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Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 1. Chemical and Physical Characterization and Isotopic Tests

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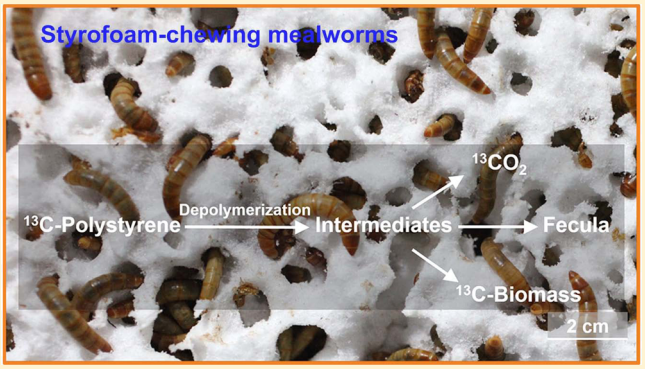
Supporting Information

ABSTRACT: Polystyrene (PS) is generally considered to be durable and resistant to biodegradation. Mealworms (the larvae of *Tenebrio molitor* Linnaeus) from different sources chew and eat Styrofoam, a common PS product. The Styrofoam was efficiently degraded in the larval gut within a retention time of less than 24 h. Fed with Styrofoam as the sole diet, the larvae lived as well as those fed with a normal diet (bran) over a period of 1 month. The analysis of fecula egested from Styrofoam-feeding larvae, using gel permeation chromatography (GPC), solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy, and thermogravimetric Fourier transform infrared (TG−FTIR) spectroscopy, substantiated that cleavage/depolymerization of long-chain PS molecules and the formation of depolymerized metabolites occurred in the larval gut. Within a 16 day test period, 47.7% of the ingested Styrofoam carbon was converted into CO_2 and the residue (ca. 49.2%) was egested as fecula with a limited fraction incorporated into biomass (ca. 0.5%). Tests with α ^{13}C- or β ^{13}C-labeled PS confirmed that the ^{13}C-labeled PS was mineralized to $^{13}CO_2$ and incorporated into lipids. The discovery of the rapid biodegradation of PS in the larval gut reveals a new fate for plastic waste in the environment.



INTRODUCTION

The current global consumption of petroleum-based synthetic plastic is approximately 299 Mt/year.[1] Polystyrene (PS), molecular formula $[-CH(C_6H_5)CH_2-]_n$, commonly known as Styrofoam, accounted for approximately 7.1% (21 Mt/year) of the total plastic consumption in 2013.[1] Although PS is considered a durable plastic, PS products are often designed for a short service time and one-time use as a result of the low cost of this material. The sharp contrast between the remarkable durability of PS and the short service time of PS products has led to the increasing accumulation of PS waste in our environment. Most of the collected PS waste is disposed along with municipal solid waste in landfills.[2] Even more problematic is that a great amount of PS debris is also dispersed as "white pollutants" in the environment, becoming a global environmental concern.[2−5]

To date, it has generally been thought that PS is not subject to biodegradation by microorganisms and soil invertebrates.[6−8] Previous investigations have used ^{14}C-labeled PS tracers added to a variety of mixed microbial consortia from soil, sewage sludge, decaying garbage, or manure.[8−10] The recovery of $^{14}CO_2$ ranged from 0.01% to less than 3% over periods of 1−4 months, which does not yet constitute convincing results of the biodegradation of PS because PS may contain a small fraction of impurities, such as styrene.[8−10] Although a few strains of pure bacteria isolated from soils were capable of colonizing PS surfaces, the isolates have not proven that these bacteria were effective in the biodegradation of PS, changing neither the physical nor chemical properties of its long-chain molecules. Further, no traces of metabolic activity were found.[11,12]

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Several soil invertebrates, including earthworms, isopods, millipedes, slugs, and snails, have also been tested to determine whether they were able to degrade PS. These soil invertebrates were fed with ^{14}C-labeled PS tracers in their normal diets.[10] No respired $^{14}CO_2$ was recovered during a 2 week test period. Some mandibulate insects, as reported previously, are able to chew and eat plastic packages, including polyvinyl chloride (PVC), polyethylene (PE), and polypropylene (PP) packaging films.[13−15] However, until recently, little was known about whether the ingested plastic could be biodegraded in the gut of the plastic-eating insect.

