Biodegradation of Plastics in *Tenebrio* Genus (Mealworms)

Shan-Shan Yang and Wei-Min Wu

Contents

S.-S. Yang (✉)
State Key Laboratory of Urban Water Resource and Environment, School of Environment, Harbin Institute of Technology, Harbin, China
e-mail: shanshanyang@hit.edu.cn

W.-M. Wu
Department of Civil and Environmental Engineering, William and Cloy Codiga Resource Recovery, Stanford, CA, USA

Center for Sustainable Development and Global Competitiveness, Stanford University, Stanford, CA, USA
e-mail: billwu@stanford.edu

Defu He and Yongming Luo (eds.), *Microplastics in Terrestrial Environments - Emerging Contaminants and Major Challenges*,
Hdb Env Chem (2020) 95: 385–422, DOI 10.1007/698_2020_457,

Abstract Most petroleum-based plastics are resistant to biodegradation in the environment. Observation of damage, penetration, and ingestion of plastics by insects and their larvae lead to research on biodegradation of plastics by insects. The larvae of darkling beetles (Coleoptera: Tenebrionidae), especially *Tenebrio molitor* and *Tenebrio obscurus* larvae, showed the capacity of rapid gut microbe-dependent degradation of polystyrene (PS). *T. molitor* larvae also degrade low-density polyethylene (LDPE). The biodegradation was evaluated on the basis of plastic mass balance, modification of ingested polymers, formation of biodegraded intermediates, as well as ^{13}C isotopic tracer tests. Ingested PS or LDPE polymer can be depolymerized by up 60–70% within 12–24 h after 1- or 2-week adaption. Ingested PS or PE supports the larvae with energy for life activities but not growth. Co-feeding normal diet (e.g., bran) enhances PS and PE consumption rate significantly. Gut microbial communities shifted after the larvae were fed with PS or PE. A few plastic-degrading gut bacterial strains have been isolated from gut of *T. molitor*, but they grow on plastics slowly. The rapid biodegradation of PS and PE is likely a result of synergistic effects of intestinal microbial activities and host digestive system, and further research is needed to understand the mechanisms.

Keywords Biodegradation, Mealworms, Microbial community, Plastics, *Tenebrio* genus

1 Introduction

1.1 Major Plastic Wastes in Environment

Ever since the first industrial-scale production of synthetic polymers (plastics) took place in the 1940s, the production, consumption, and waste generation rate of plastic solid waste (PSW) has increased considerably [1]. The global annual plastic production accounts for more than 300 million tonnes [2]. The growth of plastic production in the past decades has substantially outpaced any other manufactured materials. The same properties that make plastics so versatile in innumerable applications – durability and resistance to degradation – make these materials difficult or impossible for nature to assimilate.

Today, there is a growing scientific consensus demonstrating that PSW is a major environmental concern of increasing global significance. In 2010, the total amount of PSW produced by 192 coastal countries in the world was 275×10^6 t, of which $4.8–12.7 \times 10^6$ t finally entered the ocean, while China imported $1.32–3.53 \times 10^6$ t of PSW into the ocean, ranking first in the world. In the USA, PSW generation found in municipal solid waste (MSW) has increased from 11% in 2002 [3] to 12.1% in 2007 [4]. In China, over 59.5×10^6 t of PSW is produced in 2015, accounting for 22.9% of MSW generation. More and more generation of PSW has raised enormous

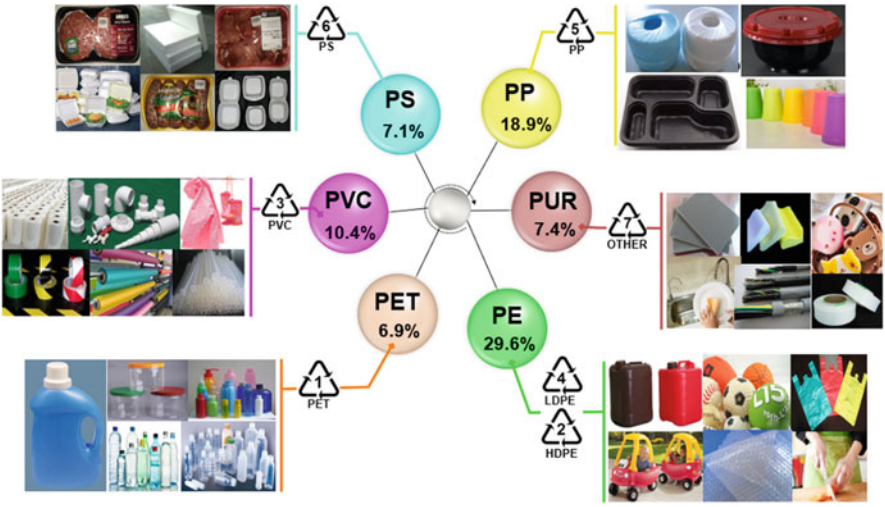

Fig. 1 The most widely used plastics on the market (figure from Yang et al. [8])

questions and challenges to the society regardless of their sustainability awareness and technological advances [1]. Moreover, the annual plastic production has been and will continue increasing in the foreseeable future [5, 6]. It is predicted that by 2025, the annual import of plastic wastes into the ocean will increase by ten times [7]. However, among the generated PSW, less than half of it was confined to discard and either contain in a managed system, such as sanitary landfills and open dumps. Major plastic polymers produced include polyethylene (PE) 29.6%, polypropylene (PP) 18.9%, polyvinyl chloride (PVC) 10.4%, polyurethane (PUR) 7.4%, polystyrene (PS) 7.1%, and polyethylene terephthalate (PET) 6.9% (Fig. 1) [9]. Thus, without a well-designed and tailor-made management strategy for end-of-life plastics, it is only reasonable to find a considerable amount of plastics wastes in the final stream of municipal solid waste.

Of particular concern, plastic pollution has the potential to poison animals and pose serious threats to human health. According to a hazard-ranking model based on the United Nations' Globally Harmonized System of Classification and Labeling of Chemicals, the chemical ingredients of more than 50% of plastics are hazardous [10]. These harmful chemicals leached from the plastic wastes or in the form of small or microplastic debris are more likely to infiltrate food webs [11] and potentially impact ecologically important species including mussels, salt-marsh grasses, and corals [11, 12]. Humans and mussels that ingested the chemicals from plastics and small or microplastic debris could accumulate in the body and harm the cells and other tissues [11, 13]. The disadvantages of plastic pollution must be carefully considered to design the best solutions to the environmental challenges posed by the enormous and sustained global growth in plastic production and use.

1.2 Biodegradation of Plastics by Microorganisms

Natural degradation is hard to get rid of plastic waste. The majority of plastics is resistant to decomposition by microorganisms [14] due to high-molecular-weight structural complexity and hydrophobic surfaces [15]. These properties make the polymer inaccessible to the microbial enzymes. The potential to decompose and degrade plastics in various environments has been studied for decades, in order to investigate the fate of plastics in the environment and to find solution to increasing accumulation of plastic wastes [16–20]. However, most of these plastics are recalcitrant to biodegradation by microorganisms, and the degradation rate is a generally very slow [21, 22]. For instance, Ohtake et al. [21] examined plastic polymer products buried under soil for 32 years and did not find any evidence of biodegradation of PS and PVC but found extremely slow biodegradation of low-density polyethylene (LDPE) film and bottle [19]. To date, slow biodegradation of LDPE, PP, and PET polymers by mixed and single microbial cultures has been reported. The mass removal or degradation is measured in periods of weeks, months, or years. Table 1 summarizes some research results of microbial degradation of major plastics, which has proved that the plastics can be degraded by several bacteria or flora from various environments, especially from soil, sludge, landfill, and other contaminated sites. The challenges to microbial biodegradation of plastics are summarized as follows:

1. Extremely poor biodegradation efficiency. The majority of previous studies focus on isolation and characterization of microbial strains in the ability of degrading PE, PS, PP, and PE (Table 1). But the isolated cultures performed poorly in both microbial growth and metabolism of target plastics.
2. Unclear mechanism of biodegradation of plastics. Most reports mainly focused on the colonization on plastic materials as well as mass loss of plastic materials added. The key metabolic genes and enzymes are rarely revealed. Therefore, searching for effective key genes and enzymatic systems for biodegrading plastics and explaining the degradation mechanisms are the key scientific questions needed to be answered.
3. Unknown intermediates and the impacts and fate of additives. The metabolic pathways and intermediates of biodegradation of plastics (PE, PS, and PP) are still unknown. The potential hazards of these degradation products have also not been investigated. In the biodegradation processes, the impacts and the fates of various additives should be addressed.

1.3 Plastic Damaging/Degradation by Insects

Since the 1950s, as plastic materials had been rapidly developed and widely applied, plastic degradation received attentions, and some research had been performed about plastic films of PE, PP, and PVC in pest insects. Most of these insects belong to

Table 1 Reported tests on microbial degradation of major plastic materials

References	Culture source	Results	Test period
Guillet et al. [23]	Activated sludge	0.7% of PS mineralized	75 days
Sielicki et al. [24]	Soil and liquids	1.5–3.0% of PS degraded	4 months
Kaplan et al. [25]	17 fungi, 5 soil invertebrates, 5 groups of microbial flora (sludge, soil, feces, garbage, corrupt plastics); 5 groups of mixed microbial flora	0–0.24% of PS degraded	35 days
		0.04–0.57% of PS degraded	5–11 months
Mor and Sivan [26]	*Rhodococcus ruber* C208	0.5% and 0.8% of PS weight loss	4–8 weeks
Atiq et al. [27]	*Paenibacillus urinalis* NA26, *Bacillus* sp. NB6, and *Pseudomonas aeruginosa* NB26	Colonization on PS. But no PS weight loss was confirmed	8 weeks
Albertsson [28]	Three *Phellinus ribis*	0.36–0.39% of PE mineralized and 0.02% of PE assimilated	2 years
Albertsson et al. [29]	Mixed culture of fungus *Japonica* and *Fusarium*	0.5% of PE mineralized	498 days
Sivan et al. [30]	*Rhodococcus* C208	0.86% of PE degraded	7 days
Tribedi and Sil [31]	*Pseudomonas* AKS2	4–6% of PE degraded	45 days
Balasubramanian et al. [32]	*Arthrobacter* GMB5 and *Pseudomonas* GMB7	12% and 15% of PE degraded	30 days
Kyaw et al. [33]	Four *Pseudomonas* strains	20%, 11%, 9%, and 1.75% of PE degraded	120 days
Harshvardhan and Jha [34]	*Kocuria palustris* M16, *Bacillus pumilus* M27, and *Bacillus subtilis* H1584	1%, 1.5%, and 1.75% of PE degraded	30 days
Yamada-Onodera et al. [35]	*Penicillium* YK	Increase of average molecular weight of PE	3 months
Cacciari et al. [36]	Microbial flora	Small molecular products of PP increased	6 months
Arkatkar et al. [37]	Soil mixed culture	0.4% of PP weight loss	1 year
Arkatkar et al. [38]	*Pseudomonas azotoformans*, *Pseudomonas stutzeri*, *Bacillus subtilis*, *Bacillus flexus*	2.5% of PP weight loss. Ultraviolet treatment improved biological accessibility of PP	12 months
Jeyakumar et al. [39]	Two fungi (F1 and F2)	Pretreatment and modification of PP effectively improved the degradation	1 year

PE polyethylene, *PP* polypropylene, *PS* polystyrene

moths in the family Pyralidae of the order Lepidoptera and darkling beetles in the family Tenebrionidae of the order Coleoptera [40–42]. Darkling beetles (*Tribolium castaneum*, *Rhizopertha*, *Lasioderma serricorne*, *Tenebrioides mauritanicus*,

Zophobas morio, etc.) in Tenebrionidae and several moths and their larvae (*Plodia interpunctella*, *Galleria mellonella*, *Ephestia cautella*) in the family Pyralidae were investigated and known to penetrate and/or consume PE, PVC, and PP films, but no efforts were made to assess the fate or biodegradation of ingested plastics [40–42]. In 2014, researchers in China reported isolation of PE-degrading bacterial strains from LDPE-eating Indian meal moth, i.e., *P. interpunctella* larvae [43], indicating that the larvae could have the capacity of degrading LDPE. Since 2017, biodegradation of PE in Pyralidae larvae has been reported in greater wax worms (*Galleria mellonella*) [44, 45] and lesser wax worms (*Achroia grisella*) [46]. Biodegradation of PS and PE in larvae of darkling beetles (Coleoptera: Tenebrionidae) has been confirmed since 2015 [47–52].

The research on plastic degradation in Tenebrionidae started as the observation of consumption of Styrofoam (or expanded PS foam) by yellow mealworms (*Tenebrio molitor* larvae) was reported by students competing in high school science fairs in the early 2000s: in 2003, Ms. Chong-Guan Chen raised yellow mealworms fed with PS foam and hypothesized that PS was biodegraded [53]; in 2009, Ms. I-Ching Tseng claimed isolation of bacterial strains from yellow mealworm gut using PS as the sole carbon source [54]. Both larvae of *Tenebrio molitor* Linnaeus 1758, commonly referred to as yellow mealworms, and *Tenebrio obscurus* Fabricius 1792, referred to as dark mealworms, belong to *Tenebrio* genus of Coleoptera within the cosmopolitan family Tenebrionidae, which is comprised of more than 20,000 species. Convincing academic evidence of PS degradation in *Tenebrio* genus was reported using *T. molitor* larvae from Beijing, China, in 2015 [47, 48]; then in the larvae from California, USA [49]; and 12 sources from China, the USA, and the UK [50]. PS degradation in *T. obscurus* larvae was reported in 2019 [51]. Ingestion and biodegradation of LDPE in *T. molitor* were also reported in 2018 [52]. In addition, *Zophobas atratus* Fabricius 1775 (Coleoptera: Tenebrionidae) larvae (commonly named as superworms, King Worms or Morio Worms) have been tested for eating PS foams by high school students at science fairs and posted on web sites for years. PS-biodegrading capability of *Z. atratus* larvae has been confirmed recently [55]. In 2010, Miao and Zhang [56] tested *Z. morio* larvae fed with LDPE, linear low-density polyethylene (LLDPE), ethylene-vinyl acetate (EVA), and PVC microplastics and Styrofoam but did not provide solid data on biodegradation. They fed the larvae with respective plastic material versus bran with a ratio of 1:1 (w/w) and then 0.5:1, 0.2:1, and finally 0:1 each week as well as with Styrofoam (PS). The larvae consumed 2.4 g PS per kg larvae per day. Based on analysis of frass egested using thermogravimetry-differential thermal synchronous analyzer (TGA-SDTA), no changes in physical properties of LDPE and EVA but changes in physical properties of residual PVC and PS were observed.

