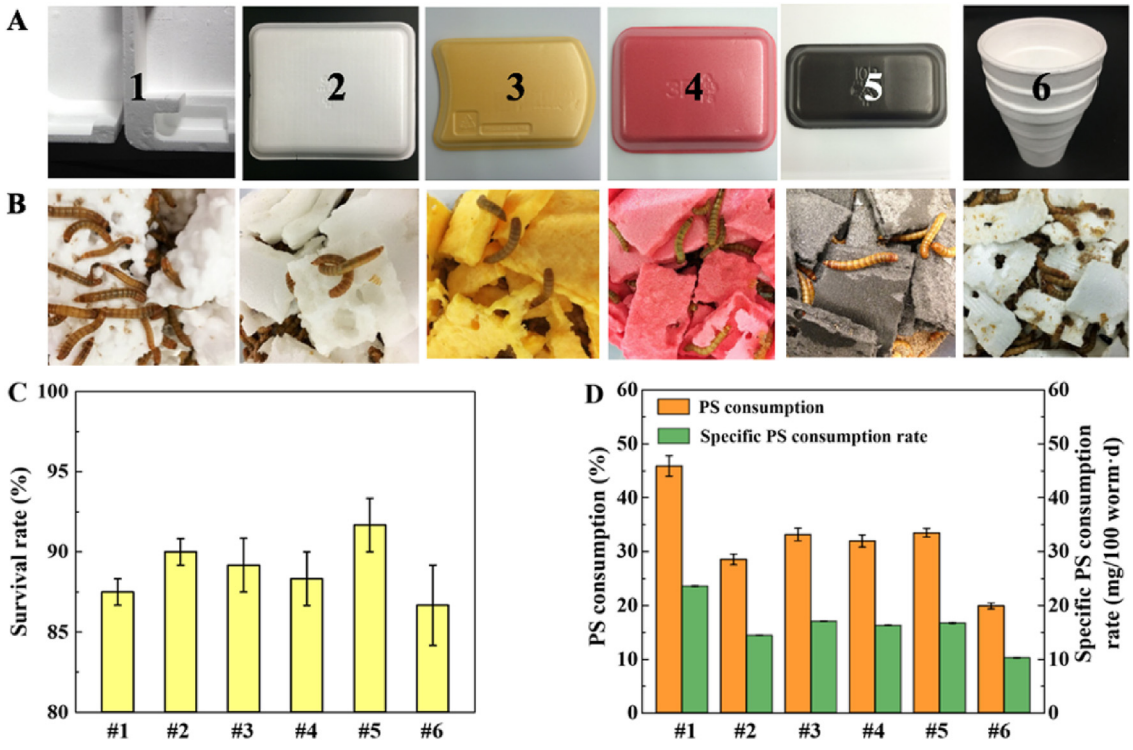

Fig. 4. Biodegradation of six different PS wastes by *T. molitor* strain CA. **A.** Images for the six PS wastes collected for tests: product 1 is electronics EPS packaging; products 2 through 5 are XPS packaging used for meat, fruits, and vegetables; product 6 is a coffee cup made from XPS foam. **B.** The mealworms consumed all the PS materials tested. **C.** Survival rate after 32 days for mealworms fed different types of PS wastes plus bran. **D.** 32-day PS consumption (%) and average specific consumption rate. These tests were performed over a 32-day period by adding materials from different PS products to incubators that initially contained 120 mealworms that were subsequently fed at a bran: PS ratio of 16:1 at 25 °C. EPS = expanded polystyrene foam; XPS = extruded polystyrene foam.