Recently, we reported that waxworms (the larvae of the Indian mealmoth or *Plodia interpunctella*) were capable of chewing and eating PE films, and two bacterial strains capable of degrading PE were isolated from the gut of the worms, i.e., *Enterobacter asburiae* YT1 and *Bacillus sp.* YP1.[16,17] During the same research period, we found that mealworms, the larvae of the mealworm beetle or *Tenebrio molitor* Linnaeus (a species of darkling beetle), which are much larger in size than waxworms (typically approximately 25 versus 12 mm in length), can eat Styrofoam as their sole diet. Mealworms are pests and have four life stages: egg, larva, pupa, and adult. They are also a profitable animal food available in many insect markets and pet stores. They can easily be reared on fresh oats, wheat bran, or grain with potato, cabbage, carrots, or apple. Here, we report evidence that biodegradation and mineralization of PS does occur in the gut of the mealworms based on the changes in chemical and physical properties of egested residues (fecula) after passage through the gut system compared to the original Styrofoam diet, with the conversion of ingested PS into CO_2 and biomass. Our results confirmed PS biodegradation in the larval gut and indicated the presence of a promising petroleum-based plastic-degrading process in the environment.

MATERIALS AND METHODS

Test Materials. The Styrofoam feedstock tested for biodegradation was obtained from SINOPEC Beijing Yanshan Company, Beijing, China. The chemical composition of the Styrofoam was identified as containing PS > 98% with the number-average molecular weight (M_n) of 40 430 and weight-average molecular weight (M_w) of 124 200 (Table S1 of the Supporting Information). No catalysts and additives were added, as per the manufacturing standard in China (QB/T 4009-2010).

Both α ^{13}C- and β ^{13}C-labeled PS samples were purchased from Sigma-Aldrich, St. Louis, MO. Their material numbers are 604445-SPEC and 604453-SPEC, respectively. The molecular weights of the two chemicals were characterized by gel permeation chromatography (GPC, Alliance V2000, Waters, Milford, MA) and were found to be 51 920 (M_n) and 133 700 (M_w) for α ^{13}C-labeled PS and 51 690 (M_n) and 159 000 (M_w) for β ^{13}C-labeled PS.

Mealworms were purchased from Daxing Insect Breeding Plant, Beijing, China, Insect Breeding Plant, Qinhuangdao, Hebei, China, and the Bug Company, Ham Lake, MN, for the investigation of Styrofoam-eating behavior (Figure S1 of the Supporting Information). The mealworms (growth age at approximately 3−4 instars) from Daxing Insects Breeding Plant were used for all tests.

Styrofoam-Feeding Tests. The mealworms purchased from various sources reared on bran were placed in a polypropylene plastic container with Styrofoam blocks. The mass loss of the Styrofoam block as a function of time caused by mealworm consumption was measured periodically. A test of the survival of mealworms reared in the laboratory solely on a Styrofoam diet in comparison to those reared on the conventional diet of bran was conducted, as described below. Mealworms (500) were reared with 5.8 g of Styrofoam blocks as a sole diet in a climate chamber (RQH-250, Shanghai, China) under controlled conditions [25 ± 1 °C, 80 ± 2% humidity, and 16:8 (light/dark) photoperiod]. During incubation, dead mealworms were removed immediately after their death. The survival curves of mealworm groups fed on Styrofoam were compared to those of the groups fed on bran using a *t* test. Triplicate incubators were prepared for each test.

Collection and Characterization of the Fecula. The mealworms were fed with Styrofoam blocks as their sole diet for 30 days. Subsequently, the mealworms were transferred to a clean box to collect the fecula every 12 h and to avoid carryover of uningested Styrofoam morsels mixing with the accumulated fecula. The collected fecula were immediately stored in liquid nitrogen for further analysis.

Fresh fecula of Styrofoam-feeding mealworms (ca. 1.0 g) were extracted with 150 mL of tetrahydrofuran (THF) as the solvent in a Soxhlet extractor at 90 °C for 12 h. Then, the extracted solution was concentrated to 5 mL. The molecular weights and molecular weight distributions of the Styrofoam and the degraded products in the fecula were determined using GPC with a 50 μL injection each time. THF was used as an eluent at a flow rate of 1.0 mL/min at 40 °C.

Solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) analysis was carried out at 100 MHz on a spectrometer (AVANCE III 400, Bruker, Billerica, MA) at ambient temperature. The operational parameters were 1.5 ms contact time, 4 s recycle delay, 0.013 s acquisition time, 4 μs 90° pulse, and 5 kHz MAS spin.

The thermal characterization was performed using a thermogravimetric (TG) analyzer (TGA-209F1, NETZSCH, Selb, Germany) interfaced with Fourier transform infrared (FTIR, Nicolet Magna IR-8700, Thermo Scientific, Waltham, MA) spectroscopy. Samples of the fecula and Styrofoam (ca. 5 mg) were analyzed at a heating rate of 20 °C/min from ambient temperature to 600 °C under high-purity nitrogen (99.999%) at a flow rate of 10 mL/min.