2 Biodegradation of Polystyrene (PS) and Polyethylene (PE)

2.1 Polystyrene Degradation

Industrial production of PS began around 1930 [57]. PS polymer, which is made from styrene monomers containing C=C bonds, possesses long hydrocarbon backbone with a benzene ring linked to every other carbon atom [14]. On the basis of structure, PS can be classified into three forms (Fig. 2a). PS containing all of the phenyl groups on one side is termed as isotactic PS. If the phenyl groups are randomly distributed, then it is called atactic PS. Syndiotactic PS is a new type of PS. The phenyl groups on the polymer chain are attached to alternating sides of the polymer backbone chain. The only commercially important form of polystyrene is atactic, in which the phenyl groups are randomly distributed on both sides of the polymer chain. This random positioning prevents the chains from aligning with sufficient regularity to achieve any crystallinity.

The PS products include (a) expanded PS (EPS), trade name Styrofoam, which is widely used for building insulation and packing; (b) extruded PS used for food containers, coffee cups, food trays, etc.; and (c) high-density PS products which commonly used as liquid containers, toys, etc. In 2014, the global market for PS materials was valued at $32 billion with a projected 2020 market valued at $42 billion [58]. 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. PS wastes are major pollutants of soils, rivers, lakes, and oceans [59] and are among the major microplastics (<5 mm) accumulating in the environment including ocean, surface water, and wastewater [9, 60].

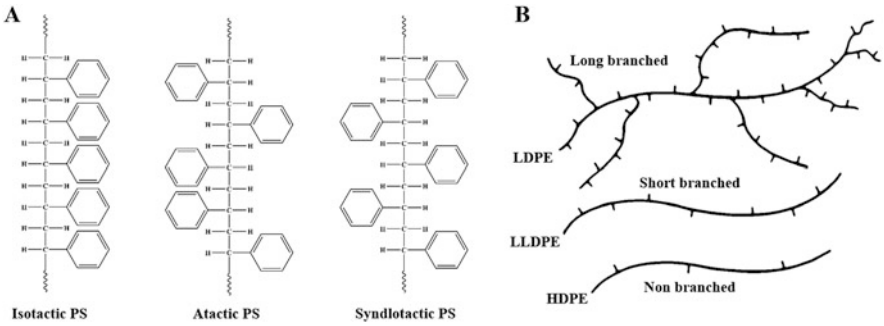

Fig. 2 The major different PS and PE polymers based on structure. (**a**) PS polymers (left) isotactic PS, (middle) atactic PS, and (right) syndiotactic PS. Commonly used PS products are atactic PS. (**b**) PE polymers. HDPE, LDPE, and LLDPE

Attempts to investigate biodegradation of petroleum-based PS pollutants can be traced back to the 1970s. Researchers have studied feasibility of PS biodegradation with microbes from soils, seawater, landfill sediment, activate sludge, and compost. Some of these studies included use of ^{14}C-labeled PS [16, 24–26, 61]. However, it has been thought that PS is not subject to efficient and rapid biodegradation by microorganisms and soil invertebrates [21, 62]. The scientific consensus was that rapid PS degradation would require photolytic or thermolytic cleavage of –C–C– bonds prior to biodegradation [57, 63, 64].

2.2 Polyethylene (PE) Degradation

PE is the most used polymers around the world, and is utilized in packaging, representing ~40% of total demand for plastic products (www.plasticseurope.org) with over a trillion plastic bags used every year [65]. As the most common petroleum-based plastic, PE is expressed as "$[CH_2-CH_2]_n$" and comprises a linear backbone of carbon atoms, which is resistant to degradation [43, 44]. Commercial PE polymers include HDPE (high-density polyethylene), LLDPE, and LDPE (Fig. 2b). HDPE is composed of linear chains which are packed closely together, with a very low level of short-chain branching, and has a high degree of crystallinity (70–95%) [66]. LDPE is characterized by a significant level of long-chain branching (typical branch length of several hundred carbon atoms) as well as short-chain branching (2–6 carbon atoms long). The short branches of LDPE hinder close packing and result in a relatively low crystallinity (45–60%). LLDPE is a linear molecule with higher level of short-chain branching than HDPE but without long chains with a middle crystallinity. The structure and physical properties of PE polymers certainly impact biodegradability. Since the early 1970s, tests on the biodegradation of virgin PE (unpretreated and without any additives), mainly LDPE, had been performed under natural environmental conditions, including soils, seawater, sludge, and compost, which harbor a multitude of diverse microbial communities [16–21, 28]. These studies concluded that the biodegradation of virgin PE was extremely slow and limited in mixtures of some microbial communities [43]. One of the well-known tests was that Ohtake et al. [19, 21] found extremely slow biodegradation of LDPE film and bottle after they were buried under soil for more than 32 years using GPC and FTIR analyses. Biodegradation of PE in the environment occurred mainly through the biological activity of microorganisms after photo- or thermo-oxidation [67, 68]. Slow (in periods of weeks/months) PE biodegradation has been observed, given appropriate conditions. For example, modest degradation of PE was observed after nitric acid treatment and incubation for 3 months in a liquid culture of the fungus *Penicillium simplicissimum* [35]. Slow PE degradation was also recorded after 4–7 months exposure to the bacterium *Nocardia asteroides* [69]. Besides, almost no biodegradation of PE through the biological activities of select microorganisms can be observed without pretreatments

[67]. However, recently, much more rapid biodegradation of PE has been found in plastic-eating insect larvae of two moth larvae [44–46] and yellow mealworms [52].

2.3 Tenebrio *Genus in Darkling Beetles*

To date, most published research results on plastic degradation by insects are reported using *Tenebrio* larvae, especially *T. molitor*. Currently, there are three extant *Tenebrio* species reported [70]; two of them, *Tenebrio molitor* and *Tenebrio obscurus*, have been observed worldwide and commercially available in China, in the USA, as well as around the world [51], while *T. opacus* Duftschmid, 1812, is only found in France [70]. Observations of *T. molitor* larvae chewing and ingesting Styrofoam (the trade marker of expanded polystyrene foam) by teenage students and then researchers lead to investigating biodegradation of PS by *T. motor* larvae. As described previously, convincing evidence of rapid PS biodegradation in *T. molitor* larvae has been reported since 2015 [47–50]. Based on the recent survey from collaborators, yellow mealworms in all 25 locations consumed PS foam, including North America (Canada, Mexico, USA), South America (Chile, Costa Rica), Asia (Cambodia, China, Japan, Indonesia, India, Iran, Israel, South Korea, Thailand), Europe (Finland, France, Germany, Poland, Slovenia, Spain, Turkey, UK), Africa (Nigeria, South Africa), and Australia [50, 71]. Detailed studies confirmed the ubiquity of PS digestion and biodegradation by 12 sources of *T. molitor* larvae: five from the USA, six from China, and one from the UK according to the study of Yang et al. [50]. As depicted in Fig. 3, 12 strains of mealworms were able to chew and burrow into block EPS. These *T. molitor* larvae were also able to chew and burrow into PE foam (Fig. 4).

3 Characterization of Plastic Biodegradation

Characterization of plastic polymer biodegradation by *Tenebrio* larvae and other insects is primarily based on (a) mass loss or mass removal of polymer fed; (b) supporting life activities by ingesting polymer as sole diet; (c) the modification of mechanical, chemical, and physical properties of egested residues (in the frass or fecula); and (d) production of CO_2 or biodegraded functional organic groups and/or intermediates. Stable isotopic tracer ^{13}C and radioisotopic tracer ^{14}C are also effective tools to prove biodegradation. These procedures were described in several review articles on microbial plastic degradation and also established in the research work on biodegradation of PS and PE in *Tenebrio* larvae [47–52, 72, 73].

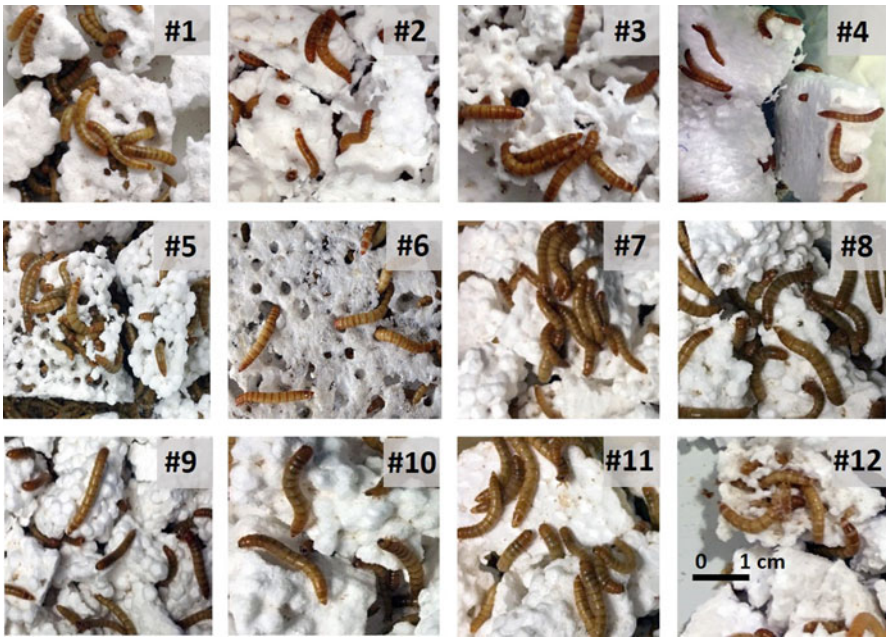

Fig. 3 *Tenebrio molitor* larvae from 12 sources have the capacity of degrading PS foam. Sources #1–#5 are from the USA; source #6 is from the Belfast, UK; and sources #7–#12 were from China (figure from Yang et al. [50])

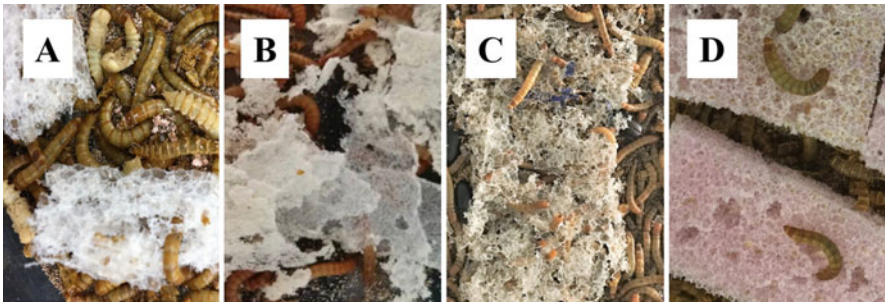

Fig. 4 *Tenebrio molitor* larvae have the capacity of ingesting and degrading LDPE foam. (**a**), (**c**), and (**d**) *T. molitor* larvae source from the USA. (**b**) *T. molitor* larvae from Harbin, China

3.1 Survival Rate and PS Consumption

Survival rate (SR) of *Tenebrio* larvae fed with PS or other plastic materials as sole diet versus that fed with normal diet bran is used as an indication to test the possibility of digestion or biodegradation of plastics [47–52] and also used for the evaluation of the effect of PE and beeswax as sole diet for greater wax worms (*Galleria mellonella*) [45] and lesser wax worms (*Achroia grisella*) [46].

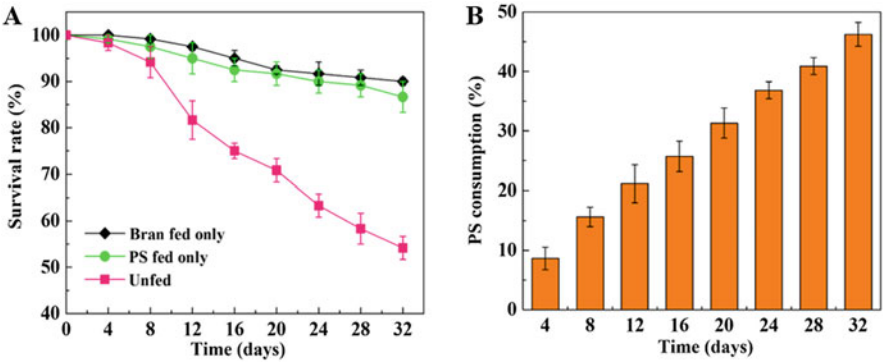

Fig. 5 *Tenebrio molitor* larvae chewed and ate PS foam for living. (**a**) Survival rates of *T. molitor* larvae fed with normal diet bran and PS only and unfed. (**b**) Accumulated PS consumption (%) over time. This test was conducted in duplicate with 120 larvae in each incubator over a 32-day period (figures from Yang et al. [49])

Most researchers use short-term SR to evaluate the effectiveness of EPS as energy source to support the life activities of *T. molitor* larvae. Yang et al. [47] reported the results for the determination of the SR of *T. molitor* larvae over 1-month period and showed that the difference between of the SR of Styrofoam-feeding larvae and the SR of conventional diet (bran)-feeding larvae was not significant (average 85%). During the 1-month rearing period, the larvae (500 in total) obtained in Beijing, China, consumed 31.0 ± 1.7% of Styrofoam with an initial weight of 5.8 g as the sole diet. In their studies, results found that almost half of the ingested PS carbon was converted into CO_2 in the mealworm gut [47]. Afterward, similar results were observed using different sources of *T. molitor* larvae around the world [49, 50]. Results showed that the SRs of *T. molitor* larvae fed with PS foam were similar to that fed with normal diet bran but significantly higher than that unfed (Fig. 5a); the PS consumption progressively increased over a 32-day rearing period with PS as the only feedstock as shown in Fig. 5b [49]. A total consumption of 0.83 ± 0.04 g PS by the end of the test was observed according to 120 mealworms. 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 at values up to 65% in the short (12–15 h) residence time of the mealworm gut. At the end of the 32-day test at 25°C, the SR of the larvae fed with 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%). Over the 32-day period of the test, starved mealworms lost 2.6 ± 0.2% of their average weight; the larvae fed with PS alone maintained a stable weight; and bran-fed larvae experienced a 32.0 ± 1.5% weight gain. Consumption of PS, PVC, and polylactide (PLA) by *T. molitor* larvae was also tested for 21 days by Bożek et al. [74]. They found that the larvae consumed respective polymers by 9%, 12%, and 3%; the larvae fed with plastics decreased

their weight by 18%, 15%, and 19%, respectively, while the weight of those fed with bran increased by 45%. This suggested that the polymers did not support larval growth.

