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REVIEW ARTICLE

Radical innovation breakthroughs of biodegradation of plastics by insects: history, present and future perspectives

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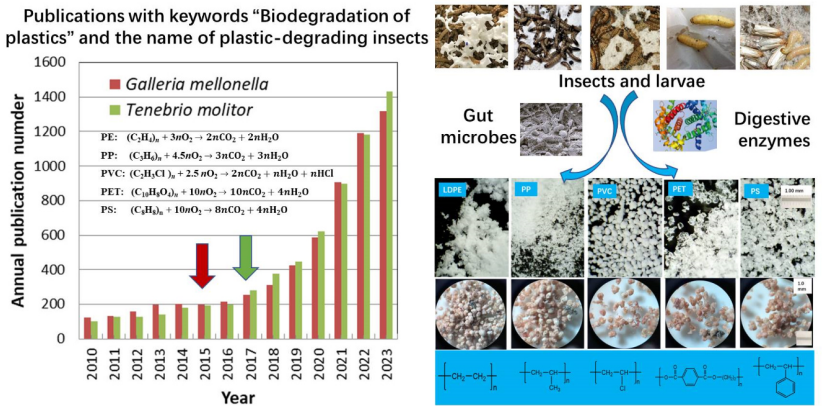
Special issue—Towards a pollution-free planet

HIGHLIGHTS

- Insect damaging and penetrating plastic materials has been observed since 1950s.
- Biodegradation of plastics by insects has become hot research frontiers.
- All major plastics can be biodegraded with half-live on hourly basis.
- The biodegradation is performed by the insect hosts together with gut microbiota.
- Future perspectives focus on biodegradation mechanisms and potential applications.

GRAPHIC ABSTRACT



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ABSTRACT

Insects damaging and penetrating plastic packaged materials has been reported since the 1950s. Radical innovation breakthroughs of plastic biodegradation have been initiated since the discovery of biodegradation of plastics by *Tenebrio molitor* larvae in 2015 followed by *Galleria mellonella* in 2017. Here we review updated studies on the insect-mediated biodegradation of plastics. Plastic biodegradation by insect larvae, mainly by some species of darkling beetles (Tenebrionidae) and pyralid moths (Pyralidae) is currently a highly active and potentially transformative area of research. Over the past eight years, publications have increased explosively, including discoveries of the ability of different insect species to biodegrade plastics, biodegradation performance, and the contribution of host and microbiomes, impacts of polymer types and their physic-chemical properties, and responsible enzymes secreted by the host and gut microbes. To date, almost all major plastics including polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyurethane (PUR), and polystyrene (PS) can be biodegraded by *T. molitor* and ten other insect species representing the Tenebrionidae and Pyralidae families. The biodegradation processes are symbiotic reactions or performed by synergistic efforts of both host and gut-microbes to rapidly depolymerize and biodegrade plastics with hourly half-lives. The digestive ezymens and bioreagents screted by the insects play an essential role in plasatic biodegradation in certain species of Tenebrionidae and Pyralidae families. New research on the insect itself, gut microbiomes, transcriptomes, proteomes and metabolomes has evaluated the mechanisms of plastic biodegradation in insects. We conclude this review by discussing future research perspectives on insect-mediated biodegradation of plastics.

1 Introduction

Since Leo Baekeland's 1907 invention of the first fully synthetic plastic, Bakelite (Bowden, 1997), plastics have been developed and then used in manufacturing goods at large scale. Global plastics production has steadily grown from 2 million tons in 1950 to 390.7 million tons in 2021 (Yang et al., 2023a). Among the many widely used plastics, the main resin types and principle contributors to environmental pollution are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyurethane (PUR), and polystyrene (PS). These resins have contributed to 26.93%, 19.3%, 12.9%, 6.2%, 5.5%, and 5.3% of global plastic produc-

tion, respectively according to the report by PlasticsEurope (Yang et al., 2023a). As a result of the consumption and use of various plastic products, post-consumer plastic wastes are generated (Yang et al., 2023a).

Plastic polymers can be classified into hydrolysable plastics (*e.g.*, PET and PUR) and non-hydrolysable plastics (PE, PP, PVC, and PS), based on physical and chemical properties. Specially, non-hydrolysable plastics are highly resistant to enzymatic depolymerization and microbial biodegradation (Inderthal et al., 2021). Biodeterioration and biodegradation were investigated at the beginning of the application of plastics in the early 1940s as fungal growth on the synthetic resin PVC was observed (Brown, 1945). Since the 1960s, it has been well known that the most commonly used plastics are resistant to biodegradation, resulting in plastic waste accumulation in environments (Yang et al., 2023a). The amount of plastic waste that enters marine environments each year was estimated to be close to 6.4 million tons (Agamuthu, 2018). Plastic wastes make up 62.31% of the marine litter composition, contributing to 49% of the total litter on the seafloor and 81% of the litter on the sea surface with potential hazardous effect on animal and human health.