Test of the Carbon Mass Balance. Carbon balance for the Styrofoam ingested by the worms was estimated using batch trails with incubators equipped with a pre-CO_2 removal and sequential CO_2 trapping system (Figure S2 of the Supporting Information). The worms were fed with Styrofoam as a sole diet in 12 glass jars (500 mL in volume) in an incubator containing 40 worms each. The incubators were sealed with rubber stoppers. Compressed air passed through two CO_2 trappers with 2 M NaOH solution (250 mL) in series to remove CO_2 from the air, which was then moisturized before entering the incubator. The off-air passed through another two CO_2 trappers in series to collect CO_2 produced from the incubator. Prior to the test, the weights of Styrofoam and mealworms added were determined. CO_2 produced from each incubator was collected in NaOH solutions and precipitated with $BaCl_2$ to $BaCO_3$, which was measured after being dried to a constant weight. The measured dry weight of $BaCO_3$ was used for the calculation of trapped CO_2. The incubation time was 4, 8, 12, and 16 days, respectively. At the end of each incubation time, three incubators as a group were sacrificed. The mass changes in Styrofoam, weight of worm biomass, CO_2 produced, and fecula egested were determined. A lifeless

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control was also used to ensure that no CO_2 was generated (Figure S2 of the Supporting Information). The carbon content of the dried worm biomass and fecula was determined using an elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). The conversion of ingested Styrofoam to CO_2 and mealworm biomass was estimated using the procedures described in detail in Figure S2 of the Supporting Information.

^{13}C-Carbon Isotope Tracer Experiments. α ^{13}C-labeled or β ^{13}C-labeled PS powder (20 mg) was mixed with bran powder (10 mg) and then wrapped in 50 mL of 3% agar jelly to feed the mealworms (Figure S3 of the Supporting Information). The jelly food contained 0.4 mg of PS/mL and 0.2 mg of bran/mL. The glass jars (500 mL in volume) were also used as incubators with 40 mealworms each. The living control group of triplicate incubators was fed only with unlabeled bran wrapped in agar jelly. $^{13}CO_2$ in off-air from the incubator sealed with a rubber stopper was trapped in the two-stage CO_2 trappers with 1 M NaOH (250 mL) and precipitated with $BaCl_2$ to $BaCO_3$, as described above. The isotopic composition (atom %) of carbon was analyzed using isotope ratio mass spectrometry (Finnigan MAT 253, Thermo Electron, Waltham, MA).

The incubation with ^{13}C-labeled PS lasted 16 days. At the end of the incubation, mealworms fed both with and without ^{13}C-labeled PS were harvested separately. The mealworms were first blown and then were washed and killed by submerging in ethanol. This step was to avoid contamination of non-metabolized or partially metabolized ^{13}C-labeled products on the exterior of the mealworms. The washed mealworms then were lyophilized to produce dried bodies. After lyophilization, the whole gut tissue (which might contain fecula) was easily removed from the lyophilized body, which was then used for lipid extraction. All lipids were extracted from the bodies using chloroform in a Soxhlet extractor for 6−8 h. The lipid−chloroform solution was then evaporated under N_2, and 100 mg dried samples were resuspended with 4 mL of MeOH/NaOH (0.5 mol/L) at 100 °C for 5 min. After cooling to room temperature, 5 mL of the mixture of MeOH/ethyl ether−boron trifluoride [(MEBT), 1:3, v/v] was added to the flask and methylated at 100 °C for 2 min. After cooling to room temperature, 8 mL of saturated NaCl aqueous solution was added. Finally, 2 mL of *n*-hexane was added to extract the methylated derivatives. Then, the extracted derivatized fatty acids (FAs) were separated by gas chromatography (GC) to produce individual FAs, which were then analyzed by combustion−isotope ratio mass spectrometry (GC−C−irMS, Thermo Electron, Waltham, MA).[18]

RESULTS AND DISCUSSION

Mealworm Styrofoam-Eating Behavior. Feeding trials with Styrofoam were performed with mealworms from Beijing and Qinhuangdao, China, and Ham Lake, MN. The Styrofoam samples used were not pretreated in any way and contained no additives (Table S1 of the Supporting Information). The mealworms from all sources ate Styrofoam as soon as it was fed (Figure 1a). The eating activity of the mealworms (20−25 cmm in length) appeared high and created hollows in the Styrofoam blocks (Figure 1a). The same observations were repeated more than 3 times, regardless of the three different sites where the mealworms were purchased (Figure S1 of the Supporting Information). Their eating activity resulted in a decrease in the mass of Styrofoam, which depended upon the test period, the number and growth stage of the mealworms, and the batch of mealworms purchased. For example, a group of 500 mealworms (n = 3 groups) from Beijing caused a total mass loss of Styrofoam accounting for 31.0 ± 1.7% of the initial mass (5.8 g) within 30 days (Figure 1b).