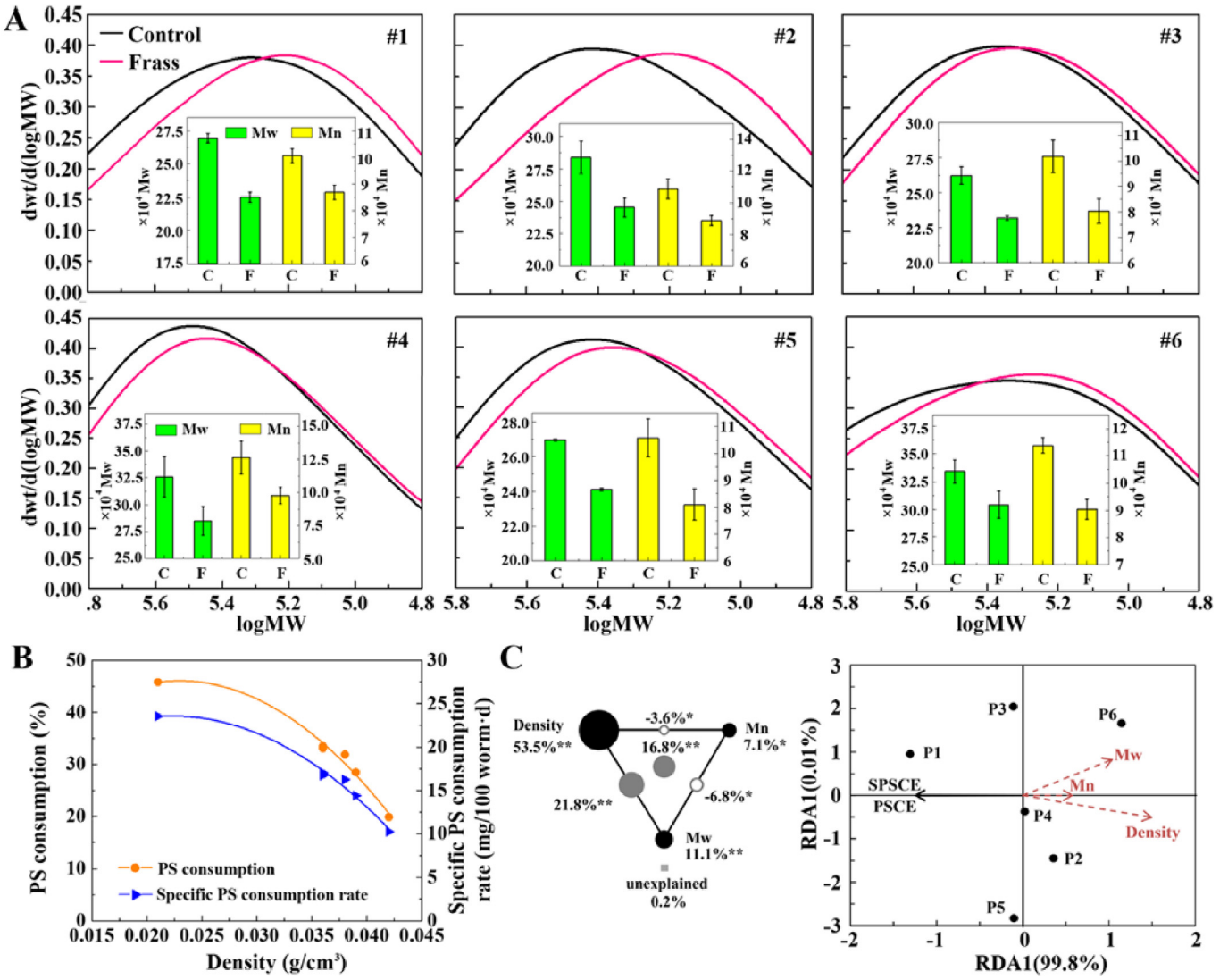

Fig. 5. Mealworm-mediated depolymerization of the six PS waste materials. **A.** Comparison of shifts in M_w, M_n, and MWD of control (feed PS) and THF extract of frass residues after 32 days of incubation. **B.** PS consumption and specific PS consumption rates correlated negatively to PS foam density. **C.** Redundancy Analysis (RDA) of the specific rate of PS consumption and PS characteristics, including density, M_n and M_w, and a Variation Partitioning Analysis (VPA) based on partial RDA. P1-P6 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.