In the terrestrial environment, plastic pollution including from microplastics (MPs, 1 μm to 5 mm) and nano-plastics (< 1 μm), mainly arises from uncollected and undisposed plastic wastes, residues of agricultural plastic mulch films, urban runoff, sewage discharge, wastewater irrigation, and sewage sludge application, *etc*. (Wu et al., 2017; Sun et al., 2022b; Wang et al., 2022a). Under various gaining conditions, *e.g.*, freeze–thaw cycling, mechanical abrasion, and UV irradiation MPs are generated in great number. For example, up to 334 million MPs particles per m^2 (> 10 μm) can be generated from aged HDPE plastic gauze (Gao et al., 2024). The contamination of MPs are widely spreaded. In a study, micro-nano plastics concentrations were estimated to be about 2.4 ± 1.3 $\times 10^5$ particles per liter of bottled water, about 90% of which were nanoplastics (Qian et al., 2024). The huge quantity of plastic waste not only causes serious pollution of landscapes, but also significantly affects the growth and development of crop roots and the movement of water and fertilizer. This can further cause a reduction in crop yields, consequently affecting deep soil and groundwater environments (Hu et al., 2022). MPs pollute the seas and negatively affect the health of aquatic organisms and animals. The data obtained in laboratory mice and rats suggest a profound negative influence of microplastics on human health (Gola et al., 2021; Sun et al., 2022b; Zolotova, et al., 2022). MPs are frequently detected in environmental and human samples. Humans are potentially exposed to microplastics through oral intake, inhalation, and skin contact. The toxic effects of microplastics on cells, organoids, and animals consist of oxidative stress, DNA damage, organ dysfunction, metabolic disorder, immune response, neuro toxicity, as well as reproductive and developmental toxicity (Li et al., 2023).

The commonly used methods for disposing of plastics, such as landfilling, mechanical recycling, and incineration, have a number of drawbacks and limitations for managing plastic waste. For this reason, biodegradation approaches have long been thought to degrade plastics made of petroleum (Shah et al., 2008) and have been investigated for more than 70 years (Yang et al., 2023a).

The terminology “biodegradation” indicates degradation of a substance by enzymes *in vivo*, and it is generally associated with microbial degradation or biodegradation, and the process by which microorganisms break down organic matters as described in Wikipedia. Degradation of recalcitrant plastics by biological means is incredibly appealing due to their affordability and eco-friendliness (Sanchez, 2020), and can be performed on site and/or via natural processes, which makes it the most desirable approach when remediating the environment. However, although researchers have been studying microbial degradation of plastic wastes since 1960s, recalcitrant petroleum-based plastics are considered to be biochemically inert due to their: 1) extensive inert C–C backbone structures devoid of any functional groups; 2) steric hindrance formed by the large molecular weight long chain structure, which makes it unable to enter microbial cells directly and be degraded by intracellular enzymes; and 3) resistant to hydrolysis by most biological enzymes, requiring high-energy oxidation reactions for degradation (Shah et al., 2008; Min et al., 2020; Inderthal et al., 2021). These properties make them extremely difficult to biodegrade. There are currently very few known microorganisms and enzymes that can significantly oxidize these high-energy structures (Chamas et al., 2020; Inderthal et al., 2021). The tests with isolated microorganisms show some kind of surface deterioration or the initiation of chemical changes of tested polymers due to bacterial activity; and however, these changes are minimal and slow (Matjašič et al., 2021). The half-life of plastics in environments is considered as long as on the basis of several years to hundreds of years (Chamas et al., 2020).

Insect damage of plastic packaging has been observed as early as the 1950s when plastics production began at industrial scales and applications (Gerhardt and Lindgren, 1954). Early publications reported observations of insects damaging plastic packaging materials made from PE, PP, and PVC. As early as in 1965, Nature reported that the larvae of *Hofmannophila pseudospretella* (the Brown House or false clothes moth) was known to eat PE, PS, and nylon films (Whalley, 1965). These observations were of both adults and larvae of Lepidoptera (*i.e.*, moths, mainly Pyralidae), Coleoptera (*i.e.*, beetles, mainly Tenebrionidae) as well as Blattodea (*i.e.*, cockroaches)

(Gerhardt and Lindgren, 1954; Cline, 1978; Highland and Wilsons, 1981; Newton, 1988; Riudavets et al., 2007).

Observation of consumption of PS foams by yellow mealworms (*Tenebrio molitor* larvae) was reported by students competing in several high school science fairs. In 2003, Ms. Chong-guan Chen raised mealworms fed PS foam and theorized that the PS biodegraded, and in 2009, Ms. I-Ching Tseng isolated bacteria from mealworm guts using PS as the sole carbon source as described previously (Yang et al., 2018a; 2018b). Due to limiting of test condition and lack of analytical tools, their discoveries were not verified by more rigorous analytical approaches.

The insects and their larvae damaging and penetrating plastic foams and films have also been attracted research attempts for decades. Plastic-eating/degrading insects have been discoveried during last ten years (Fig. 1). Yang et al. (2014) reported that Indian meal moth larvae (*Plodia interpunctella*) ate LDPE film and first time isolated LDPE-degrading gut bacterial strains. *P. interpunctella* larvae thus were assumed as a plastic-degrading insect. The significant research breakthroughs have been achieved since 2015. Researchers have verified that members of the families Pyralidae and Tenebrionidae can consume and/or biodegrade all major plastics like PE, PP, PS, PVC, PUR and PET. To date, the plastics-degrading larvae of Tenebrionidae (Coleoptera) include *Tenebrio molitor* (Yang et al., 2015a; 2015b; Brandon et al., 2018; Yang et al., 2018a; 2021b; Peng et al., 2019, 2020a; Liu et al., 2022a; Zhu et al., 2022; He et al., 2023), *Tenebrio obscurus* (Peng et al., 2019; Yang et al., 2021a; Ding et al., 2024), *Zophobas atratus* (Peng et al., 2020a; Kim et al., 2020; Yang et al., 2020), *Tribolium castaneum* (Wang et al., 2020), *Plesiophthalmus davidis* (Woo et al., 2020), *Alphitobius diaperinus* (Cucini et al., 2020; 2022), and *Uloma* sp. (Kundungal et al. 2021). Larvae of Pyralidae (Lepidoptera) that also biodegrade plastics include *Plodia interpunctella* (Yang et al., 2014; Mahmoud et al., 2021), *Galleria mellonella* (Bombelli et al., 2017; Vasileva et al., 2018; Kong et al., 2019; Kundungal et al., 2019b; Billen et al., 2020; Peydaei et al. 2021; Cassone et al., 2022; Sanluis-Verdes et al., 2022; Serrano-Antón et al., 2023; Spínola-Amilibia et al., 2023), *Achroia grisella* (Kundungal et al., 2019a) and *Corcyra cephalonica* (Stainton) (Kesti and Thimmappa, 2019). Especially, *G. mellonella* was discovered to sythesize polyethylene degrading enzymes (Boschi et al., 2023). In 2019, Warnke et al. (2019) listed the discoveries of degradation of plastics in insects as one of 100 radical innovation breakthroughs for the future in a report by European Commission. The biodegradation of plastics by insects has become an attractive research area, which have been reviewed by several authors (An et al., 2023; Yang et al., 2023a). Insects have been listed as cardidate organisms for biodegradation of plastics together with natural miroorganisms and artificially derived microganisms, fungi, and algae (Wu et al., 2023).