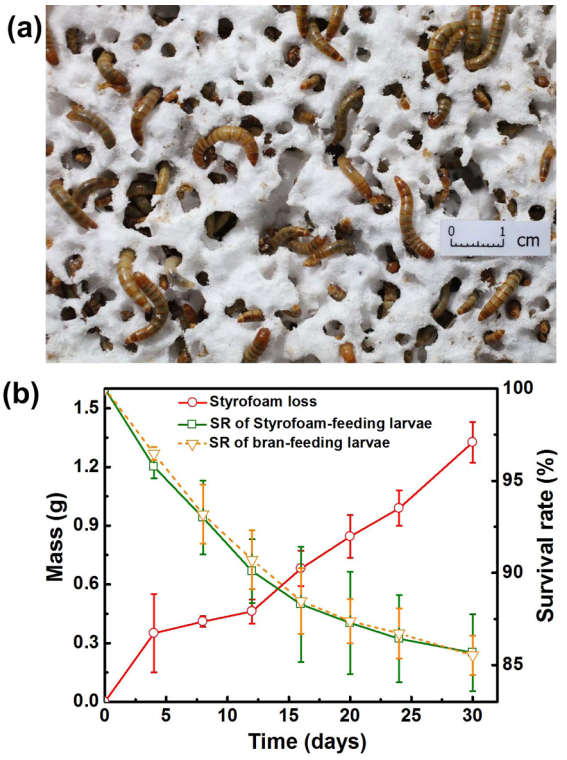

Figure 1. Styrofoam-eating behavior of mealworms (*T. molitor*). (a) Larvae of *T. molitor* chew and eat the Styrofoam block. (b) Styrofoam mass loss caused by a group of mealworms eating and the SR of Styrofoam-fed and conventional diet (bran)-fed mealworm populations over 30 days [mean ± standard deviation (SD); n = 3 groups for each condition; 500 mealworms for each group]. Survival curves are illustrated by the proportional shift in surviving mealworms over time. No significant difference (t test; p = 0.944 > 0.05) in the survival curves between the Styrofoam- and the bran-feeding mealworms was observed.

A test for the determination of the survival rate (SR) over a 1 month period using the same batch of mealworms from Beijing showed that the difference between the SR of Styrofoam-feeding mealworms (500 mealworms as a group; n = 3 groups) and the SR of conventional diet (or bran)-feeding mealworms was not significant (500 mealworms as a group; n = 3 groups; t test; p = 0.944 > 0.05) (Figure 1b). These Styrofoam-feeding mealworms survived for 1 month more until they stopped eating to become pupae, which then emerged as adult beetles within 2 weeks. These observations imply that Styrofoam feeding did not pose a negative impact on the survival capabilities of the mealworms.

Changes in the Chemical Structure and Composition of Ingested Styrofoam. According to our observation, the mealworms began to egest fecula 12−24 h after ingestion of Styrofoam (inset of Figure 2a), suggesting a short retention time (<24 h) for the Styrofoam held in the gut. Fresh fecula were collected and analyzed to determine whether changes in chemical structure and composition of the ingested Styrofoam had occurred after passage through the gut.

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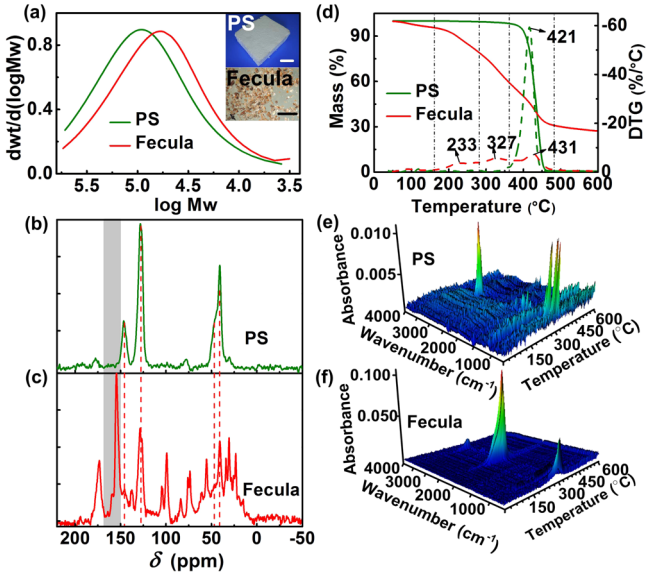

Figure 2. Changes in the chemical structure and composition of Styrofoam after passage through the mealworm gut as fecula. (a) Molecular weight distribution shift of the fecula extract versus the control PS. The inset picture is the control (up; scale bar = 1 cm) versus the fecula (down; scale bar = 1 mm). (b and c) ^{13}C CP/MAS NMR spectra of the control and the fecula. The new appearance of phenyl derivatives at the δ 150−160 ppm resonance regions in the fecula was indicated with a gray column. (d) TG/DTG curves of the control PS and the fecula (TG curves are solid lines, and DTG curves are dashed lines). (e and f) Three-dimensional infrared (IR) spectra of gaseous compounds produced in the TG equipment during thermal decomposition of the control and the fecula.