In the long term, the change in SR is different. A test was performed with *T. molitor* larvae from the UK for 98 days under three feeding conditions: unfed, EPS alone, and EPS plus bran. The SRs of the larvae fed with EPS alone matched those fed with EPS plus bran during the initial 35 days (95.5% versus 98.0%) and then dropped to low levels, like those of unfed controls. Further investigation revealed that both the unfed larvae and larvae fed with PS alone engaged in cannibalism. The 98-day SR was 11.8% for unfed larvae and 11.5% for larvae fed with PS alone. By contrast, the 98-day SR for mealworms fed with bran plus PS was 81.5% [50]. Because PS contains only hydrogen and carbon, it does not provide adequate nutrition (N, P, Na, K, trace elements, amino acids, etc.) for a long-term survival and growth. The positive effect of PS on SR does not last for a long time due to the lack of nitrogen sources and other nutrients. The addition of bran relieved this constraint. In the absence of added bran, however, PS-fed mealworms survived by consuming dead mealworms and their molts [50].

3.2 *Factors Influencing Plastic Consumption by* T. molitor *Larvae*

Physical and chemical properties are essential factors influencing the consumption and digestibility of the polymers by *T. molitor* larvae. Till now, most research has been done using Styrofoam or EPS [47, 49–52, 71, 74]. More research is needed to test different materials with various additives and polymer structures.

Studies indicated that supplementation of nutrient-containing co-diet can enhance PS consumption and degradation by mealworms (Fig. 6). Wheat bran (WB) is a normal feedstock for *T. molitor* larvae and can be obtained from agricultural or food processing industries. Soy protein is a widely used food additive for humans and animals. When the larvae were fed with soy protein or WB in the presence of PS, they first ate the protein or WB and then PS. All feed conditions resulted in higher SR values than the unfed control (60.8%). SR values were similar for larvae fed with PS alone (87.5%) and for mealworms fed with PS plus soy protein (89.2%) or WB (90.8%) (Fig. 6a). Adding soy protein or WB significantly increased rates of PS degradation compared to PS alone. The 32-day PS consumption rate was 39.1% for PS alone, 76.8% for PS plus soy protein, and 67.6% PS plus WB (Fig. 6b). The weight gain of larvae fed with PS plus soy protein was 6.3% greater than that of mealworms fed with PS alone, and the weight gain of larvae fed with PS plus bran was 33.5% greater than that of larvae fed with PS alone. A long-term test over 1 year performed at Stanford University further indicated that when *T. molitor* larvae were fed with PS plus co-diets, WB could provide all nutrients for mealworms to complete their life cycle, but soy protein did not.

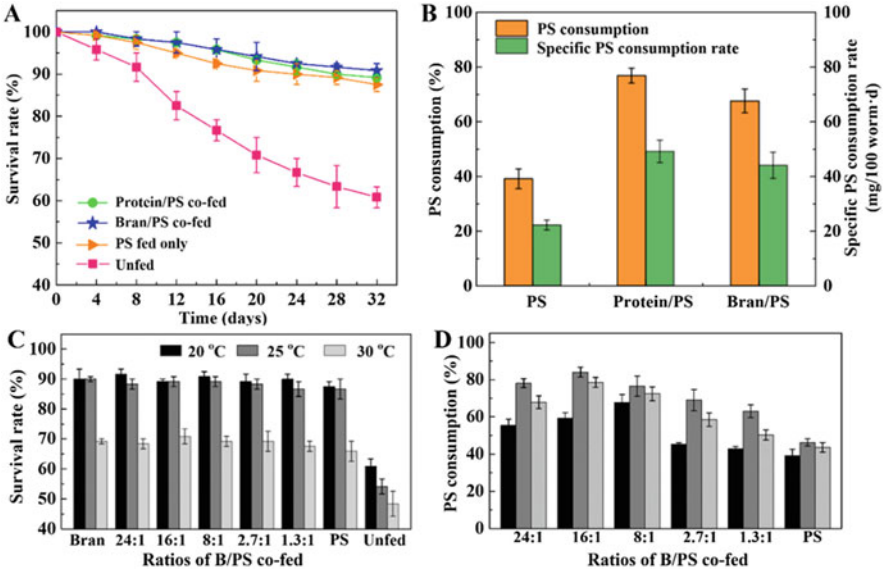

Fig. 6 Effects of co-diets and temperature on PS consumption by *T. molitor* larvae. (**a**) Comparison of SRs for larvae with different diets. (**b**) PS consumption (%) and specific PS consumption rates for unfed larvae compared to the larvae fed with soy protein plus PS or bran plus PS over a 32-day period. (**c**) SRs for the larvae fed with various ratios of bran versus PS at 20°C, 25°C, and 30°C over 32 days. (**d**) PS consumption (%) for the larvae fed with PS alone and various ratios of bran versus PS (w/w) at 20°C, 25°C, and 30°C over 32 days (mean ± standard deviation) (figures from Yang et al. [49])

The combined effects of rearing temperature (20, 25, and 30°C) and WB/PS ratios on SR values and PS consumption rates were evaluated over a 32-day period (Figs. 6c, d). Highest 32-day percentages of PS consumed were 84.0% at 25°C for a WB/PS ratio of 16:1, 78.5% at 30°C for a WB/PS ratio of 16:1, and 67.6% at 20°C for a WB/PS ratio of 8:1. Visibly less PS residue remained in incubators fed with WB plus PS than in incubators fed with PS alone. Besides, rearing temperature had a significant impact on SR values. For the same WB:PS ratio, SRs were significantly lower at 30°C than at 20°C or 25°C. At 20°C and 25°C, SRs were similar regardless of feed ratio, but sensitive to temperature. The effects of temperature on SR 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 [75]. The effect of temperature is likely strain-dependent since *T. molitor* larvae in Indonesia grow well at above 30°C [71].

Similarly, Brandon et al. [52] found that at the end of the 32-day experiment, the SR of the larvae fed with PE was 98.3%, a value that was not significantly different ($p = 0.92$) from that of the bran-fed controls (96.3%). There was also no significant difference in SR of mealworms fed with PE alone and mealworms fed with PE plus bran (95.0%). This indicated that PE supported the life activities during the 32-day

experiment. Consumption of PE and PS increased throughout the experiment. From the initial 1.80 g PE, the total mass loss at the end of the experiment was 0.87 g by mealworms fed with PE. For mealworms fed with PS, the total PS mass loss was 0.57 g. For both PE- and PS-fed mealworms, the mass loss was significantly greater when the mealworms received bran as a co-feed. For PE plus bran, the mass loss was 1.10 g, and for PS plus bran, the mass loss was 0.98 g. Specific rates of plastic consumption (mg plastic consumed per 100 worms per day) followed the same pattern.

3.3 Reproduction of T. molitor *Fed with PS*

Long-term tests indicated that provision of added nutrition (wheat bran) enabled *T. molitor* to reproduce and mate and could therefore enable selective breeding [49]. The first generation of mealworms fed with PS plus WB completed their life cycle (Fig. 7), developing into pupae and then beetles in 2 weeks at 28°C, and produced a second generation of yellow mealworms. A new generation of mealworms was then reared for 3 months with PS and WB; this generation appeared to have a higher affinity for PS materials. Rearing at 25°C, 120 s generation juvenile mealworms weighing ~30 mg per mealworm had a specific PS consumption rate of 16.9 mg PS/100 larvae per day or 5.6 mg PS/1,000 mg mealworms per day on a weight basis. These values fall within the range of values measured for the mature first-generation PS-degrading *T. molitor* larvae that weighed 75–85 mg per larva. Rearing with PS plus WB as their diets, the second-generation juveniles' mealworms

Fig. 7 The first generation of *T. molitor* larvae fed with PS plus wheat bran completed their life cycle and can digest various PS foam products (figure from Yang et al. [49])

eventually grew to be mature larvae (weighing 90 mg or higher, like the first generation) and then developed into pupae and beetles. The larvae fed with WB and PS completed all their life cycle stages (larvae, pupae, beetles, egg), and the second generation had a favorable PS degradation, opening the door for selective breeding. Further tests indicated that both generations of *T. molitor* larvae have similar capacity of ingesting and biodegrading various PS foams (Fig. 7).

3.4 PS Degradation by Tenebrio *obscurus Larvae*

Another member of *Tenebrio* genus, *Tenebrio obscurus* larvae (dark mealworms), also has the capacity of ingesting and biodegrading PS foam. A comparison study demonstrated the ability for PS degradation within the gut of *T. obscurus* larvae even at greater rates than *T. molitor* larvae from the same source [51]. *T. obscurus*, Fabricius 1792 larvae, obtained from Shandong, Sichuan, and Henan provinces, China, and Colorado, USA, chewed and ingested PS foam (Fig. 8). It is speculated that the chewing and ingestion of PS foam is likely an adaptive behavior intrinsic to *T. obscurus*. *T. obscurus* larvae behaved similarly to each other but differently from *T. molitor* larvae. They were all sensitive to light and mostly hid below PS foam in clusters. The larvae of *T. molitor* were less sensitive to light and spread themselves on the foam surface or penetrated the inside matrix. *T. obscurus* larvae like corn flour but do not prefer bran diet, while *T. molitor* prefer both. A test was performed to compare the PS consumption performance of *T. obscurus* versus *T. molitor* larvae; initial larvae (410) were randomly selected and placed in a food grade polypropylene container (volume of 3,300 mL) under controlled conditions (25 $\pm$ 1°C, 70 $\pm$ 5% humidity, and dark environment). To assess the capacity of consuming PS initially, PS blocks (7.2 g) were added. Co-diet treatments were PS plus bran (1.2 g) for *T. molitor* larvae and PS plus corn flour (1.2 g) for *T. obscurus* larvae. An additional 1.2 g of the co-diet was supplemented every 5 days to reach a final ratio of PS to co-diet of 1.0:1.0 at the end of the test. During the 31-day test with PS as the only diet, the PS mass consumption by the *T. obscurus* larvae was 55.4%, while that by *T. molitor* was 41.5% (Table 2). The PS consumption increased when co-diets were added, i.e., the *T. obscurus* consumed 67.1% of PS and *T. molitor* consumed 56.8 $\pm$ 1.9%. At the end of the 31-day test at 25°C, the SRs of both species fed with EPS alone were 91.5% and 89.3%, respectively, significantly greater than those of unfed controls (67.6% and 62.0%) and not significantly less than corn flour-fed and bran-fed larvae (95.0% and 93.2%). Results showed that the *T. obscurus* were capable of rapid PS consumption at rates which were even greater than those of *T. molitor*.

In addition, both *T. obscurus* larvae from China and the USA chewed and ate LDPE foam. However, their capacity of biodegrading LDPE has not been examined.

Fig. 8 *T. obscurus* larvae from various sources can chew and ingest PS foam. The larvae from (**a**) *T. obscurus* from Harbin, China (14 days rearing period). (**b**) *T. obscurus* from Harbin, China (28-day rearing period). (**c**) *T. obscurus* from Shandong, China. (**d**) *T. obscurus* from Sichuan, China. (**e**) *T. obscurus* from Henan, China. (**f**) *T. obscurus* from Colorado, USA (figures (**c**)–(**f**) from Peng et al. [51])

4 Methods for Evaluation of Plastic Biodegradation

4.1 Residual Polymers in Frass

An effective approach to assess plastic biodegradation is to examine modification of polymers after passage of insect gut using a solvent to extract residual polymer from insect frass or fecula of the insects or larvae fed with plastic. The frass of *T. molitor* larvae contained remaining PS particles, modified PS polymers, and other residues, such as undigested exoskeletons (Fig. 9a). Tetrahydrofuran (THF) is commonly used to extract PS (or PVC) polymer from the frass of *T. molitor* larvae (Fig. 9b). In one study, *T. molitor* larvae were fed with PS foam as sole diet for 32 days. The percentage of undigested PS residue in the frass (w/w, %) decreased from 66.2% on day 4 to 35.2% by day 24, stabilizing thereafter (Fig. 9c). The polymer residue remaining after evaporation was weighed to determine the THF extractable fraction,

Table 2 Summary of PS biodegradation by *T. obscurus* and *T. molitor* larvae (data from Peng et al. [51])

Mealworm source	Initial weight, mg larva^{-1}	Feed	Weight change (%) at the end of test	Survival rate, %	Specific PS consumption rate[a]	
					mg PS 100 larvae^{-1} day^{-1}	mg PS g larvae^{-1} day^{-1}
T. obscurus	66.15 ± 2.13	PS + C	+15.9 ± 4.1	94.9 ± 1.7	39.24 ± 1.73	5.94 ± 0.26
		PS	−8.1 ± 3.7	91.5 ± 1.5	32.44 ± 0.51	5.06 ± 0.08
		Unfed	−13.2 ± 2.7	67.6 ± 2.2	Nd	Nd
T. molitor	62.41 ± 1.72	PS + B	+14.6 ± 1.8	93.2 ± 1.0	33.23 ± 0.80	5.33 ± 0.13
		PS	−8.6 ± 1.2	89.3 ± 2.7	24.30 ± 1.34	3.89 ± 0.47
		Unfed	−18.2 ± 6.0	62.0 ± 2.9	Nd	Nd

[a]Specific PS consumption rates were calculated on the basis of the mass of PS consumed over the test period (31 days). *PS* polystyrene, *C* corn flour, *B* bran, *Nd* not determined. Test temperature at 25°C