Although the current definition of plastic biodegradation is strictly connected to the idea of microorganisms in the environment, the insects bring another level of complexity and potentiality to this scenario. In fact, while the symbiotic microbiome provides a pool of microorganisms of interest, the animals are not a simple container of bacteria isolated from the environments. Microbes are fully embedded in their hosts, and many of their potentialities cannot be understood out of their physiologic context. In this case the environment is represented by the animal tissues, and microorganisms add their own contributions to any metabolic activity the host is engaged on. This translates into a multiplication of the ways a material of choice can be degraded and disposed of. Once the molecular details of these complex reactions have been dissected, we can take full advantage of the tools this multiplicity will provide. Although the word "biodegradation" traditionally refers to degradation by microorganisms only, it has been used extensively when referred to degradation by insects.

Based on above discoveries, in this review article, we consider that the term of "biodegradation of plastics" should be expanded from processes of breakdown of plastics performed by bacteria and fungi *via* enzymes to the biological processes performed by all orgasms including bacteria, fungi, insects as well as other macroinvertebrates *e.g.*, land snails *Achatina fulica* (Song et al., 2020) and even vertebrates if any were found. Fig. 1 presents an overview of the main research articles on the biodegradation of different plastics in insects over the last eight years. Besides insects, other invertebrates have also been observed to ingest or biodeteriorate plastics, but little further research has to be done on them. For example, land snail *Achatina fulica* (Férussac, 1821) was found to be capable of degrading expanded PS (EPS) (Song et al., 2020).

To date, published research reports on biodegradation of plastics by insects have been focused mainly on the larvae of *T. molitor, T. obscurus,* and *Z. atratus* (Tenebrionidae), and *Galleria mellonella* (Pyralidae), probably and partially, due to their high commercial availability. LDPE and PS were the main plastics targeted in these studies. In this review, we present a comprehensive discussion and appraisal of significant controversies, research gaps, the conclusions and recommendation of future perspectives in the biodegradation of plastics by insects.

2 Methods for characterization of biodegradation of plastics

During the last eight years, researchers have established methodology for the characterization of biodegradation of plastics by insects and their gut microbiomes (Wu and

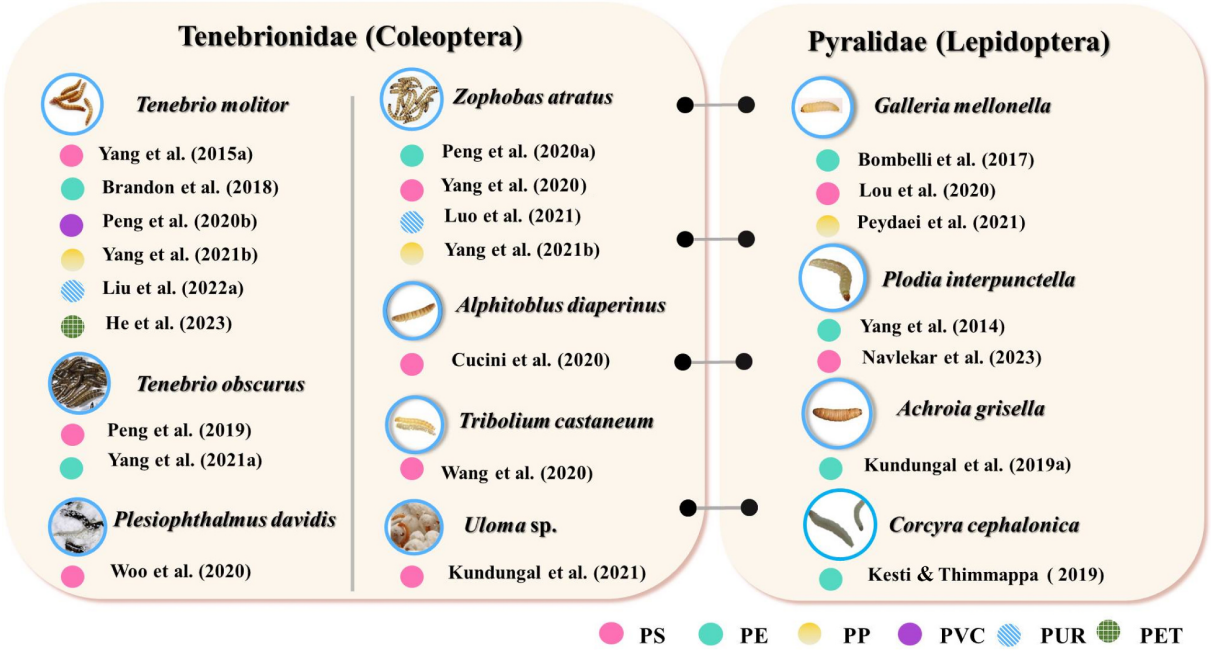

Fig. 1 The milestones of the reports of biodegradation of six major plastics by insect species in families Tenebrionidae and Pyralidae.