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

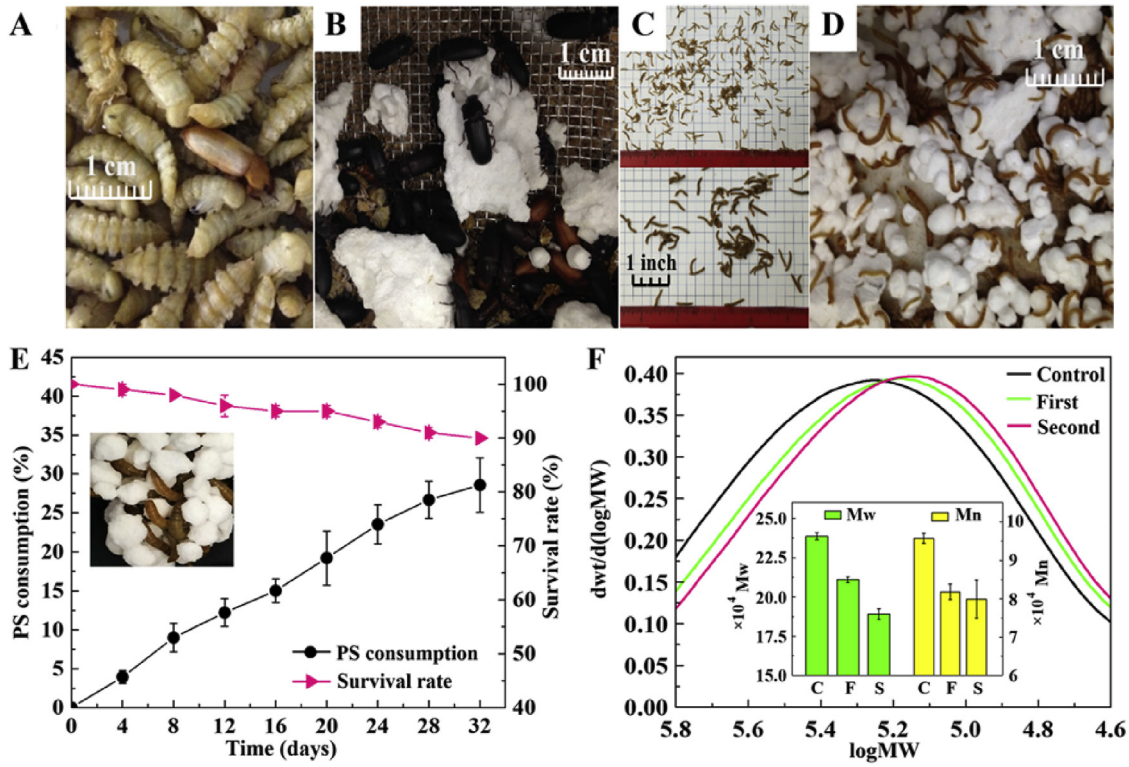

Fig. 6. Reproduction of PS-degrading mealworms. **A-D**. Life cycle stages for a second generation of PS-bran fed mealworms, showing (**A**) pupae, (**B**) beetles, **(C**) juvenile mealworms at 4 weeks (top panel) and 6 weeks (bottom panel), (**D**) PS-eating second generation. **E.** SR and the cumulative PS consumption efficiency when fed PS alone for 32 days. **F.** Changes in MWD and decrease in M_n and M_w for THF extracts of frass from the parent as compared to those of a second generation fed PS alone for 32 days. C= Control PS, F= First parent generation, S= Secondary generation.

mealworms at 20 °C is not significantly greater than those at 25 °C ($p > 0.05$). However, mealworms grown at a lower temperatures did appear 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 temperature 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 (M_w and M_n) 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, ^{1}H NMR, ^{13}C 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 affected 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

Albertsson, A.-C., Erlandsson, B., Hakkarainen, M., Karlsson, S., 1998. Molecular weight changes and polymeric matrix changes correlated with the formation of degradation products in biodegraded polyethylene. J. Environ. Polym. Degrad. 6, 187–195.