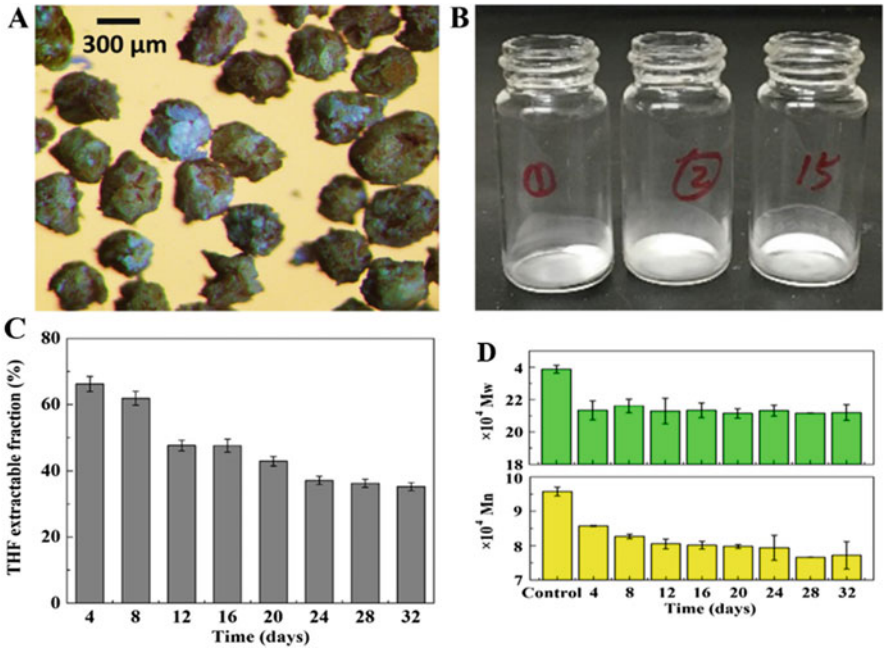

Fig. 9 (**a**) Frass (black) with embedded white polymer residuals. (**b**) Residual polymer extracted from frass. (**c**) Progressive decrease of THF extractable fraction of the frass of *T. molitor* larvae fed with PS foam as sole diet over a 32-day period. (**d**) GPC analysis shows decrease in M_w and M_n of residual PS polymer extracted from the frass during the 32-day period (figures from Yang et al. [49])

a measure of residual PS in the frass (Fig. 9b). The results suggested that the PS degradation activity increased gradually and stabilized after a 16- to 24-day adaptation period [49]. The residual PS polymers were further analyzed using GPC to examine the molecular weights (Fig. 9d). When the larvae were fed with PS plus other co-diets, the THF extract may contain other extractable components except for PS residue. Pre-extraction with ethanol and/or even water to remove impurities may be needed.

For the larvae fed with PE, the extraction of residual PE is performed with dichloromethane (DCM) solvent. The procedure of extraction of PE from the frass of *T. molitor* larvae fed with PE foam was similar but slightly different from that fed with PS as described by Brandon et al. [52]. In tests, the results showed that less than 40% of residual LDPE polymer was detected in the frass after *T. molitor* larvae were fed with LDPE for 2 weeks, indicating a rapid depolymerization and biodegradation occurred [52].

4.2 Major Analytical Methods

The evidences of biodegradation of PS and PE can be provided via analysis of egested residues of PS in the frass characterized by GPC, TGA, FTIR, solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C-CP/MS NMR), liquid-state ^{1}H NMR (^{1}H-NMR) analysis, differential scanning calorimetry (DSC), as well as other methods.

GPC analysis provides the information of the number-average molecular weight (M_n) and the weight-average molecular weight (M_w), which have been considered as a major indication of depolymerization and degradation of polymers [76]. GPC analysis provides information on three key indicators of depolymerization and degradation of plastic materials: M_n, M_w, and molecular weight distribution (MWD). The changes in M_n and M_w of the residual polymer in frass generally decreased significantly from those of the original PS material, indicating depolymerization or modification occurs. A typical example is illustrated in Fig. 9d. *T. molitor* larvae were fed with PS as sole diet for 32 days. The M_w in frass was much lower than that in PS feedstock. The M_n showed progressive decreasing trend. The MWD of residual polymer shifts to lower molecular weight, indicates significant and broad depolymerization [47, 48]. The ubiquity of PS biodegradation in *T. molitor* larvae was supported by the evidence of PS depolymerization within the guts of 12 sources from China, the USA, and Northern Ireland, with significant decreases in M_n and M_w accompanied by shifts in MWD to lower molecular weights (in Table 3). These results suggested that depolymerization/cleavage of the long-chain structure of PS took place and lower molecular weight fragments were newly formed in the mealworm gut. Effects of nutrient supplements and impacts of temperature on PS degradation by a *T. molitor* strain from the USA are exhibited in Fig. 10. Residue PS within frass fed with PS only and PS plus bran showed significant decrease in M_w and M_n in comparison with control (PS feedstock); and differences between different temperatures and co-feeding PS were also not statistically significant (Figs. 10a, b). All samples exhibited similar changes in MWD, with shifts to lower molecular weights than those of PS feed (Fig. 10c).

Similar results were observed during PS degradation in *T. obscurus* larvae. Peng et al. [51] compared *T. molitor* larvae from the same location; frass samples from *T. obscurus* larvae fed with PS only contained polymer extracts with M_n values that were 26.0% lower than the feedstock and M_w values that were 59.2% lower than the feedstock (PS feedstock with M_n of 107,000; M_w of 345,000). Frass samples from *T. molitor* had M_n values that were 11.7% lower and M_w values that were 29.8% lower than the feedstock. These decreases in M_n and M_w were significant for all sources (*t* test, $p < 0.05$), indicating depolymerization and degradation of PS feedstock were ubiquitous across both species. The result also suggested that *T. obscurus* larvae tested had superior PS depolymerization and biodegradation than *T. molitor* larvae test. In addition, except for the macromolecular peak, some low-molecular-weight peaks (molecular weighs between 200 and 1,400) were also

Table 3 The decrease in average molecular weights (M_n and M_w) of the residual PS polymers in the frass of the mealworms fed with bran plus PS (data from Yang et al. [50])

Mealworms	M_n	M_w	M_n reduction compared with control PS (%)	M_w reduction compared with control PS (%)
PetCo Pet Store Chain, Mountain View, California (#1)	81,535 ± 1,588	211,190 ± 512	9.40 ± 0.72	7.19 ± 0.48
PetSmart Pet Store Chain, Sunnyvale, California (#2)	77,738 ± 2,040	203,006 ± 5,928	13.58 ± 3.53	10.78 ± 2.57
Timberline Fisheries, Marion, Illinois (#3)	77,945 ± 2,979	202,813 ± 8,199	13.34 ± 4.61	10.88 ± 3.24
Exotic Nutrition Pet Company, Newport News, Virginia (#4)	75,894 ± 3,836	205,549 ± 3,977	15.64 ± 4.73	9.66 ± 2.14
Rainbow Mealworms, Compton, California (#5)	77,151 ± 1,512	204,113 ± 5,533	14.27 ± 0.13	10.29 ± 2.73
A pet store in Belfast, Northern Ireland, UK (#6)	83,958 ± 4,584	182,105 ± 9,327	5.37 ± 0.19	12.10 ± 4.51
A pet store in Beijing (#7)	78,397 ± 3,770	214,922 ± 3,164	12.92 ± 2.51	5.55 ± 1.12
A store in Harbin, Heilongjiang Province (#8)	77,800 ± 2,062	212,239 ± 1,133	13.50 ± 4.08	6.72 ± 0.79
A mealworm farm in Tai'an County, Shandong Province (#9)	81,849 ± 1,535	212,780 ± 5,798	9.05 ± 0.18	6.50 ± 2.06
A pet store in Xi'an City, Shaanxi Province (#10)	81,448 ± 3,553	214,145 ± 2,717	9.53 ± 2.17	5.89 ± 1.28
A pet store in Shanghai (#11)	77,325 ± 2,279	215,754 ± 3,410	14.08 ± 1.48	5.18 ± 1.72
A pet store in Shenzhen City, Guangdong Province (#12)	82,531 ± 1,512	230,797 ± 1,960	11.19 ± 1.48	9.02 ± 1.09

Mealworms #1–#5, #7–#11, PS feedstock $M_n = 89{,}996 \pm 1{,}855$, $M_w = 227{,}545 \pm 1{,}180$; mealworms #6, PS feedstock $M_n = 88{,}725 \pm 19{,}710$, $M_w = 207{,}155 \pm 2{,}437$; mealworms #12, PS feedstock $M_n = 92{,}949 \pm 2{,}534$, $M_w = 253{,}675 \pm 914$

detected in the frass samples from *T. obscurus* and *T. molitor* fed with PS only, suggesting that some oligomer products might be generated. However, further confirmation test is needed to determine whether the difference of the performance by the two larvae was case-specific or generically different.

To evaluate PE depolymerization in *T. molitor* larvae, the residual PE in frass was extracted with DCM, and the samples were analyzed using high-temperature GPC

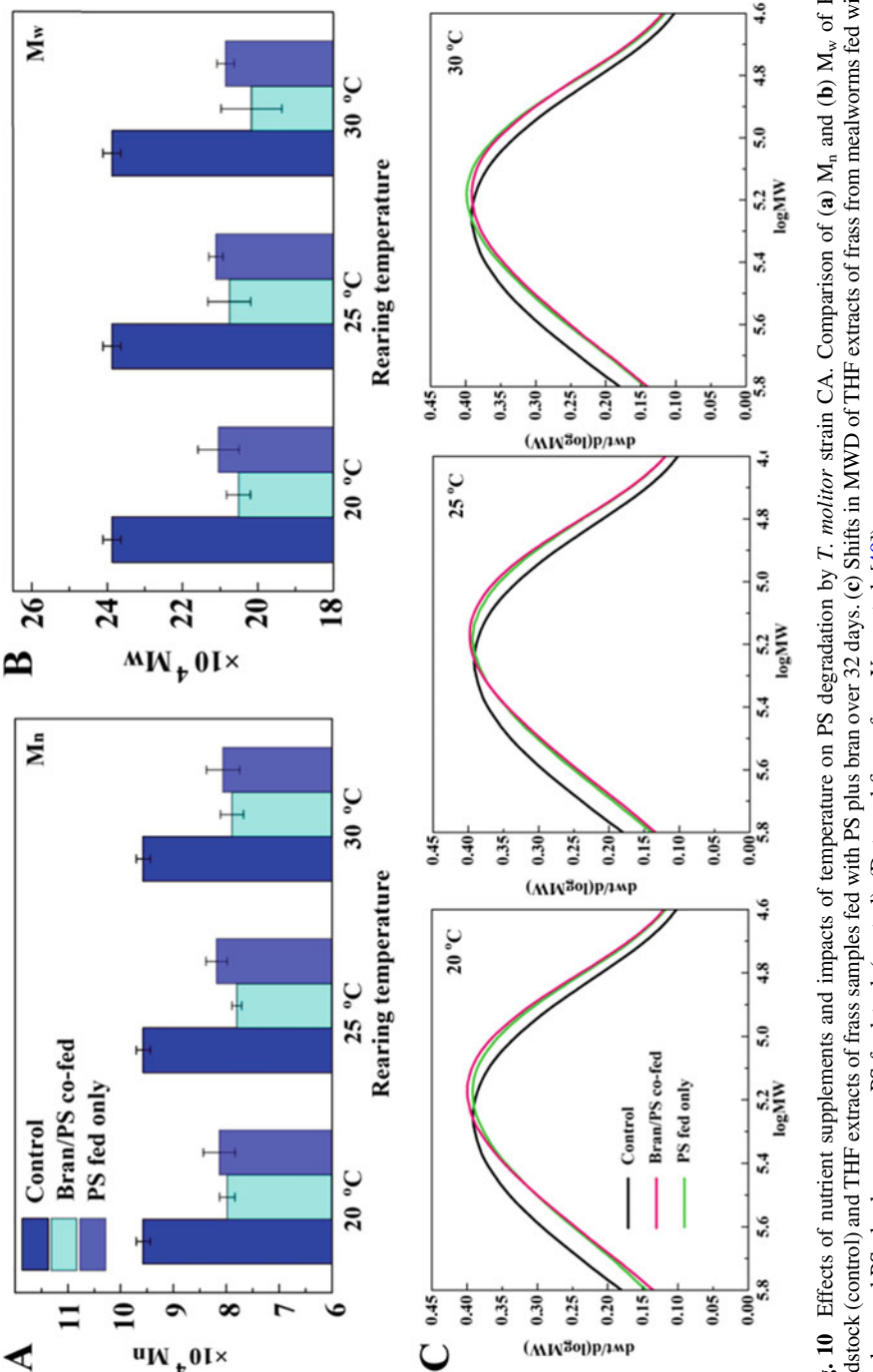

Fig. 10 Effects of nutrient supplements and impacts of temperature on PS degradation by *T. molitor* strain CA. Comparison of (**a**) M_n and (**b**) M_w of PS feedstock (control) and THF extracts of frass samples fed with PS plus bran over 32 days. (**c**) Shifts in MWD of THF extracts of frass from mealworms fed with PS alone and PS plus bran versus PS feedstock (control). (Data and figure from Yang et al. [49])

(HT-GPC) [52]. HT-GPC analysis of the residual polymers from the larvae fed with PE and PE plus bran showed a significant decrease in weight-averaged (M_w) and number-averaged (M_n) molecular weight compared to the PE feedstock (M_w 184,600, M_n 27,500). The residual polymer from PE-fed mealworms showed an average reduction in M_w of 61.3% and reduction in M_n of 40.15%. The residual polymer from mealworms fed with PE plus bran showed an average reduction in M_w of 51.8% and reduction in M_n of 47.6%, indicating significant depolymerization of PE occurred within the gut of the mealworms fed with PE and PE plus bran. Limited depolymerization patterns were also found during plastic biodegradation e.g. a decrease in M_w and increase in M_n occurred during PUR degradation by a mixed microbial culture [77] and increases in both M_w and M_n was observed during PS degradation by *Galleria mellonlla* larvae [78]. The mechanisms of the limited depolymerization remains unknown. More studies are needed to understand the factors influencing and/or controlling the pattern of depolymerization.