Criddle, 2021). Mass loss, chemical and physical alteration, chemical structure changes, and identification of biodegraded intermediates and products are some of the most common analytical approaches used to characterize the biodegradation of plastics by insects. Furthermore, gut microbiome characterization is an essential aspect in relation to biodegradation of plastics. The biodegradation of plastics by insects were first confirmed by investigation with *T. molitor* larvae in 2015 (Yang et al., 2015a, 2015b) followed by *G. mellonella* larvae (Bombelli et al., 2017; Kong et al., 2019). These studies primarily established procedures and methods for the characterization of plastics biodegradation in insects (Wu and Criddle, 2021; Yang et al., 2021a; Peng et al., 2023a) as mainly illustrated in Fig. 2.

2.1 Plastics-degradation tests

For gravimetric determination of weight loss of plastics (consumption rate and removal rate), monitoring physiology of plastics-fed larvae (weight change, survival rate *etc.*), and collection of frass for analyses of residual plastics, mass reduction or weight loss is a common and crucial biodegradation indicator. The mass reduction efficiency is commonly calculated as Eq. (1):

$$R_p(\%) = (W_{pi} - W_{pf})/W_{pi} \times 100\%, \quad (1)$$

where R_p is plastic mass reduction (%); W_{pi} is weight of plastic ingested by insects (g); W_{pf} is the total mass of residual plastics in excrement (egested frass or feces) of the insects (g). Commonly, the mass of residual polymers in the excrement is estimated using the mass of solvent extract. The following solvents are commonly used for respective polymers as THF (PS and PVC), 1,2,4-TCB (PE, PP), DMF (PUR), HFIP (PET), and chloromethane (CF) (PLA). Prior to solvent extraction, pretreatment of the excrement should be conducted by extraction with water, ethanol or other solvents which do dissolve the target plastics is needed to avoid interference by impurities.

Half-life ($t_{1/2}$) is the time required for a quantity of substance to reduce to half of its initial value. The term is commonly used in nuclear physics to describe how quickly unstable atoms undergo radioactive decay or how long stable atoms survive, and a biological half-life or elimination half-life is the time it takes for a substance (drug, radioactive nuclide, or other) to lose one-half of its pharmacologic, physiologic, or radiological activity. The term has ben also used to express the degradation rank of plastics (Chamas et al., 2020; Lott et al., 2021). Using the acquired data based on the mass of ingested plastics, the plastic content in the frass, the mass of the frass, and the detention time of the diet in the gut, the reduction rate constant (K) of plastics can be estimated using the following equation Eq. (2):

$$K = -\frac{1}{t}\left(\ln\frac{W_{pi}}{W_{pf}}\right), \quad (2)$$

where t denotes the degradation time or detention time in the gut (hour or day). The result (K) is used to determine the half-life ($t_{1/2}$) of the treated plastic using the following formula Eq. (3):