The change in the long-chain structure of PS molecules was investigated by analyzing the whole molecular weight distributions and average molecular weights of the degraded products in the fecula and the control PS using GPC. The degraded products were extracted from the collected fecula (ca. 1.0 g) with THF. The whole molecular weight distribution curve of the fecula extract shows a shift toward lower molecular weight compared to the molecular weight distribution of the control PS (Figure 2a). M_n and M_w for the fecula extract also decrease compared to the control PS (M_n, 32 260 versus 40 430; M_w, 98 330 versus 124 200). These results suggest that depolymerization/cleavage of the long-chain structure of PS took place and lower molecular weight fragments were newly formed in the mealworm gut. The observation of the decrease in M_n and M_w is a major indication of depolymerization and degradation of polymers,[19] which has been reported during biodegradation of PE films by the two bacterial strains isolated from the guts of waxworms in our laboratory.[16,17]

The chemical compositions of Styrofoam and fecula (residues of the Styrofoam egested through the gut of the mealworms) were characterized using solid-state ^{13}C CP/MAS NMR and thermal analysis. Analysis of the ^{13}C CP/MAS NMR is usually applied to identify directly the native composition of the solid substrate without separation of components.[20−22] As shown in Figure 2b, only four resonance signals were detected in the spectrum of the control PS. Two resonance signals at δ 146 and 128 were assigned to non-protonated and protonated aromatic carbons, and two resonance signals at δ 41 and 46 corresponded to the methylene and methyl (aliphatic) carbons.

In the spectrum of the fecula (Figure 2c), some new resonance signals were detected in the spectrum of the fecula (Figure 2c). The newly appearing alkyl- and methyl-C resonance signals (δ 10−40) could be assigned to aliphatic hydrocarbons.[21] The newly emerging resonance signals at δ 175, 104, 99, 84, 75, 73, 61, 55, and 23 were attributed to chitin from the insect cuticle.[21] The new aromatic C (δ 140, 154, and 160) resonance signals could be ascribed to phenyl derivatives, as reported by Gilardi et al.[22] The phenyl derivatives are possible proxies for the fragments or smaller molecules produced during depolymerization/oxidation of PS.[8]

Thermal analysis can be used to compare the changes in chemical composition of the solid substrate by analyzing the gaseous compounds produced during substrate pyrolysis under anoxic conditions. TG coupling with the FTIR spectroscopy method is based on the precise study of the weight loss (thermal decomposition) of the sample during programmed temperature and online analysis of the evolved gaseous compounds produced during thermal decomposition. TG/differential thermogravimetric (DTG) profiles during the thermal decomposition of the fecula and the control Styrofoam as a function of the temperature were shown in Figure 2d.

For the control, 98.0% of weight loss occurred during only one stage, which ranged from 360 to 480 °C, and the maximum decomposition rate occurred at 421 °C. In contrast, the fecula showed three weight loss stages, stage 1 of 15.8% at 175−275 °C, stage 2 of 23.4% at 275−360 °C, and stage 3 of 26.6% at 360−480 °C. The maximum decomposition rates during the three stages occurred at 233, 327, and 431 °C, respectively.

Under the same heating program, the fecula decomposed in more stages than the control, indicating that the fecula contained not only PS but also other new components produced during digestion in the mealworm gut. During stage 3, the weight loss of fecula was obviously less than the weight loss of the control, demonstrating the depletion of PS content in the fecula.

Gaseous compounds produced in the TG process were analyzed using FTIR. The three-dimensional (3D) FTIR profiles (panels e and f of Figure 2), compiled over the entire temperature range of thermal decomposition, show that the evolved gaseous compounds generated from the control Styrofoam and the fecula give different IR absorption.

For the control, the obvious absorptions were generated in the temperature range from 360 to 480 °C (Figure 2e). A representative FTIR spectrum at 421 °C shows that all absorbance peaks are attributable to styrene, which represents the main decomposition product of PS (Figure S4a of the Supporting Information).

For the fecula, the obvious absorptions were generated in the temperature range from 175 to 480 °C (Figure 2f). Representative FTIR spectra at 233, 327, and 431 °C show that the strongest absorbance peaks at 2000−2250 and 2268−2395 cm^{-1} could be assigned to carbon monoxide and carbon dioxide (Panels b to d of Figure S4 of the Supporting Information), respectively, which often represent the decomposition products of newly produced components in the fecula. The absorbance peaks attributed to styrene, the main decomposition product of PS, were very weak, substantiating the depletion of PS content in the fecula (panels b−d of Figure S4 of the Supporting Information).