Analysis of frass extracts by FTIR and ^{1}H NMR is another approach to confirm modification of egested PS associated with degradation [47, 49]. FTIR spectra provide useful information of formation of new functional groups as evidence of biodegraded intermediates. Figure 11a is an example. At the end of a test with 120 mealworms at 25°C, incorporation of oxygen was seen in the increase in signals associated with carbonyl groups in residual PS from the frass [50]. By comparing the FTIR spectra of the feed PS and PS in egested frass, it revealed bond changes and the incorporation of oxygen previously associated with plastic degradation via aging, irradiation, and biotransformation [79–81]. 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, 1,550–1,610 and 1,800–2,000 cm^{-1}) were dampened in frass samples, providing evidence of ring cleavage. Further evidence of degradation was the decrease in intensities of peak characteristic for PS [81] and the appearance of carbonyl groups (C=O stretch, 1,700 cm^{-1}) [43]. PS oxidation was most extensive for frass from mealworms co-fed with bran. The broadening of peaks at 2,500–3,500 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.

^{1}H NMR spectra also provide information on biodegradation. Comparison of ^{1}H NMR spectra for PS to the spectra of frass extracts revealed new peaks in the frass from mealworms fed with PS only and PS plus bran (Fig. 11b). 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.

Thermal analysis characterized by using a TGA coupling with the FTIR spectroscopy method was used for characterization of PS biodegradation by *T. molitor* larvae [47]. A typical analysis was identification of PS degradation by *T. obscurus* versus *T. molitor* larvae [51]. Thermal modifications of ingested PS in *T. molitor* and *T. obscurus* larvae fed with PS as sole diet were detected using TGA to compare the

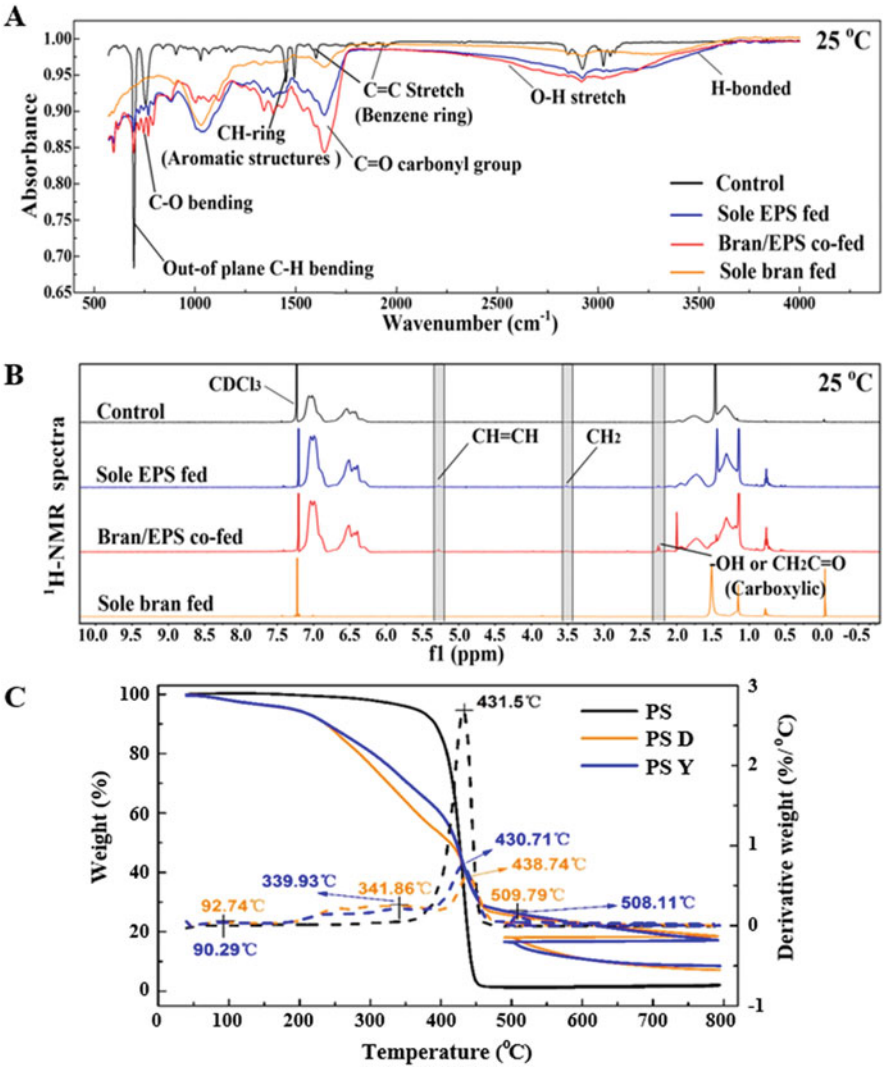

Fig. 11 Spectral analysis for the evaluation of PS degradation. (**a**) FTIR spectra and (**b**) ^{1}H NMR spectra of control (feedstock) and frass samples for mealworms fed with PS, bran plus PS, and bran alone. Samples were obtained on day 32. During the test, a final B:PS ratio was 16:1 g/g with 120 mealworms at 25°C (figures from Yang et al. [48]). (**c**) TGA spectra of PS feedstock and frass of *T. molitor* and *T. obscurus* larvae fed with PS only. Weight curve in solid line (left axis). Derivative weight curve in dash line (right axis). *Y T. molitor*, *D T. obscurus*, *AF* antibiotics, *B* bran, *CF* corn flour, *PS* polystyrene (figure from Peng et al. [51])

PS feedstock and residual PS in frass (Fig. 11c). Only one maximum decomposition rate (about 435°C) was detected in the PS sample. In contrast, for frass from *T. obscurus* fed with PS only (PS D) and from *T. molitor* (PS Y), four maximum decomposition rates (three under N_2 ambience and one under air ambience) appeared at 92.74°C, 341.86°C, 438.74°C, and 509.79°C, respectively (Fig. 11c). The decomposed part under 100°C was possibly classified as volatile organics (gut secretion, carboxylic acids compounds from PS biodegradation, etc.), while decomposed parts from 100°C to 360°C might be attributed to other biological wastes and biodegradation residue. The frass from both species decomposed in the same way, suggesting production of new organic intermediates with different thermal properties in the guts of the larvae. On the other hand, the mass loss ratio of the frass of *T. obscurus* larvae in the stage of 360°C to 480°C was 35.15%, while that of *T. molitor* larvae was 41.03%, in comparison with the PS feedstock of 96.32%. This result implied that the PS polymer structure deteriorated as it passed via the guts and that more PS was depleted or biodegraded in *T. obscurus*, suggesting that larvae of *T. obscurus* worked more efficiently in PS biodegradation than larvae of *T. molitor.*

The biodegradation of LDPE foam in *T. molitor* was confirmed using FTIR and ^{1}H NMR analyses [52]. Evidence of chemical modifications in the residual PE polymer was obtained by FTIR analysis. FTIR spectra from the residual polymers from the larvae fed with PE and PE plus bran revealed incorporation of oxygen as indicated by the appearance of peaks associated with C–O stretching (1,000–1,200 cm^{-1}) and alcohol groups (R–OH bend, 1,300–1,450 cm^{-1}; R–OH stretching, 3,000–3,500 cm^{-1}). Using ^{1}H-NMR analysis, comparison of the control PE spectra to the spectra of the residual polymer from the larvae fed with PE and PE plus bran revealed a new peak around 5.3 ppm in a region associated with alkene bonds (C=C–H). The results indicated formation of intermediates due to biodegradation.

In addition, due to limited reports on the research on biodegradation of PVC, PP, and PET, the analytical methods for evaluation of their biodegradation are still under development.

4.3 Stable Isotopic Tracer

Stable isotopic ^{13}C tracer is a useful tool to investigate biodegradation. Isotopic studies using ^{13}C-labeled PS materials have shown that PS was mineralized to $^{13}CO_2$ and incorporated into lipids [47]. The *T. molitor* larvae 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. The mean δ ^{13}C values of CO_2 released by mealworms fed with α and β ^{13}C-labeled PS diets were 3.3% and 3.9%, respectively. The released ^{13}C-labeled CO_2 from the mealworms fed with α ^{13}C-labeled and β ^{13}C-labeled PS further confirmed the partially biodegradation and mineralization of PS at the end of the 16-day period. Analysis of the ^{13}C CP/MAS NMR, which is usually applied to identify directly the native composition of the solid substrate without separation of

components [82, 83], was further conducted to identify functional groups indicative of depolymerization and oxidation. Compared with the spectrum of the PS feedstock, the new aromatic C (δ 140, 154, and 160) resonance signals could be ascribed to phenyl derivatives, as reported by Gilardi et al. [83]. The phenyl derivatives are possible proxies for the fragments or smaller molecules produced during depolymerization/oxidation of PS [62].

^{14}C tracer was widely used for the research on biodegradation of plastics during the 1970s–1980s [62]. However, this technique has been limited by the availability of radioisotope materials such as ^{14}C PE and ^{14}C PS.

5 Plastic-Degrading Microbial Communities and Functional Bacteria

5.1 The Role of Gut Microbes in Plastic Degradation

It is important to understand whether gut microflora play an indispensable role in the biodegradation of plastics, i.e., gut microbe-dependent or gut microbe-independent biodegradation. Antibiotics, such as gentamicin, nystatin, and ampicillin, suppress gut microbiota in mealworms and provide insight into the role that gut microbiota play in digestive processes, such as the digestion of cell walls and glucoside detoxification [84]. In the studies to test PS degradation, gentamicin has been used to depress or inhibit gut microbes in *T. molitor* and *T. obscurus* larvae [48–51]. Gentamicin is effective to treat mostly Gram-negative bacteria and some Gram-positive bacteria. The results showed that the microbial communities were inhibited and depressed by 10^2–10^3 in the presence of gentamicin (Fig. 12a), and the larvae fed with antibiotic gentamicin almost lost their ability to depolymerize PS (Fig. 12b), indicating that the gut bacteria impaired the ability of the mealworm to depolymerize long-chain PS molecules. GPC analyses indicated inhibition of depolymerization when *T. molitor* larvae from five sources in the USA were fed with gentamicin-containing WB, but depolymerization remained elevated in the control treatment (without gentamicin addition). No statistically significant differences were observed in M_w and M_n values between PS feedstock and residual polymers extracted from frass samples of mealworms receiving the gentamicin treatment for all five US sources (Fig. 12b, c). On the other hand, significant differences were observed in M_w and M_n values between PS feedstock and residual polymers extracted from control and gentamicin treatments. In tests to investigate PS degradation by *T. obscurus* larvae versus *T. molitor* larvae (Fig. 12d), gentamicin depression of gut microbes also inhibited PS depolymerization in *Tenebrio obscurus* larvae [51]. This is evidence that gentamicin suppressed gut microbiota and inhibited PS depolymerization. The depolymerization of PS is likely gut microbe dependent.

The effect of antibiotics on PE degradation in *Tenebrio* larvae has not yet been reported. However, addition of antibiotics did not inhibit the metabolism of beeswax

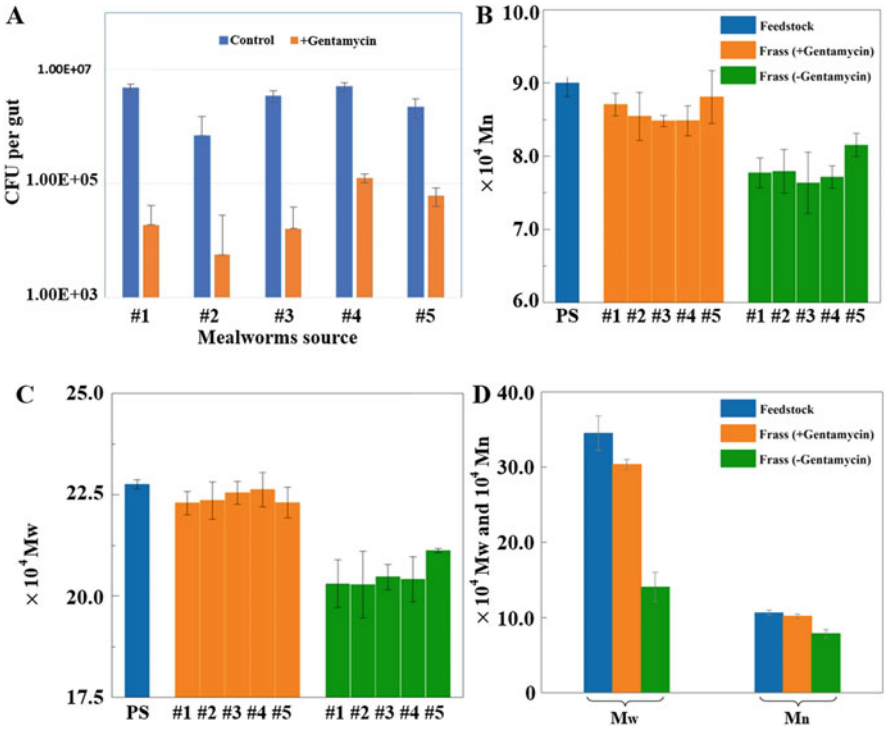

Fig. 12 Effect of antibiotics gentamicin on depolymerization of PS by *Tenebrio* larvae. (**a**) Depression of gut microbes by counting CFU in guts of five *T. molitor* larvae fed with gentamicin for 7 days in comparison with control. (**b**) Comparison of M_n in the PS residues from frass of five *T. molitor* larvae. (**c**) Comparison of M_w in the PS residues from frass of five *T. molitor* larvae. The five *T. molitor* larval sources are described in Table 3 (*#1–#5*). (**d**) Comparison of M_w and M_n of PS feedstock. PS polymers extracted from frass of *T. obscurus* fed with PS only without and with gentamicin. The larval source was from Shandong, China (data from Peng et al. [51])

and LDPE film by greater wax moth larvae (*Galleria mellonella*), indicating gut microbe independence [45]. Research is needed to identify whether biodegradation of PE in *Tenebrio* larvae is dependent or independent on gut microbes.