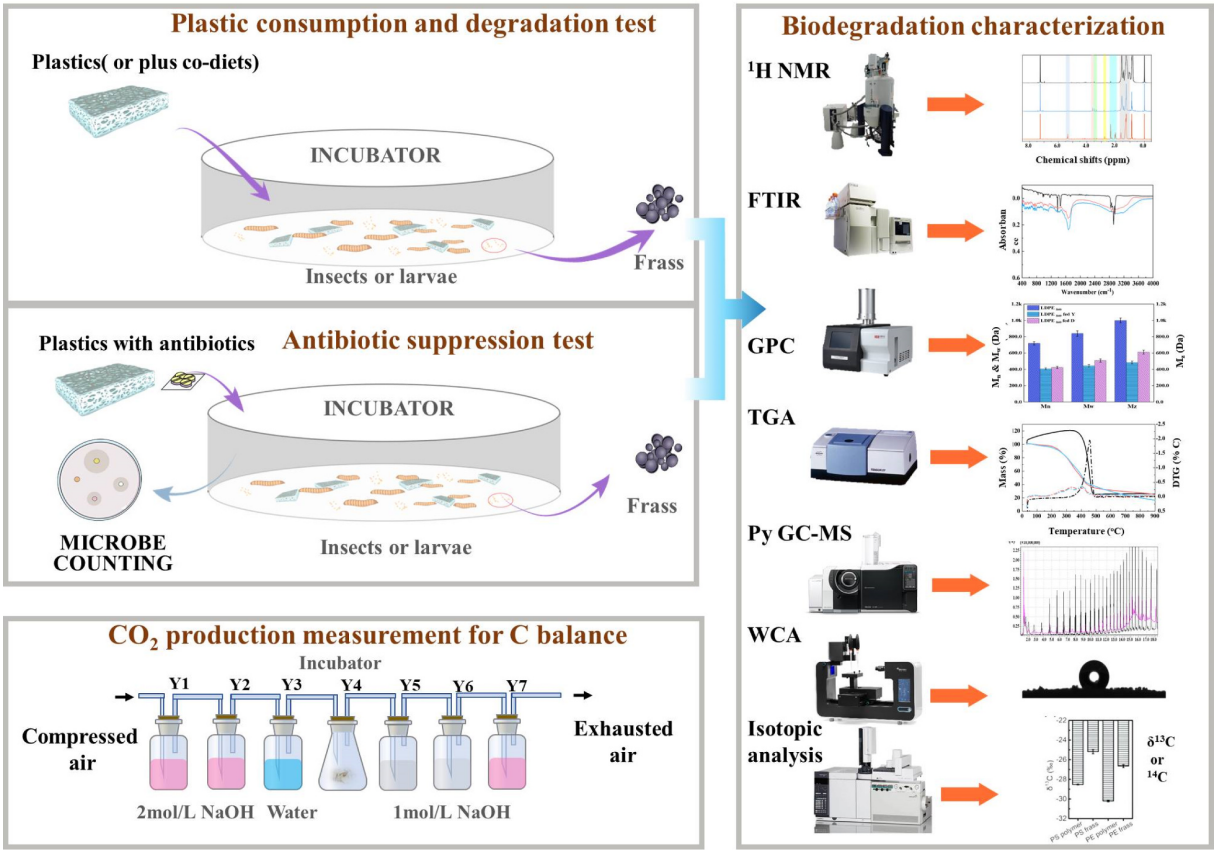

Fig. 2 The procedures and analytical methods for the characterization of plastic biodegradation by insect larvae and/or adults. Note: the batch trails for CO_2 production include two pre-CO_2 removalers, a water trapper, an incubator, and three CO_2 trappers.

$$t_{1/2} = 0.693/K. \quad (3)$$

2.2 Chemical and structure modification analysis

The molecular weight distribution (MWD) and molecular weight of samples of polymer are commonly determined using GPC and high-temperature GPC (HT-GPC). The GPC results include the weight average molecular weight (M_w), the number average molecular weight (M_n), the viscosity average molecular weight (M_v), and the size average molecular weight (M_z). The decrease in M_w means the decrease in the strength, toughness, brittleness, and chemical resistance of the polymer and the increase in solubility.

Dispersity (or polydispersity index, PDI) is calculated as Eq. (4)

$$\mathrm{PDI} = M_w/M_n \quad (4)$$

PDI describes broadness of polymer chains. GPC is calibrated using narrow standards (PDI less than ~1.10) with broad standards (PDI > 1.1) *i.e.*, the same polymer as the sample to be analyzed. Biodegradation results in either PDI increase or decrease, depends on depolymerization rates of polymers with different molecular weights. The increase of PDI means the decrease in the strength and the increase in the toughness and solubility of the polymer after biodegradation. GPS is an essential analytical tool to explore plastic biodegradation and depolymerization and has been used for the research of plastic biodegradation for more than two decades (Otake et al., 1995).

FTIR and An-FTIR have been most widely utilized to analyze chemical modifications of polymer structures as well as chemical changes on the surface of polymers (Matjašič et al., 2021). The FTIR and or An-FTIR spectra reveal the development of functional groups as a result of biodegradation by insects, microbes or enzymatic action (Yang et al., 2018a; Yin et al., 2020; Sandt et al., 2021).

The chemical changes of plastic polymers caused by biodegradation have been investigated employing proton nuclear magnetic resonance (^{1}H NMR). This technique uses NMR spectroscopy to analyze hydrogen-1 nuclei within a substance's molecules to ascertain the chemical structure. The emergence of new peak(s) in sample spectra following biotreatment is utilized to evaluate plastic biodegradation (Brandon et al., 2018; Yang et al., 2018a).

A polymer's thermal stability was determined by thermogravimetric analysis (TGA). The results in the changes of the physical properties are used as an indicator

of degradation of the polymer (Yang et al., 2015a; Peng et al., 2019).

Depending on test settings, researchers have also employed alternative analytical techniques for assessing the biodegradation of plastics, such as X-ray photoelectron spectroscopy (XPS), which has been utilized to confirm changes in functional group composition and surface chemical components (Yang et al., 2015b); solid-state ^{13}C cross-polarization/magic angle spinning NMR (CP/MAS NMR) to determine newly emerging functional groups in solid materials (Yang et al., 2015a).

The crystallinity significantly impacts on biodegradability of plastics (Min et al., 2020; Inderthal et al., 2021). X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) have been used for the measurement of crystallinity of polymers (Kong and Hay, 2002; Aboelkheir et al., 2019).

Water contact angle (WCA) measurements are conducted to evaluate hydrophobicity changes on polymer surfaces following biodegradation. Reduced hydrophobicity is a sign that plastic polymers have been biodegraded by microbes and digestive enzymes (s) (Yang et al., 2014, 2015b; Lou et al. 2022). This method is commonly used for the characterization of plastics after microbial degradation. Prior to test, the surface of plastic sample should be cleaned up *e.g.*, using sodium dodecyl sulfate (SDS) solution (Yin et al., 2020).

Scanning electron microscopic (SEM) and microscopic observation are commonly used to examine the surface of plastic materials before and after biodegradation as well as attached microbes.

2.3 Carbon balance and CO_2 production

Commonly used system is batch trails with an incubator equipped with two pre-CO_2 removalers and two sequential CO_2 trappers (Fig. 2). The released CO_2 in the headspace is trapped using NaOH or KOH solution and then measured by BaCl precipitation or determined using TOC analyzer. Carbon balance test can be tested by collecting CO_2 released from plastic-fed larvae versus the control (unfed larvae). The carbon balance is calculated on the basis of total CO_2–C released, total C of ingested plastics, total C in frass, initial total C in biomass, and final total C in biomass to estimate the conversion rate of C of ingested plastics by the larvae (Yang et al., 2015a). Ali et al. (2023) compared CO_2 production of LDPE-fed *Achroia grisella* larvae versus unfed larvae to estimate the conversation of consumed LDPE to CO_2. This test is useful for *T. molitor*, *G.mellonela* and most insects but could be interfered with if cannibalism is present among the larvae, especially in the case of the larvae of *Z. atratus* and *T. obscurus* (Peng et al., 2019, 2020a).