As indicated by the NMR spectra (panels b and c of Figure 2) and thermal analysis (panels d−f of Figure 2), both native compositions and chemical components of the evolved gaseous compounds produced during thermal decomposition were different between the control and the fecula, indicating that the

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Table 1. Carbon Balance Estimates of the Ingested Styrofoam Converted into Biomass, CO_2, and Fecula in the Batch Styrofoam-Feeding Trials with Different Incubation Periods[a]

incubation time (days)	item	initial carbon (mg)	final carbon (mg)	Δ = final − initial (mg)	percentage of ingested styrofoam recovered (%)
4	styrofoam	592.1 ± 19.0	501.3 ± 24.0	−90.8	
	biomass	933.0 ± 22.0	933.5 ± 16.0	0.5	0.6
	CO_2	0.0	18.8 ± 0.4	18.8	20.7
	fecula	0.0	66.8 ± 14.8	66.8	73.6
	total recovery				94.9
8	styrofoam	610.0 ± 22.0	500.0 ± 39.0	−110.0	
	biomass	896.0 ± 16.0	896.6 ± 32.0	0.6	0.5
	CO_2	0.0	39.2 ± 1.0	39.2	35.6
	fecula	0.0	65.7 ± 15.7	65.7	59.7
	total recovery				95.8
12	styrofoam	720.0 ± 5.0	563.0 ± 26.0	−157.0	
	biomass	794.0 ± 24.0	795.0 ± 23.0	1.0	0.6
	CO_2	0.0	65.0 ± 12.0	65.0	41.4
	fecula	0.0	89.0 ± 16.0	89.0	56.7
	total recovery				98.7
16	styrofoam	826.0 ± 54.0	609.0 ± 47.0	−217.0	
	biomass	815.0 ± 36.0	817.0 ± 6.0	1.0	0.5
	CO_2	0.0	103.6 ± 3.0	103.6	47.7
	fecula	0.0	106.7 ± 10.0	106.7	49.2
	total recovery				97.4

[a] n = 3 incubators for each incubation time, with 40 mealworms for each incubator. The carbon contents of Styrofoam, biomass, and fecula were calculated using their dry weight and carbon contents measured by an elemental analyzer.

degradation of ingested Styrofoam and production of degraded products took place in the guts of the mealworms.

Mineralization of Ingested Styrofoam. The conversion of the carbons of Styrofoam to CO_2, mealworm biomass, and fecula residues was assessed by a series of carbon mass balance tests with different incubation periods of 4, 8, 12, and 16 days with 40 mealworms in each incubator (Figure S2 of the Supporting Information). The results showed that total carbon recovery efficiencies were greater than 95% (Table 1). The carbon balance estimates showed that the carbon of the ingested Styrofoam recovered as CO_2 was increased from 20.7 to 47.7% and the carbon of the ingested Styrofoam egested as fecula was decreased from 73.6 to 49.2% from day 4 to day 15 (Figure 3a and Table 1), suggesting that the activity for the digestion of ingested Styrofoam increased progressively.

The mineralization of PS to CO_2 was further verified through determination of the production of $^{13}CO_2$ by the mealworms fed either α ^{13}C- or β ^{13}C-labeled PS-containing diet (Figure S3 of the Supporting Information). The mealworms were continuously fed a 3% solidified jelly containing each of two ^{13}C-labeled PS (0.4 mg/mL) and bran (0.2 mg/mL) over a 16 day period. For the control, mealworms were fed on bran. CO_2 released in the off-air was trapped in 1 M NaOH solution and recovered as $BaCO_3$ for analysis. The mean δ ^{13}C value of CO_2 released by the mealworms fed on bran was −8.2 ‰, while the mean δ ^{13}C values of CO_2 released by mealworms fed on α and β ^{13}C-labeled PS diets were 3.3% and 3.9‰, respectively (Figure 3b), indicating that, in comparison to the control mealworms fed with bran, significant ^{13}C enrichment ($p < 0.05$) was observed in the CO_2 released from ^{13}C-labeled PS-feeding mealworms at the end of the 16 day period, confirming that ^{13}C-labeled PS was partially mineralized in $^{13}CO_2$.