5.2 *Plastic-Degrading Microbial Communities*

Microbial communities of *T. molitor* in relation to PS degradation have been investigated [50, 52]. Comparing the great variations in different original bacterial communities between source populations due to differences in diet at different geographic locations (China, UK, and USA) and strain-specific properties, the microbial community analyses demonstrated significant taxonomic shifts for mealworms fed with diets of PS alone and WB plus PS [50]. The dominant bacterial

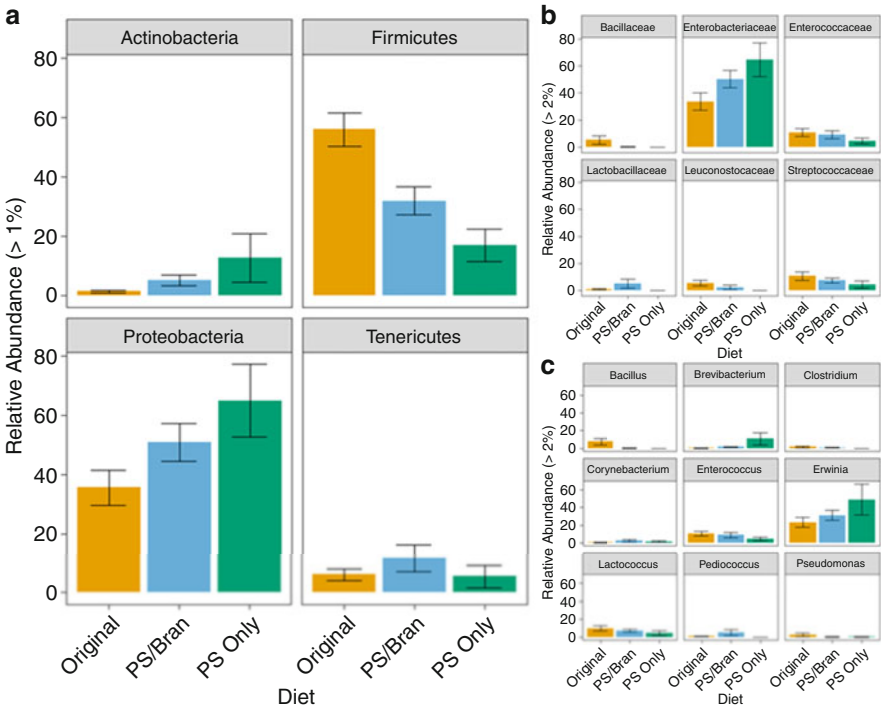

Fig. 13 Relative abundances of dominant (**a**) phyla, (**b**) families, and (**c**) genera in internal microbial communities from original mealworms versus those fed with PS and bran plus PS. Error bars indicate standard deviations for multiple *T. molitor* larval samples from China, the UK, and the USA (data and figures from Yang et al. [50])

phyla across mealworm samples fed with PS and WB plus PS, representing greater than 99% average relative abundance in the measured bacterial communities, were *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Tenericutes* (Fig. 13a). The six most abundant families (>98% total relative abundance on average) were *Bacillaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostocaceae*, and *Streptococcaceae*, representing a diverse range of mostly aerobic and/or facultative bacteria (Fig. 13b). At the genus level (Fig. 13c), when the diet of the mealworms shifted from bran to PS only or PS plus bran, the gut microbiota shifted to a community with improved capabilities for PS degradation.

Brandon et al. [52] performed differential abundance analysis of the gut microbiome in *T. molitor* larvae and found that several minority OTUs strongly associated with the plastic diets (Fig. 14). The larvae were fed with respective diets, i.e., PE, PS, bran, and PE plus bran, for 32 days. This analysis revealed that two OTUs were strongly associated ($p < 0.05$) with both the plastic diets (PE and PS): *Citrobacter* sp. and *Kosakonia* sp. Both OTUs are members of the *Enterobacteriaceae*, a family known to contain PE-degrading member *Enterobacter*

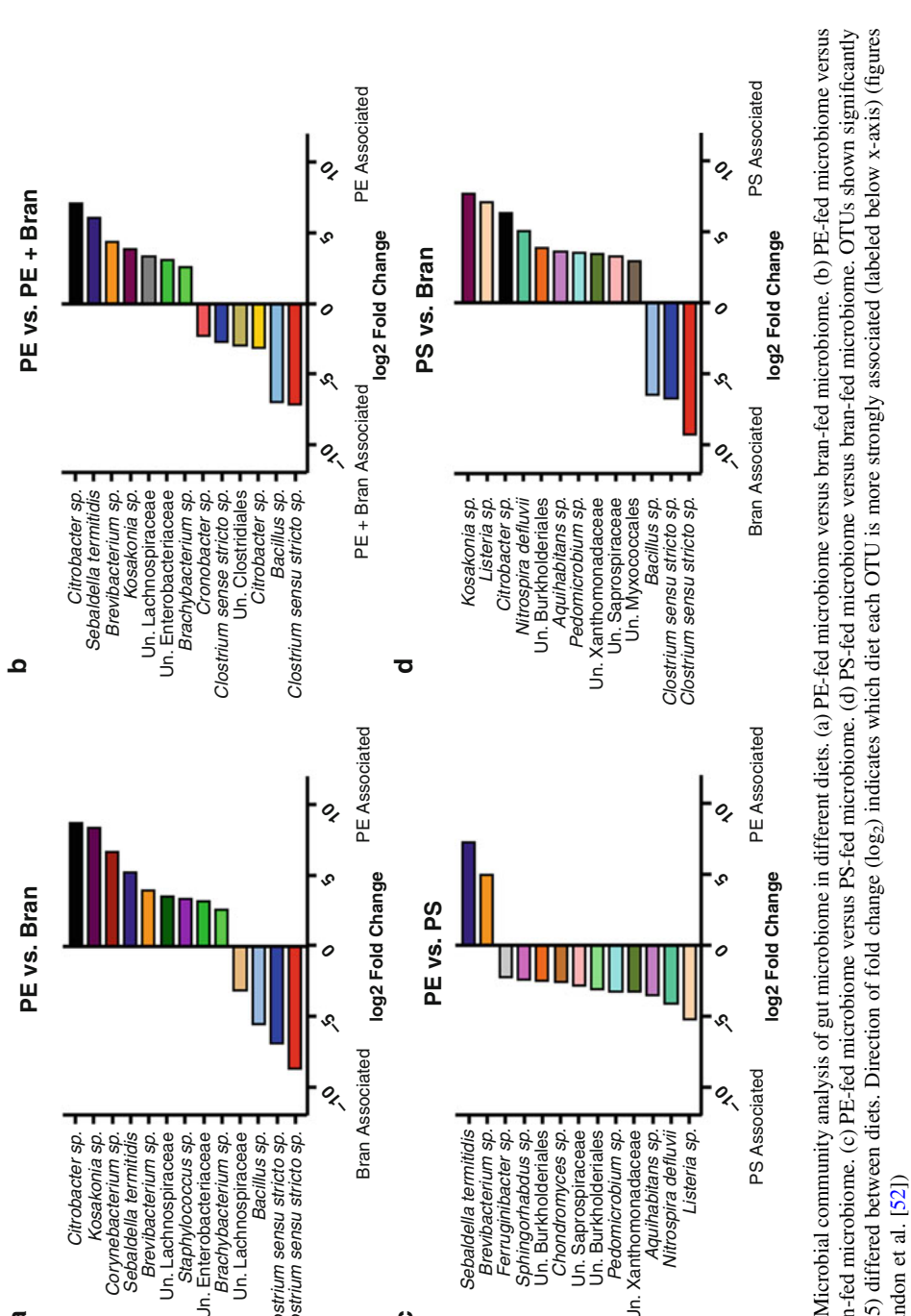

Fig. 14 Microbial community analysis of gut microbiome in different diets. (a) PE-fed microbiome versus bran-fed microbiome. (b) PE-fed microbiome versus PE + bran-fed microbiome. (c) PE-fed microbiome versus PS-fed microbiome. (d) PS-fed microbiome versus bran-fed microbiome. OTUs shown significantly ($p < 0.05$) differed between diets. Direction of fold change ($\log_2$) indicates which diet each OTU is more strongly associated (labeled below x-axis) (figures from Brandon et al. [52])

asburiae YT1 isolated from the gut of the larvae of Indian meal moth [43, 85]. Both OTUs can utilize oxygen (*Citrobacter* sp. are aerobic; *Kosakonia* sp. are facultative anaerobic), which could be further evidenced for their involvement in plastic degradation, as incorporation of oxygen is key in the accelerated biodegradation of both PE and PS [22, 57, 68, 72]. Both *Citrobacter* sp. and *Kosakonia* sp. were more abundant (based on relative abundance) in both of the plastic-only diets than the plastic plus bran fed diets and were also more abundant than the other OTUs identified via differential abundance analysis.

Two OTUs, both minority members of the microbial community, were significantly associated ($p < 0.05$) with PE-fed microbiomes: *Sebaldella termitidis* and *Brevibacterium* sp. (Figs. 14a–c). *Sebaldella termitidis* is phylogenetically isolated within the phylum *Fusobacteria*, is anaerobic, and is a known inhabitant of the posterior end of the termite gut track. *Brevibacterium* sp. is aerobic bacteria known to be associated with hydrocarbon degradation, including n-alkanes.

Seven OTUs, all minority members of the microbial community, were significantly associated ($p < 0.05$) with the PS-fed gut microbiome: *Listeria* sp., *Nitrospira defluvii*, *Pedomicrobium* sp., *Aquihabitans* sp., unclassified *Xanthomonadaceae*, unclassified *Saprospiraceae*, and unclassified *Burkholderiales* (Figs. 14c, d). Most of these PS-associated OTUs are aerobic, which is important when considering their possible role in the degradation of polystyrene. The increase in OTUs associated with the PS microbial community could be indicative of a more diverse suite of daughter products created in PS degradation, likely due to the more complex chemical composition of PS and the presence of benzene rings that could degrade into a variety of daughter products. It is still unknown that the changes in the PS microbial community were also affected by the presence of trace amounts (<1%) of a chemical flame retardant which is present in most commercially available PS products.

The gut microbial community of *T. obscurus* that shifted to that of high PS degradation capacity compared to *T. molitor* was proved in a recent study [51]. A ternary analysis suggested that the families *Enterococcaceae*, *Spiroplasmataceae*, and *Enterobacteriaceae* were strongly associated with the PS diet in *T. obscurus*, which was consistent with the result in relative abundance distributions. At present, however, it is difficult to prove which microbial genera or families are responsible for enhanced PS degradation because only a few PS-degrading bacteria have been isolated.

5.3 Plastic-Degrading Gut Microbes

PS-degrading bacterial strains have been isolated from *T. molitor* larval gut. A PS-degrading bacterial strain (*Exiguobacterium* sp. strain YT2, phylum: *Firmicutes*) was isolated from the larvae fed with PS as diet and was verified to degrade PS polymer [48]. *Exiguobacterium* is a genus of bacilli and a member of the low GC phyla of *Firmicutes*. This bacterial strain YT2 grew on the PS film by forming

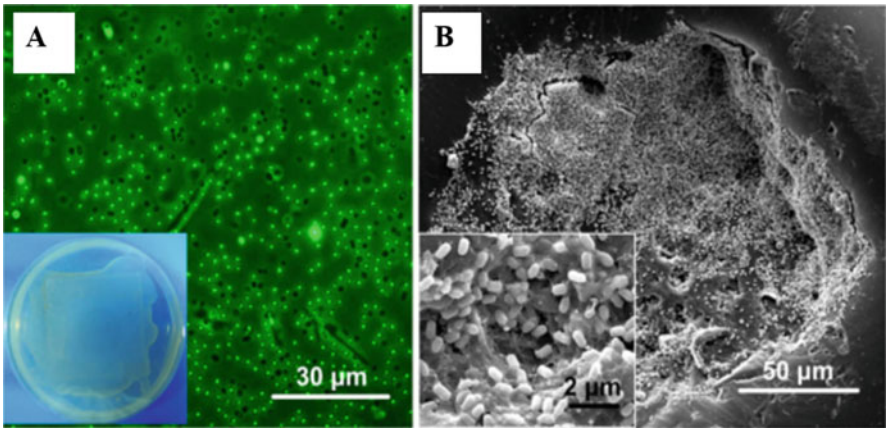

Fig. 15 Biofilm formation and deterioration of PS film surface topography after a 28-day incubation with strain YT2. (**a**) Fluorescent microscopic image of biofilm showed the presence of active cells after a 28-day incubation. Live cells are green, and dead cells are red. (**b**) SEM observations of the physical surface topography of the PS film incubated with strain YT2 after 28 days (figures from Yang et al. [48])

opaque colonies visible, but did not grow on the agar medium (Fig. 15). After a 28-day incubation, the molecular weight of the residual PS pieces was decreased based on GPC analysis, and the release of unknown water-soluble daughter products was detected on gas chromatography/mass spectrometry (GC/MS). The single culture of strain YT2 (10^8 cells/mL) removed PS by $7.4 \pm 0.4\%$ in liquid medium containing 2,500 mg/L PS pieces during a 60-day period. Analyses of GPC, X-ray photoelectron spectroscopy (XPS) scanning, and C 1s spectra of residual PS confirmed PS depolymerization by strain YT2. This proved that PS-degrading microorganisms are present in the gut *T. molitor* larvae and supported the gut microbe dependence of PS biodegradation. However, the isolated bacterial strain YT2 outside the living host appears to show much lower PS degradation efficiency than that demonstrated in the gut system. This suggests that the mechanism of PS degradation in mealworms' gut could be more complicated than gut microbial activities.

Isolation of plastic-degrading bacteria from *T. molitor* larvae was also reported by Suh and Lee [86]. They examined colonization on four different plastic (PS, PET, PP, and PVC) films as indication of plastic biodegradation and claimed ten isolated bacterial strains belonging to *Escherichia fergusonii*, *Bacillus toyonensis*, and *Klebsiella oxytoca*. Tang et al. [87] isolated unknown aerobic strains TM1 and ZM1 from *T. molitor* and *Z. morio* using yeast extract on agar plates and claimed their growth on PS plates which were prepared by adding PS emulsion (in chloroform solvent) into agar basal medium.