2.4 Biodegradation intermediates

Determination of biodegradation intermediates is widely used tool to study plastic degradation. GC and GC-MS have been used in biodegradation of plastics for decades. They are effective tools to determine the biodegraded intermediates in insect excrement and gut intestinal content. Gas chromatograph coupled to quadrupole time-of-flight mass spectrometry (Q-TOF-MS) (GC-TOF-MS) system was used to analyze metabolites in the intestine of *T. molitor* larvae fed PS (Tsochatzis et al., 2021). Liquid chromatography triple quadrupole mass spectrometry (LC-QQQ-MS) was used to analyze metabolites of *G. mellonella* larvae fed PLA polymers (Shah et al., 2023).

Pyrolysis gas chromatography mass spectrometry (Py-GCMS) has been widely used to characterize the structure of synthetic organic polymers and copolymers, polymer blends, biopolymers and natural resins. Py-GCMS analysis is used to identify degraded products or fragments by comparing the sample of treated polymer with virgin polymer (Lou et al., 2020). This method is also used for the determination of nanoplastics eg., PS NPs in human blood animal tissues and frass (Leslie et al., 2022; Peng et al., 2023d).

2.5 Isotopic analyses

Isotopic analyses include isotopic tracer test and stable isotopic ratio assay. The isotopic tracer using ^{14}C-labeled plastic polymers was used to assess the mineralization ad biodegradation of plastics mostly in 1970–1980s (Albertsson, 1978, 1980). The formation of $^{14}CH_4$ and $^{14}CO_2$ during degradation (if evaluated anaerobically) is a direct indication of mineralization. The production of intermediate can be monitored by determining soluble ^{14}C containing organics. Recently, ^{14}C-labeled graphere oxide (GO) was used for the biodegradation of GO by *T. molitor* larvae (Liu et al., 2022b).

Using ^{13}C labeled isotopic polymers as tracer is useful to examine biodegradation and mineralization. The increase in $\delta^{13}C$ in the CO_2 produced and ^{13}C in biomass components such as fatty acids was used as indications of mineralization and assimilation following the feeding of *Tenebrio molitor* larvae with ^{13}C-plastics (Yang et al., 2015a).

During biodegradation, organisms preferentially take up light ^{12}C, and thus the ^{13}C isotopes in residual organic compounds increase. Monitoring $^{13}C/^{12}C$ ratio has been successfully applied for biodegradation tests *e.g.*, *in vitro* biodegradation of hydrocarbon vapor (Stehmeier et al., 1999). Detection of the ratio of $^{13}C/^{12}C$ of the residual plastics from insect frass versus the virgin plastics in delta (δ) notation in parts per thousand (‰) was calculated as follows (Eq. (5)):

$$\delta^{13}C\,(‰) = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1\right] \times 1000, \quad (5)$$

where the $\delta^{13}C$ value is calibrated by the international carbon isotope standard Vienna Pee Dee Belemnite (VPDB). The overall precision of the ^{13}C measurement was ± 0.20‰. Research results demonstrate that significant increase in $\delta^{13}C$ value was observed in the residual plastic polymers (PS, LDPE, PET and PP) versus virgin plastics and confirmed biodegradation (Peng et al., 2023d; He et al., 2024a; 2024b). Similarly, the change in ratio of $^{37}Cl/^{35}Cl$ can be also used to monitor the biodegradation of PVC. Biodegradation results in increase in $\delta\ ^{37}Cl$ in residual PVC after biotreatment (Xu et al., 2023). The changes in ratio of $^{15}N/^{14}N$ can be used to estimate the activity of the contribution of nitrogen-fixation to the protein or biomass of the insects fed with nitrogen-deficient diet *i.e.* plastics (Yang et al., 2023b) because N-fixation trends to decrease $\delta^{15}N$ value in biomass.

Polyethylene isotopically labeled with deuterium (PED4) was also used as tracer to detect if deuterated molecules were metabolized by *G. mellonella* (Réjasse et al., 2022).

2.6 Antibiotic suppression test

Antibiotic suppression test is used to characterize the contribution of gut microbes to depolymerisation/biodegradation of plastics together with GPC analysis. The studies using single antibiotics (*e.g.*, gentamicin, which inhibits mostly Gram-negative and some Gram-positive bacteria) to inhibit gut microbes (Yang et al., 2015b; Yang et al., 2018a; 2021b), as well as mixed antibiotics (*e.g.*, gentamicin, rifampicin and streptomycin at 3:2:6, w/w) did this in order to enhance the inhibition of gut microbes (Wu and Criddle, 2021). If depolymerization of plastics stops or weakens under antibiotic suppression, the depolymerisation/biodegradation trends gut microbe-dependent. If not, the depolymerisation is gut microbe-independent or less gut microbe-dependent.

2.7 Isolation and characterization of gut microbes

The procedures of isolation of plastics-degrading bacteria are basically the same to those for the isolation of plastics-degraders from environments (Wu and Criddle, 2021). These procedures include 1) enrichment of microbial cultures in liquid carbon free basal medium (LCFBM) with target plastic powders or small sheets as a sole carbon source; 2) spreading highly enriched cultures on Luria-Bertaini (LB) medium agar plates to form single colonies at 25 °C, with the LB medium containing (per 1000 mL deionized water) 10 g of bacteriological trypton, 5 g of yeast extract to support the growth of heterotrophic bacteria to which most plastics-degraders belong; 3) picking colonies and transferring them in LCFBM with target plastics powder or sheets; 4) checking morphotype under a microscope as cells grow (increase in turbidity); 5) repetition of the above steps until a single culture or same morphotype cells are observed; 6) characterizing isolated plastics-degrading cultures using established methods, including mass loss of plastics, growth curve, change in WCA, microscopic and SEM observation, and analyses of residual polymers versus virgin polymers with GPC, FTIR, ^{1}N NMR, TGA, XPS, *etc*. (Fig. 3). The increase in the $\delta^{13}C$ value of residual polymer is also an effective indication of microbial biodegradation. Because commercial plastic products contain a variety of additives, it is advisable to use high purity plastic materials for the enrichment and isolation.