Assimilation of ^{13}C-PS by Styrofoam-Feeding Mealworms. Carbon mass balance estimates showed that the carbon of the ingested Styrofoam recovered as mealworm biomass remained at only approximately 0.5% and the biomass

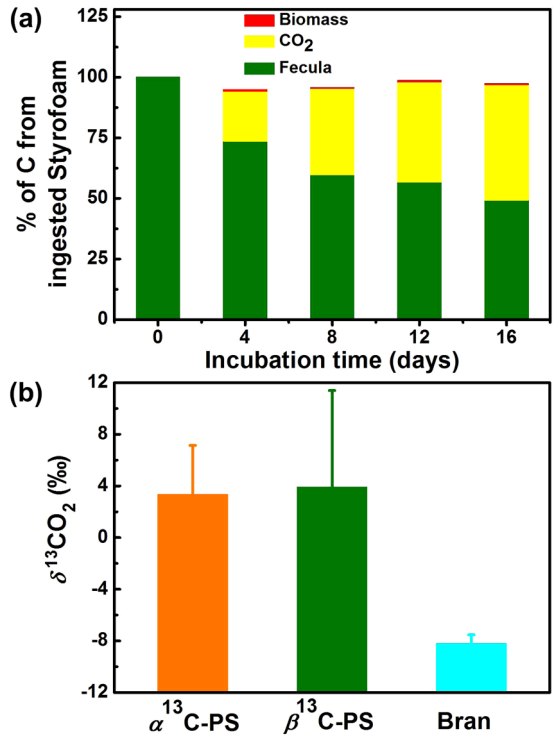

Figure 3. Conversion of PS into CO_2. (a) Carbon proportion of the ingested Styrofoam recovered as CO_2, mealworms biomass, and fecula residues based on the carbon balance estimates over different incubation times of 4, 8, 12, and 16 days (mean value; n = 3 groups for each condition; 40 mealworms for each group). Detailed calculations are shown in Figure S2 of the Supporting Information. (b) ^{13}C signatures of CO_2 produced by the mealworms fed with ^{13}C-labeled PS (α or β ^{13}C-PS) versus unlabeled bran over a 16 day incubation period (mean ± SD; n = 3 groups for each condition; 40 mealworms for each group).

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weight of Styrofoam-feeding mealworms remained almost unchanged (increased by ca. 0.2%) after the 16 day test period (Figure 3a and Table 1).

A compound-specific stable isotopic technique with FAs as biomarkers has been applied to determine carbon assimilation in insects.[18] We analyzed the δ ^{13}C value in individual FAs of the mealworms fed on a ^{13}C-labeled PS diet or bran using GC−C−irMS. Figure 4 shows that the δ ^{13}C values of individual FAs

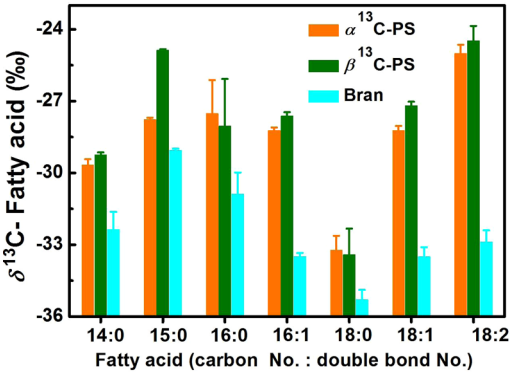

Figure 4. ^{13}C signatures of individual FAs extracted from the mealworms fed with ^{13}C labeled PS (α or β ^{13}C-PS) versus unlabeled bran after a 16 day incubation period (mean ± SD; n = 3 groups for each condition; 40 mealworms for each group). The paired t test was used to evaluate the difference of ^{13}C signatures of individual FAs between the bran-feeding mealworms and the α or β^{13}C labeled PS-feeding mealworms (p = 0.004 and 0.002 < 0.05, respectively). The δ ^{13}C values are assigned relative to the Pee Dee Belemnite (PDB) standard. C14:0, myristic acid; C15:0, pentadecanoic acid; C16:0, palmitic acid; C16:1 (Δ9), palmitoleic acid; C18:0, stearic acid; C18:1 (Δ9), oleic acid; and C18:2 (Δ9 + Δ12), linoleic acid.

were significantly higher (paired t test; p = 0.004 and 0.002 < 0.05, respectively) in the mealworms fed with either α ^{13}C- or β ^{13}C-labeled PS than the controls fed with bran, especially in the unsaturated FAs. Nevertheless, the δ ^{13}C values still stayed negative, suggesting that ^{13}C from the ^{13}C-labeled PS was assimilated into mealworm biomass, but the fraction was limited.