6 Mechanism on Biodegradation of Plastics in Insects and Research Prospects

The mechanism of rapid biodegradation of PS and PE in *Tenebrio* larva is still under investigation. Recent studies indicated that *T. molitor* larvae also have unique capacity of degrading lignin material in wheat straw, rice straw, and corn straw [88, 89]. Lignin is a class of complex cross-linked phenolic polymers and resistant to biodegradation. The mechanism of biodegradation of lignin in *T. molitor* larvae is also unknown since we do not know if the gut microbes or the larvae or both could secret ligninolytic enzymes (heme peroxidases, laccase, etc.). Through the action of unidentified enzymes in the termite gut, lignocellulose polymers are broken down into sugars and are transformed into hydrogen. The bacteria within the gut turn the sugar and hydrogen into cellulose acetate, an acetate ester of cellulose on which termites rely for energy. It is not clear whether the capacity of degrading lignocellulose in the larvae also works on various plastics.

Based on the research results on biodegradation of plastics in insects reported and the conceptual model proposed by Yang et al. [47], a primary schematic diagram for symbiotic degradation of plastics (PS and PE) in *Tenebrio* larvae is proposed in Fig. 16 as follows. First, plastic materials (foam, film, powders, or fragments) are chewed into small particles and ingested into the gut. Chewing reduces the size of plastic particles and increases the contact surface area of particle exposure to microbes, bacterial extracellular enzymes, and digestive enzymes. During the ingestion, oxygen in air also enters the intestinal tract and then serves as electron acceptor for aerobic and facultative microbes as well as enzymatic reactions. In the gut, the ingested plastic particles are further fragmented due to mixing, stirring, and moving in the intestinal tract. The fragmented particles are further mixed with gut microbiota that excretes extracellular enzymes and digestive enzymes from the insect to catalyze the depolymerization of the particles into small molecule products. Biodegraded intermediates are further produced via various enzymatic oxidations after depolymerization. Some biodegraded intermediates were further mineralized into CO_2 and H_2O by multiple functional microbes and/or the mealworm host. Limited carbons of the intermediates are further incorporated into biomass. A part of H_2O produced is utilized by *T. molitor* larvae as water source. The biodegraded products including intermediates and CO_2 and undigested residual plastic polymers with some gut microbes are egested as frass. The frass contains plastic-degrading microbes and can be recycled back to the intestinal tract again.

The discovery of biodegradation of plastics, especially PS and PE in insects and their larva, has opened a new door to reach the fate of plastic wastes in the environment and the potential solutions to plastic pollution. To date, the published results of plastics degradation are still limited to PS and LDPE. The feasibility of biodegradation of other recalcitrant plastics such as PP, PVC, and PET in insects should be investigated. The fate of additives in plastics such as plasticizer and flame retardant should also be addressed. Fundamental research topics on biodegradation of plastics with *Tenebrio* larvae and other insects should be considered including

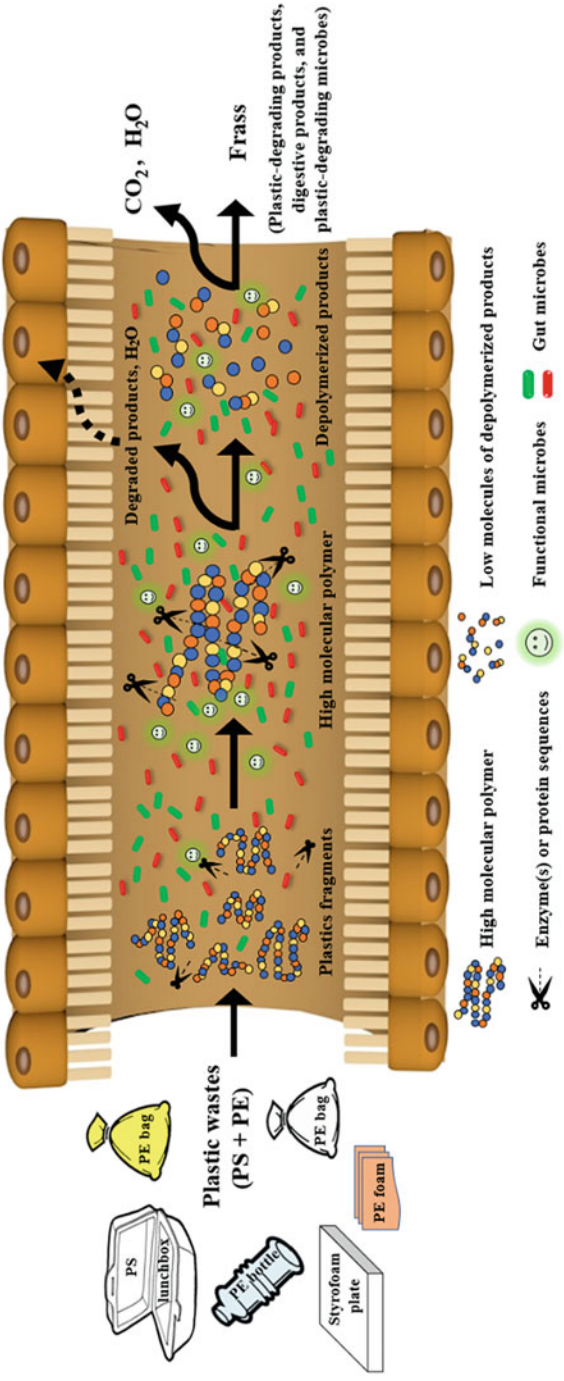

Fig. 16 Conceptual model for biodegradation of plastics in insect intestinal tract

(a) factors impacting and limiting biodegradation of plastics (polymer types, molecular weight, and structure); (b) the enzyme(s), protein sequences, and genes of functional microbes related to plastic degradation; (c) the interaction or synergistic effect between host intestinal tract and gut microbes; and (d) the plastic degradation-related or assistant digestive enzyme(s), protein sequences, cofactor(s), and genes of the insects. Understanding the mechanisms of the insect-related plastic biodegradation could greatly benefit to management of plastic wastes and recovery of resource from used plastics, production of new generation of plastics products, as well as development of innovative technologies for bioremediation of existing plastics pollution sites.

Acknowledgments The authors were supported by Harbin Institute of Technology, Harbin, China, and Stanford University, Stanford, USA. The authors gratefully acknowledge the financial support by the Woods Institute for Environment at Stanford University (award 1197667-10-WTAZB), the National Natural Science Foundation of China (Grant No. 51708154), and the Open Project of State Key Laboratory of Urban Water Resource and Environment (Grant No. ES201906). We appreciate the contributions of data, figures, and tables by our long-term research team members: Professor Craig S. Criddle and Ms. Anja M. Brandon, Stanford University, USA; Professor Jun Yang and Dr. Yu Yang, Beihang University, China; Professor Yalei Zhang and Mr. Boyu Peng, Tongji University, China; Professor Defeng Xing and Professor Nanqi Ren, Harbin Institute of Technology, China; Professor Debra Phillips, Queen's University of Belfast, UK; and Mr. Joseph P. Receveur and Professor Mark Eric Benbow, Michigan State University, USA.

References

1. Al-Salem SM, Lettieri P, Baeyens J (2009) Recycling and recovery routes of plastic solid waste (PSW): a review. Waste Manag 29(10):2625–2643. https://doi.org/10.1016/j.wasman.2009.06.004
2. Plastics Europe. Plastics-the-facts-2018. http://staging-plasticseurope.idloom.com/index.php?cID=179
3. USEPA (2002) Municipal solid waste in the United States: 2000 facts and figures. Executive summary. Office of solid waste management and emergency response (5305W), EPA530-S-02-001, June. Available at: www.epa.gov
4. USEPA (2008) Municipal solid waste in the United States: 2007 facts and figures. Executive summary. Office of solid waste management and emergency response (5306P), EPA530-R-08-010, November. Available at: www.epa.gov
5. Rochman CM, Browne MA, Halpern BS, Hentschel BT, Hoh E, Karapanagioti HK, Rios-Mendoza LM, Takada H, Teh S, Thompson RC (2013) Policy: classify plastic waste as hazardous. Nature 494(7436):169–171. https://doi.org/10.1038/494169a
6. Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R, Law KL (2015) Plastic waste inputs from land into the ocean. Science 347(6223):768–771. https://doi.org/10.1126/science.1260352
7. Thompson RC, Moore C, Saal FV, Swan SH (2009) Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc B 364(1526):2153–2166. https://doi.org/10.1098/rstb.2009.0053
8. Yang SS, Brandon AM, Xing DF, Yang J, Pang JW, Criddle CS, Ren NQ, Wu WM (2018) Progresses in polystyrene biodegradation and prospects for solutions to plastic waste pollution. IOP Conf Ser Earth Environ Sci 150:1–9. https://doi.org/10.1088/1755-1315/150/1/012005

9. Wu WM, Yang J, Criddle CS (2017) Microplastics pollution and reduction strategies. Front Environ Sci Eng 11:6–7. https://doi.org/10.1007/s11783-017-0897-7
10. Lithner D, Larsson A, Dave G (2011) Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ 409:3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038
11. Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC (2008) Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L). Environ Sci Technol 42:5026–5031. https://doi.org/10.1021/es800249a
12. Uhrin AV, Schellinger J (2011) Marine debris impacts to a tidal fringing-marsh in North Carolina. Mar Pollut Bull 62:2605–2610. https://doi.org/10.1016/j.marpolbul.2011.10.006
13. Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, Streck RJ (1998) Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol Biomarkers Prev 7:419–428. https://doi.org/10.1023/A:1008833422577
14. Atiq N (2011) Biodegradability of synthetic plastics polystyrene and styrofoam by fungal isolates. Doctor of philosophy in microbiology, Quaid-i-Azam University
15. Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F, Nava-Saucedo JE (2008) Polymer biodegradation: mechanisms and estimation techniques-a review. Chemosphere 73:429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064
16. Jones PH, Prasad D, Heskins M, Morgan MH, Guillet JE (1974) Biodegradability of photodegraded polymers. I. Development of experimental procedures. Environ Sci Technol 8:919–923. https://doi.org/10.1021/es60095a010
17. Albertsson AC, Karlsson S (1988) The three stages in degradation of polymers—polyethylene as a model substance. J Appl Polym Sci 35(5):1289–1302. https://doi.org/10.1002/app.1988.070350515
18. Pegram JE, Andrady AL (1989) Outdoor weathering of selected polymeric materials under marine exposure conditions. Polym Degrad Stab 26(4):333–345. https://doi.org/10.1016/0141-3910(89)90112-2
19. Ohtake Y, Kobayashi TA, H M N, Ono K (1998) Oxidative degradation and molecular weight change of LDPE buried under bioactive soil for 32–37 years. J Appl Polym Sci 70 (9):1643–1648. https://doi.org/10.2324/gomu.66.756
20. Artham T, Sudhakar M, Venkatesan R, Madhavan NC, Murty KVGK, Doble M (2009) Biofouling and stability of synthetic polymers in sea water. Int Biodeterior Biodegrad 63 (7):884–890. https://doi.org/10.1016/j.ibiod.2009.03.003
21. Ohtake Y, Kobayashi T, Asabe H, Murakami N, Ono K (1995) Biodegradation of low—density polyethylene, polystyrene, polyvinyl chloride, and urea formaldehyde resin buried under soil for over 32 years. J Appl Polym Sci 56(13):1789–1796. https://doi.org/10.1002/app.1995.070561309
22. Gautam R, Bassi AS, Yanful EK (2007) A review of biodegradation of synthetic plastic and foams. Appl Biochem Biotechnol 141(1):85–108. https://doi.org/10.1007/s12010-007-9212-6
23. Guillet JE, Regulski TW, Mcaneney TBJ (2002) Biodegradability of photodegraded polymers. II. Tracer studies of biooxidation of Ecolyte PS polystyrene. Environ Sci Technol 8 (10):923–925. https://doi.org/10.1021/es60095a011
24. Sielicki M, Focht DD, Martin JPJ (1978) Microbial degradation of [$C^{14}C$]polystyrene and 1,3-diphenylbutane. Can J Microbiol 24(7):798. https://doi.org/10.1139/m78-134
25. Kaplan DL, Hartenstein R, Sutter J (1979) Biodegradation of polystyrene, poly(methyl methacrylate), and phenol formaldehyde. Appl Environ Microbiol 38(3):551–553
26. Mor R, Sivan A (2008) Biofilm formation and partial biodegradation of polystyrene by the actinomycete *Rhodococcus ruber*. Biodegradation 19(6):851–858. https://doi.org/10.1007/s10532-008-9188-0
27. Atiq N, Ahmed S, Ali MI, Andleeb S, Ahmad B, Robson G (2010) Isolation and identification of polystyrene biodegrading bacteria from soil. Afr J Microbiol Res 4(14):1537. https://doi.org/10.1016/j.mrgentox.2010.05.014