Al-Kadhemy, M.F.H., Rasheed, Z.S., Salim, S.R., 2016. Fourier transform infrared spectroscopy for irradiation coumarin doped polystyrene polymer films by alpha ray. J. Rad. Res. Appl. Sci. 9, 321–331.

Bombelli, P., Howe, C.J., Bertocchini, F., 2017. Polyethylene bio-degradation by caterpillars of the wax moth Galleria mellonella. Curr. Biol. 27, R1–R3.

Bovera, F., Piccolo, G., Gasco, L., Marono, S., Loponte, R., Vassalotti, G., Mastellone, V., Lombardi, P., Attia, Y.A., Nizza, A., 2015. Yellow mealworm larvae (*Tenebrio molitor*, L.) as a possible alternative to soybean meal in broiler diets. Brit. Poult. Sci. 56, 569–575.

Burkart, K., 2009. High School Girl Discovers Styrofoam-eating Bacterium. Mother Nature Network. http://www.mnn.com/green-tech/research-innovations/blogs/high-school-girl-discovers-styrofoam-eating-bacterium (last accessed 08.02.2017).

Cline, L.D., 1978. Penetration of seven common flexible packaging materials by larvae and adults of eleven species of stored product insects. J. Eco. Entomol. 71, 726–729.

Gerhardt, P.D., Lindgren, D.L., 1954. Penetration of packaging films: film materials used for food packaging tested for resistance to some common stored-product insects. Calif. Agric. 8, 3–4.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782 https://doi.org/10.1126/sciadv.1700782, 2017.

Guillet, J.E., Regulski, T.W., McAneney, T.B., 1974. Biodegradability of photodegraded polymers II: tracer studies of biooxidation of Ecolyte PS polystyrene. Environ. Sci. Technol. 8, 923–925.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.

Jones, P.H., Prasad, D., Heskins, M., Morgan, M.H., Guillet, J.E., 1974. Biodegradability of photodegraded polymers. I. Development of experimental procedures. Environ. Sci. Technol. 8, 919–923.

Kaplan, D.L., Hartenstein, R., Sutter, J., 1979. Biodegradation of polystyrene, poly(-methyl methacrylate), and phenol formaldehyde. Appl. Environ. Microbiol. 38, 551–553.

Krueger, M.C., Harms, H., Schlosser, D., 2015. Prospects for microbiological solutions to environmental pollution with plastics. Appl. Microbiol. Biotechnol. 99, 8857–8874.

Li, L.Y., Zhao, Z.R., Liu, H., 2013. Feasibility of feeding yellow mealworm (Tenebrio molitor L.) in bioregenerative life support systems as a source of animal protein for humans. Acta. Astronaut. 92, 103–109.

Löbl, I., Merkl, O., Ando, K., Bouchard, P., Egorov, L.V., Iwan, D., Lillig, M., Masumoto, K., Nabozhenko, M., Novák, V., Petterson, R., Schawaller, W., Soldati, F., 2008. Family Tenebrionidae Latreille: 30-45, 105-352 and 467-645. In: Löbl, I., Smetana, A. (Eds.), Catalogue of Palaearctic Coleoptera, Tenebrionoidea. Stenstrup, vol. 5. Apollo Books, Stenstrup, Denmark, p. 670.

Marchetti, G.M., Drton, M., Sadeghi, K., 2015. Ggm: Functions for Graphical Markov Models. R Package Version 2.3. https://CRAN.R-project.org/package=ggm (last accessed 08.02.2017).