A test with 40 mealworms as a group in triplicate was performed to determine the weight change under three different conditions after 16 days. By comparison, the biomass dry weight of the bran-feeding mealworms increased by 33.6%, but that of starving mealworms decreased by 24.9% after a 16 day period (Figure S5 of the Supporting Information). The Styrofoam-feeding mealworms did not appear to increase their biomass dry weight (increased by ca. 0.2%) to the same extent as bran-feeding mealworms. Styrofoam, unlike the bran, does not have the proper water content and necessary growth nutrients, such as proteins, phosphorus, vitamins, and minerals. Therefore, the lack of nutrients and relatively poor biodegradability of Styrofoam resulted in the mineralization of ingested Styrofoam to CO_2, providing a limited energy source for biomass synthesis or growth. Similarly, the study of Butler and Buckerfield on digestion of synthetic lignin hydrocarbons by termites in the absence of other nutrients indicated that 8.5−32.4% ^{14}C from ^{14}C-labeled lignin was converted to CO_2, while only a limited fraction (0.002−0.004%) of ^{14}C was assimilated into termite bodies after a 50 day test period.[23]

However, it is obvious that the starving mealworms were dependent upon the endogenous metabolism of their body as an energy source for life activities, resulting in their biomass weight loss of 24.9%. Therefore, the host mealworms received a marginal benefit from the mineralization of the ingested Styrofoam into CO_2, which provided an energy source for life activities. Otherwise, the weight of the Styrofoam-feeding group would have declined as significantly as the starving group.

Additional studies are needed to examine the effect of these nutrients on the Styrofoam digestion and mealworm growth when fed a Styrofoam diet. The metabolic pathway of PS degradation will be further investigated in detail.

Implications. This work presents convincing evidence that effective biodegradation and mineralization of PS or Styrofoam, which have not been previously reported, occur in the gut tract of mealworms. The mealworms are the first reported insect larvae that are capable of degrading and mineralizing a common persistent petroleum-based plastic PS.

In our companion paper (10.1021/acs.est.5b02663), we further reported that the gut microbiota play an essential role in the biodegradation of PS or Styrofoam. The mealworm gut can be considered an efficient bioreactor. Physical and biochemical "treatment" (by chewing, ingesting, mixing, reacting with gut contents, microbial degradation by gut microbial consortia, taking up metabolic products by host, etc.) are possibly critical for the success of rapid PS degradation in the bioreactor. The PS-degrading microbial communities ubiquitously colonize in the guts of the mealworms. PS degradation is thus analogous to microbial degradation of cellulose in ruminating mammals and wood in termites for the mutual benefit of the metabolism of microbial consortia and host. More research will be conducted to fully understand the interplay between the worm metabolism and microbial metabolism and gut contents.

We propose a primary schematic diagram for this symbiotic degradation of Styrofoam (or PS) in the gut of *T. molitor* (Figure 5): (step 1) Styrofoam is chewed into small fragments

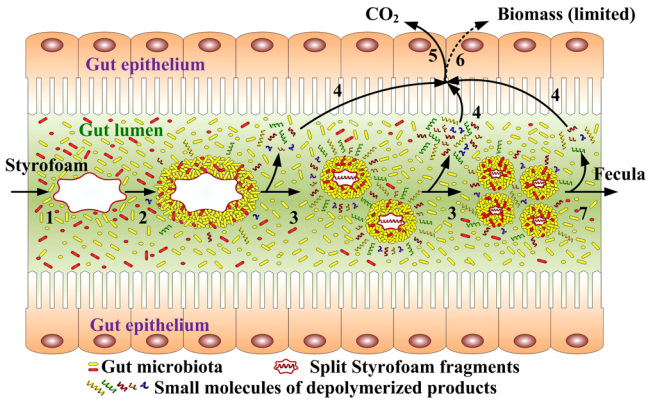

Figure 5. Schematic diagram of the proposed system for PS degradation in the gut.

and ingested into the gut; chewing reduces the size of the plastic and increases the contact surface area of PS fragments with microbes and extracellular enzymes; (steps 2 and 3) ingested fragments are mixed with gut microbiota that excrete extracellular enzymes to catalyze the depolymerization of the fragments into small-molecule products; (steps 4−6) products are mainly degraded or mineralized into CO_2 by multiple functional microbes and/or the mealworm host, and limited carbons of the products are further assimilated into biomass; and (step 7) residual Styrofoam fragments and other

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intermediates with some gut microbes are egested as fecula, where further degradation could continue.

Our discovery that mealworms can degrade PS will provide considerable enthusiasm for prospecting the gut system for new bacterial strains, key enzymes, and system conditions that contribute to the depolymerization and biodegradation of PS as well as other petroleum-based plastics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02661.

Styrofoam-eating mealworms from three different sources (Figure S1), procedures and calculations used to estimate the carbon balance of Styrofoam loss, fecula residues, CO_2, and biomass in batch Styrofoam-feeding trials (Figure S2), procedures for ^{13}C stable carbon isotope tracer experiments (Figure S3), TGA−FTIR thermograms (Figure S4), impact of the feeding condition on the biomass dry weight of mealworms after the 16 day test period (Figure S5), and characterization of Styrofoam feedstock (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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