28. Albertsson AC (1978) Biodegradation of synthetic polymers. II. A limited microbial conversion of ^{14}C in polyethylene to $^{14}CO_2$ by some soil fungi. J Appl Polym Sci. https://doi.org/10.1002/app.1978.070221207
29. Albertsson AC, Bánhidi ZG, Beyer-Ericsson LL (1978) Biodegradation of synthetic polymers. III. The liberation of $^{14}CO_2$ by molds like Fusarium redolens from ^{14}C labeled pulverized high density polyethylene. J Appl Polym Sci 22(12):3435–3447. https://doi.org/10.1002/app.1978.070221208
30. Sivan A, Szanto M, Pavlov V (2006) Biofilm development of the polyethylene-degrading bacterium *Rhodococcus ruber*. Appl Microbiol Biotechnol 72:346–352. https://doi.org/10.1007/s00253-005-0259-4
31. Tribedi P, Sil AK (2013) Low-density polyethylene degradation by *Pseudomonas* sp AKS2 biofilm. Environ Sci Pollut R 20(6):4146–4153. https://doi.org/10.1007/s11356-012-1378-y
32. Balasubramanian V, Natarajan K, Hemambika B, Ramesh N, Sumathi CS, Kottaimuthu R, Rajesh KV (2010) High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of Mannar, India. Lett Appl Microbiol 51(2):205–211. https://doi.org/10.1111/j.1472-765X.2010.02883.x
33. Kyaw BM, Champakalakshmi R, Sakharkar MK, Lim CS, Sakharkar KR (2012) Biodegradation of low density polythene (LDPE) by *Pseudomonas* species. Indian J Microbiol 52:411–419. https://doi.org/10.1007/s12088-012-0250-6
34. Harshvardhan K, Jha B (2013) Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. Mar Pollut Bull 77(1–2):100–106. https://doi.org/10.1016/j.marpolbul.2013.10.025
35. Yamada-Onodera K, Mukumoto H, Katsuyaya Y, Saiganji A, Tania Y (2001) Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK. Polym Degrad Stab 72(2):323–327. https://doi.org/10.1016/S0141-3910(01)00027-1
36. Cacciari I, Quatrini P, Zirletta G, Mincione E, Vinciguerra V, Lupattelli P, Giovannozzi SG (1993) Isotactic polypropylene biodegradation by a microbial community: physicochemical characterization of metabolites produced. Appl Environ Microbiol 59(11):3695–3700. https://doi.org/10.1002/bit.260420917
37. Arkatkar A, Juwarkar AA, Bhaduri S, Uppara PV, Doble M (2009) Degradation of unpretreated and thermally pretreated polypropylene by soil consortia. Int Biodeterior Biodegradation 63(1):106–111. https://doi.org/10.1016/j.ibiod.2008.06.005
38. Arkatkar A, Juwarkar AA, Bhaduri S, Uppara PV, Doble M (2010) Growth of *Pseudomonas* and *Bacillus* biofilms on pretreated polypropylene surface. Int Biodeterior Biodegradation 64(6):530–536. https://doi.org/10.1016/j.ibiod.2010.06.002
39. Jeyakumar D, Chirsteen J, Doble M (2013) Synergistic effects of pretreatment and blending on fungi mediated biodegradation of polypropylenes. Bioresour Technol 148:78–85. https://doi.org/10.1016/j.biortech.2013.08.074
40. Gerhardt PD, Lindgren DL (1954) Penetration of packaging films: film materials used for food packaging tested for resistance to some common stored-product insects. J Econ Entomol 47(2):282–287. https://doi.org/10.1111/j.1529-8817.1988.tb04232.x
41. Cline LD (1978) Penetration of seven common flexible packaging materials by larvae and adults of eleven species of stored product insects. J Econ Entomol 71:726–729. https://doi.org/10.1093/jee/71.5.726
42. Newton J (1988) Insects and packaging-a review. Int Biodeterior 24:175–187. https://doi.org/10.1016/0265-3036(88)90047-4
43. Yang J, Yang Y, Wu WM, Zhao J, Jiang L (2014) Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environ Sci Technol 48(23):13776–13784. https://doi.org/10.1021/es504038a
44. Bombelli P, Howe CJ, Bertocchini F (2017) Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*. Curr Biol 27:R1–R3. https://doi.org/10.1016/j.cub.2017.02.060
45. Kong HG, Kim HH, Chung JH, Jun JH, Lee S, Kim HM, Jeon S, Park SG, Bhak J, Ryu CM (2019) The *Galleria mellonella* hologenome supports microbiota-independent metabolism of

long-chain hydrocarbon beeswax. Cell Rep 26:2451–2464. https://doi.org/10.1016/j.celrep.2019.02.018
46. Kundungal H, Gangarapu M, Sarangapani S, Patchaiyappan A, Devipriya SP (2019) Efficient biodegradation of polyethylene (HDPE) waste by plastic eating lesser waxworm (*Achroia grisella*). Environ Sci Pollut R 26(18):18509–18519. https://doi.org/10.1007/s11356-019-05038-9
47. Yang Y, Yang J, Wu WM, Zhao J, Song YL, Gao LC, Yang RF, Jiang L (2015) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 1. Chemical and physical characterization and isotopic tests. Environ Sci Technol 49(20):12080–12086. https://doi.org/10.1021/acs.est.5b02661
48. Yang Y, Yang J, Wu WM, Zhao J, Song YL, Gao LC, Yang RF, Jiang L (2015) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 2. Role of gut microorganisms. Environ Sci Technol 49(20):12087–12093. https://doi.org/10.1021/acs.est.5b02663
49. Yang SS, Brandon AM, Flanagan JCA, Yang J, Ning DL, Cai SY, Fan HQ, Wang ZY, Ren J, Benbow E, Ren NQ, Waymouth RM, Zhou JZ, Criddle CS, Wu WM (2018) Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. Chemosphere 191:979–989. https://doi.org/10.1016/j.chemosphere.2017.10.117
50. Yang SS, Wu WM, Brandon AM, Fan HQ, Receveur JP, Li Y, Wang ZY, Fan R, McClellan RL, Gao SH, Ning D, Phillips DH, Peng BY, Wang H, Cai SY, Li P, Cai WW, Ding LY, Yang J, Zheng M, Ren J, Zhang YL, Gao J, Xing D, Ren NQ, Waymouth RM, Zhou J, Tao HC, Picard CJ, Benbow ME, Criddle CS (2018) Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). Chemosphere 212:262–271. https://doi.org/10.1016/j.chemosphere.2018.08.078
51. Peng BY, Su Y, Chen Z, Chen J, Zhou X, Benbow ME, Zhang Y (2019) Biodegradation of polystyrene by dark (*Tenebrio obscurus*) and yellow (*Tenebrio molitor*) mealworms (Coleoptera: Tenebrionidae). Environ Sci Technol 53(9):5256–5265. https://doi.org/10.1021/acs.est.8b06963
52. Brandon AM, Gao SH, Tian R, Ning DL, Yang SS, Zhou JZ, Wu WM, Criddle CS (2018) Biodegradation of polyethylene in mealworms (larvae of *Tenebrio molitor* Linnaeus): depolymerization, mineralization, and effects on the gut microbiome. Environ Sci Technol 52:6526–6533. https://doi.org/10.1021/acs.est.8b02301
53. Sina (2003) Mealworms can digest plastics. (in Chinese). http://news.sina.com.cn/c/2003-12-30/16251466914s.shtml. Accessed 8 Feb 2017
54. 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. Accessed 8 Feb 2017
55. Yang Y, Wang JL, Xia ML (2020) Biodegradation and mineralization of polystyrene by plastic-eating superworms Zophobas atratus. Sci Total Environ 708:135233. https://doi.org/10.1016/j.scitotenv.2019.135233
56. Miao SJ, Zhang YL (2010) Feeding and degradation effect on plastics of Zophobasmorio. J Environ Entomol 32(4):435–444.. (in Chinese). https://doi.org/10.3969/j.issn.1674-0858.2010.04.001
57. Krueger MC, Harms H, Schlosser D (2015) Prospects for microbiological solutions to environmental pollution with plastics. Appl Microbiol Biotechnol 99(21):8857–8874. https://doi.org/10.1007/s00253-015-6879-4
58. Market Research Store (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. https://www.marketresearchstore.com/report/polystyrene-expandable-polystyrene-market-for-building-z37708. Accessed 8 Feb 2017

59. Zhou P, Huang CG, Fang HD, Cai WX, Li DM, Li XM, Yu HS (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. https://doi.org/10.1016/j.marpolbul.2011.06.018
60. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M (2012) Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol 46:3060–3075. https://doi.org/10.1021/es2031505
61. Råberg U, Hafrén J (2008) Biodegradation and appearance of plastic treated solid wood. Int Biodeterior Biodegrad 62:210–213. https://doi.org/10.1016/j.ibiod.2007.12.006
62. Guillet JE, Regulski TW, McAneney TB (1974) Biodegradability of photodegraded polymers II: tracer studies of biooxidation of Ecolyte PS polystyrene. Environ Sci Technol 8:923–925. https://doi.org/10.1021/es60095a011
63. Motta O, Proto A, Carlo FD, Caro FD, 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. https://doi.org/10.1016/j.ijheh.2007.09.014
64. Geyer R, Jambeck JR, Law KL (2017) Production, use, and fate of all plastics ever made. Sci Adv 3(7):e1700782. https://doi.org/10.1126/sciadv.1700782
65. The economist (2016) Single-use refuse. http://www.economist.com/news/international/21670516-over-trillion-plasticbags-are-used-every-year-does-chargingthem-help-single-use-refuse
66. Lambert S, Wagner M (2018) Microplastics are contaminants of emerging concern in freshwater environments: an overview. In: Freshwater microplastics. Springer, Cham, pp 1–23. https://doi.org/10.1007/978-3-319-61615-5
67. Albertsson AC, Andersson SO, Karlsson S (1987) The mechanism of biodegradation of polyethylene. Polym Degrad Stab 18(1):73–87. https://doi.org/10.1016/0141-3910(87)90084-X
68. Tokiwa Y, Calabia BP, Ugwu CU, Aiba S (2009) Biodegradability of plastics. Int J Mol Sci 10(9):3722–3742. https://doi.org/10.3390/ijms10093722
69. Bonhomme S, Cuer A, Delort AM, Lemaire J, Sancelme M, Scott C (2003) Environmental biodegradation of polyethylene. Polym Degrad Stab 81:441–452. https://doi.org/10.1016/S0141-3910(03)00129-0
70. Calmont B, Soldati F (2008) Ecologie et biologie de *Tenebrio opacus* Duftschmid, 1812 Distribution et détermination des espèces françaises du genre *Tenebrio* Linnaeus, 1758 (Coleoptera, Tenebrionidae). R A R E, T XVII(3):81–87
71. Nukmal N, Umar S, Amanda SP, Kanedi M (2018) Effect of Styrofoam waste feeds on the growth, development and fecundity of mealworms (*Tenebrio molitor*). J Biol Sci 18(1):24–28. https://doi.org/10.3844/ojbsci.2018.24.28
72. Shah AA, Hasan F, Hameed A, Ahmed S (2008) Biological degradation of plastics: a comprehensive review. Biotechnol Adv 26(3):246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005
73. Raddadi N, Fava F (2019) Biodegradation of oil-based plastics in the environment: existing knowledge and needs of research and innovation. Sci Total Environ 679:148–158. https://doi.org/10.1016/j.scitotenv.2019.04.419
74. Bożek M, Hanus-Lorenz B, Rybak J (2017) The studies on waste biodegradation by *Tenebrio molitor*. In: E3S web of conferences, vol 17, p 00011. https://doi.org/10.1051/e3sconf/20171700011
75. Roberson WH (2005) Urban insects and arachnids, a handbook of urban entomology. Cambridge University Press, Cambridge, pp 126–127. https://doi.org/10.1017/CBO9780511542718
76. Albertsson AC, 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 Polym Environ 6:187–195. https://doi.org/10.1023/a:1021873631162

77. Jeon HJ, Kim MN (2016) Isolation of mesophilic bacterium for biodegradation of polypropylene. Int Biodeter Biodegr 115:244–249. https://doi.org/10.1016/j.ibiod.2016.08.025
78. Lou Y, Ekaterina P, Yang SS, Lu BY, Liu BF, Ren NQ, Corvini PFX, Xing DF (2020) Biodegradation of polyethylene and polystyrene by greater wax moth larvae (*Galleria mellonella* L.) and the effect of co-diet supplementation on the core gut microbiome. Environ Sci Technol 54(5):2821–2831. https://doi.org/10.1021/acs.est.9b07044
79. Mecozzi M, Pietroletti M, Monakhova YB (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. https://doi.org/10.1016/j.marpolbul.2016.03.012
80. Al-Kadhemy MFH, Rasheed ZS, Salim SR (2016) Fourier transform infrared spectroscopy for irradiation coumarin doped polystyrene polymer films by alpha ray. J Radiat Res Appl Sci 9:321–331. https://doi.org/10.1016/j.jrras.2016.02.004
81. Sekhar VC, Nampoothiri KM, Mohan AJ, Nair NR, 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. https://doi.org/10.1016/j.jhazmat.2016.07.008
82. Hopkins DW, Chudek JA, Bignell DE, Frouz J, Webster EA, Lawson T (1998) Application of 13C NMR to investigate the transformations and biodegradation of organic materials by wood and soil-feeding termites, and a coprophagous litter-dwelling dipteran larva. Biodegradation 9:423–431. https://doi.org/10.1023/a:1008313309557
83. Gilardi G, Abis L, Cass AEG (1995) Carbon-13 CP/MAS solid state NMR and FT-IR spectroscopy of wood cell wall biodegradation. Enzym Microb Technol 17:268–275. https://doi.org/10.1016/0141-0229(94)00019-n
84. Genta FA, Dillon RJ, Terra WR, Ferreira C (2006) Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor* larvae. J Insect Physiol 52:593–601. https://doi.org/10.1016/j.jinsphys.2006.02.007
85. Yang Y, Chen JW, Wu WM, Zhao J, Yang J (2015) Complete genome sequence of *Bacillus* sp. YP1, a polyethylene-degrading bacterium from waxworm's gut. J Biotechnol 200:77–88. https://doi.org/10.1016/j.jbiotec.2015.02.034
86. Suh DE, Lee DY (2016) Polyethylene terephthalate, polyvinyl chloride, polystyrene, polypropylene, polyethylene biodegrading microbes and its use for plastic recycling processes. J Int STEAM 1(1):1–6
87. Tang ZL, Kuo TA, Liu HH (2017) The study of the microbes degraded polystyrene. Adv Technol Innov 2(1):13–17
88. Yang SS, Chen YD, Kang JH, Xie TR, He L, Xing DF, Ren NQ, Ho S-H, Wu WM (2019) Generation of high-efficient biochar for dye adsorption using frass of yellow mealworms (larvae of *Tenebrio molitor* Linnaeus) fed with wheat straw for insect biomass production. J Clean Prod 227:33–47. https://doi.org/10.1016/j.jclepro.2019.04.005
89. Yang SS, Chen YD, Zhang Y, Zhou HM, Ji XY, He L, Xing DF, Ren NQ, Ho S-H, Wu WM (2019) A novel clean production approach to utilize crop waste residues as co-diet for mealworm (*Tenebrio molitor*) biomass production with biochar as by product for heavy metal removal. Environ Pollut 252:1142–1153. https://doi.org/10.1016/j.envpol.2019.06.28