Market Research Store, Nov-2015. Polystyrene (PS) & Expandable Polystyrene (EPS) Market for Building & Construction, Electrical & Electronics, Packaging and Other Applications: Global Industry Perspective, Comprehensive Analysis, and Forecast, 2014-2020. http://www.marketresearchstore.com/report/polystyrene-expandable-polystyrene-market-for-building-z37708 (last accessed 08.02.2017).

Mecozzi, M., Pietroletti, M., Monakhova, Y.B., 2016. FTIR spectroscopy supported by statistical techniques for the structural characterization of plastic debris in the marine environment: application to monitoring studies. Mar. Pollut. Bull. 106, 155–161.

Mor, R., Sivan, A., 2008. Biofilm formation and partial biodegradation of polystyrene by the actinomycete Rhodococcus ruber. Biodegradation 19, 851–858.

Motta, O., Proto, A., Carlo, F.D., Caro, F.D., Santoro, E., Brunetti, L., Capunzo, M., 2009. Utilization of chemically oxidized polystyrene as co-substrate by filamentous fungi. Int. J. Hyg. Environ. Health 212, 61–66.

Newton, J., 1988. Insects and packaging-a review. Int. Biodeterior. 24, 175–187.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. Vegan: Community Ecology Package. R Package Version 2.4-2. 2017. https://CRAN.R-project.org/package=vegan (last accessed 08.02.2017).

Oonincx, D.G., de Boer, I.J., 2012. Environmental impact of the production of mealworms as a protein source for humans-A life cycle assessment. PLoS One 7 (12), e51145.

PlasticsEurope, 2016/10/20. Plastics-the Facts 2016. http://www.plasticseurope.org/Document/plastics—-the-facts-2016-15787.aspx?FolID (last accessed 08.02.2017).

Råberg, U., Hafrén, J., 2008. Biodegradation and appearance of plastic treated solid wood. Int. Biodeterior. Biodegrad. 62, 210–213.

Roberson, W.H., 2005. Urban Insects and Arachnids, a Handbook of Urban Entomology. Cambridge University Press, Cambridge, UK, pp. 126–127.

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

Shang, J., Chai, M., Zhua, Y., 2003. Solid-phase photocatalytic degradation of polystyrene plastic with TiO_2 as photocatalyst. J. Solid. State. Chem. 174, 104–110.

Sina, 2003. Mealworms Can Digest Plastics (in Chinese). http://news.sina.com.cn/c/2003-12-30/16251466914s.shtml (last accessed 08.02.2017).

Sielicki, M., Focht, D.D., Martin, J.P., 1978. Microbial degradation of [^{14}C] polystyrene and 1, 3-diphenylbutane. Can. J. Microbiol. 24, 798–803.

Stevenson, L., Phillips, F., O'Sullivan, K., Walton, J., 2012. Wheat bran: its composition and benefits to health, a European perspective. Int. J. Food Sci. Nutr. 63, 1001–1013.

Team, R., 2017. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (last accessed 08.02.2017).

Wu, W.M., Yang, J., Criddle, C.S., 2017. Microplastics pollution and reduction strategies. Front. Environ. Sci. Eng. 11, 6–7.

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, Y., Yang, J., Wu, W.M., Zhao, J., Song, Y.L., Gao, L.C., Yang, R.F., Jiang, L., 2015a. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests. Environ. Sci. Technol. 49, 12080–12086.

Yang, Y., Yang, J., Wu, W.M., Zhao, J., Song, Y.L., Gao, L.C., Yang, R.F., Jiang, L., 2015b. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 2. Role of gut microorganisms. Environ. Sci. Technol. 49, 12087–12093.

Zhou, P., Huang, C.G., Fang, H.D., Cai, W.X., Li, D.M., Li, X.M., Yu, H.S., 2011. The abundance composition and sources of marine debris in coastal seawaters or beaches around the northern South China Sea (China). Mar. Pollut. Bull. 62, 1998–2007.