For public access, the isolated bacterial cultures (as well plastic degrading fungal cultures) or their sequences should be deposited to at least an internationally recognized culture center or Genbank prior to publication in academic journals.

3 Plastic-degrading insect species

To date, a total of 11 insect species have been confirmed to biodegrade plastics, and their taxa belong to Tenebrionidae and Pyralidae families (Figs. 1 and 4).

3.1 Tenebrionidae (Coleoptera)

Family Tenebrionidae (or darkling beetles) is the seventh most speciose taxon in the order Coleoptera, which has over 20000 species in 2300 genera globally (Slipinski et al., 2011; Cheng et al., 2022). The investigation on plastic degradation in Tenebrionidae began with the discovery that yellow mealworms (*T. molitor* larvae) chewed and consumed Styrofoam (or PS foam), which led student science fairs in the 2000s to hypothesize that mealworms biodegrade PS. Superworms, *Zophobas atratus* (or *Z. morio*) larvae were also tested for their ability to eat Styrofoam at science fairs in 2000s.

3.1.1 *Tenebrio molitor*

Tenebrio molitor Linnaeus 1758 (yellow mealworms) is member of family Tenebrionidae (Figs. 4(A) and 4(B)). This species is observed around the world and is naturally present in rotting wood of forests (Calmont and Soldati, 2008), where they eat dried leaves and lignocellulosic materials (Peng et al., 2019, 2020a; Yang et al., 2019a,2019b). Yellow mealworms are commercialized as animal feed all over the world and have also been suggested as a potential sustainable replacement for food protein for both human consumption and animal feed (Bovera et al., 2015). To date, this species has been most widely investigated for plastic degradation around world.

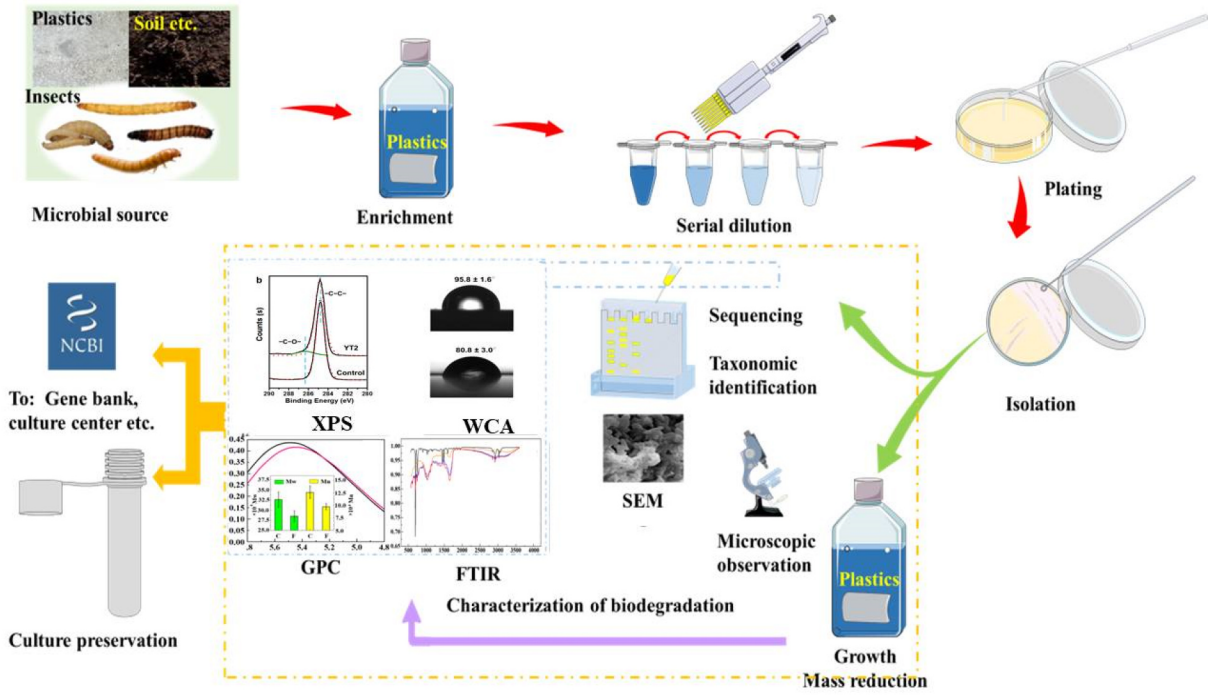

Fig. 3 Procedures for the enrichment, isolation, and characterization of plastic-degrading microorganisms from plastic-degrading insects and environmental samples.

The biodegradation of plastic polymers by *T. molitor* larvae was first reported for PS (Yang et al., 2015a), but also has been studied for LDPE (Brandon et al., 2018), PVC (Peng et al., 2020a), PP (Yang et al., 2021b; He et al., 2024b), PUR (Liu et al., 2022a), HDPE (Yang et al., 2022b; Ding et al., 2024), PET (He et al., 2023, 2024a), vulcanized styrene-butadiene rubber (SBR) and tire crumb (Aboelkheir et al., 2019), biodegradable polymers PLA (Peng et al., 2021) and PBAT (Peng et al., 2023b), graphere oxide (Liu et al., 2022b), and Nylon 11 (Leicht and Masuda, 2022).

The breakthrough report on plastic biodegradation in *Tenebrio molitor* was published in 2015. Using GPC analysis, Yang et al. (2015a) found that *T. molitor* larvae (500 individuals) consumed 31.0% ± 1.7% of PS with an initial mass of 5.8 g as their sole diet during one-month period, with 47.7% of carbon of the ingested PS converted into CO_2. The M_w and M_n of ingested PS were reduced by 20.8% and 20.2%, respectively, while TG-FTIR analysis indicated the formation of oxidized functional groups on residual PS. Tracer tests with α ^{13}C- or β ^{13}C-labeled PS showed that the ^{13}C of the ^{13}C-labeled PS was turned into $^{13}CO_2$ and added to lipids, indicating PS mineralization and assimilation.

The widespread biodegradation of PS in *T. molitor* larvae was confirmed by surveys from collaborators, demonstrating that yellow mealworms from around the world consumed PS foam: these countries included Mexico, Canada, and USA in North America; Costa Rica and Chile in South America; China, Cambodia, Japan, India, Indonesia, Israel, Iran, Republic of Korea, Malaysia, and Thailand in Asia; France, Germany, Finland, Slovenia, Poland, UK, Spain, and Turkey in Europe; Nigeria and South Africa in Africa, and Australia in Oceania (Nukmal et al., 2018; Yang et al., 2018a; 2018b). Yang et al. (2018b) confirmed PS biodegradation in *T. molitor* larvae from 12 sources of the USA, China, and the UK.

Brandon et al. (2018) first time showed that *T. molitor* degraded LDPE using a 32-d experiment. The survival rate (SR) of the larvae fed with LDPE foam alone was not considerably different from that of the controls fed WB and larvae co-fed with LDPE and WB. The results indicated that the LDPE depolymerization extent by WB co-fed larvae was slightly higher than those fed only LDPE; M_n reduction was 47.61% and 40.10%, while M_w reduction was 51.84% and 61.27%, respectively. It was found that PS and LDPE underwent widespread depolymerization, simultaneously reducing both M_w and M_n.

Peng et al. (2020b) confirmed biodegradation of PVC in *T. molitor* larvae by feeding the larvae with rigid PVC microplastics powders. The larvae survived with PVC as their sole diet with an SR of 80% over five weeks. However, the larvae in this study did not complete their life cycle and survival rate dropped by 39% after three months. *T. molitor* larvae performed broad depolymerization of PVC, *i.e.*, M_w, M_n, and M_z decreased by 33.4%, 32.8%, and 36.4%, respectively. Mass balance of Cl element indicated that although 65.4% of ingested PVC

Fig. 4 Photos of plastic degrading insect larvae and adults. (A) *Tenebrio molitor* larvae fed PS foam; (B) *Tenebrio molitor* larvae ingest LDPE foam; (C) *Tenebrio obscurus* larvae fed PE foam; (D) *Zophobas atratus* larvae fed PE foam; (E) *Plesiophthalmus davidis* larvae fed PS foam; (F) *Uloma* sp. fed PS foam; (G) *Tribolium castaneum* fed LDPE foam; (H) *Alphotobius diaperinus* larvae fed PS foam; (I) *Plodia Interpunctella* larvae chew LDPE film; (J) *Galleria mellonella* larvae chew PS foam; (K) *Achroia grisella* larvae chew HDPE film; (L) *Corcyra cephalonica* (Staiton) larvae and adults.

was biodegraded, chloride released during PVC degradation was only 2.9% of the PVC ingested, while 62.51% accumulated as chlorinated organic intermediates and 34.6% residual PVC was directly excreted and found in collected frass, indicating that PVC mineralization was limited. Also, Peng et al. (2023b) found that the residual PVC microplastic particles were more difficult to depurate and excrete for the mealworms compared to the residual PE and PS particles. The levels of oxidative stress responses, including reactive oxygen species, antioxidant enzyme activities, and lipid peroxidation, were also highest in the PVC-fed mealworms.

Biodegradation of PP was tested using *T. molitor* versus *Z. atratus* larvae fed with PP foam with M_z, M_w, and M_n of 765.0, 356.2, and 109.8 kDa, respectively, over the course of a 35-d experiment (Yang et al., 2021b). The amount of PP consumed by *T. molitor* and *Z. atratus* larvae fed with PP foam as their only diet was 1.0 ± 0.4 and 3.1 ± 0.4 mg 100 larvae^{-1}·d^{-1}, respectively. The PP consumption rates of the larvae fed the PP foam plus WB were enhanced by 68.11% and 39.70%, respectively. The residual PP polymers in the frass of *T. molitor* and *Z. atratus* larvae fed PP only showed reduction of M_w by 20.4% ± 0.8% and 9.0% ± 0.4%; M_n by 12.1% ± 0.4% and 61.5% ± 2.5%; and increase in M_z by 33.8% ± 1.5% and 32.0% ± 1.1%. The findings indicate depolymerization to a limited extent.

The biodegradation of PUR by *T. molitor* larvae was confirmed by Liu et al. (2022a). After 35 d, the larvae (108 individuals) had an SR similar to that of bran-fed larvae despite the significant mass reduction (67% of 1 g foam) caused by PUR foam consumption. Fragments of polyether-PUR were detected in the frass, suggesting that intestinal biodegradation of polyether-PUR was incomplete. The biodegradation was confirmed using FTIR, XPS, and GPC analysis. Wang et al. (2022c) also tested ingestion of PUR and PS versus bran in *T. molitor* larvae. The larvae fed PUR and PS only showed the same

SRs and weight changes. ATR-FTIR analysis supported oxidation of ingested polymers. Zhu et al. (2023) found the biodegradation of waste refrigerator PUR by *T. molitor* larvae exerted a considerable effect on their gut microorganism.

The biodegradation of PET by *T. molitor* larvae was confirmed by He et al. (2023). Both host larvae and gut microbiota contributed enzyme repertoire to PET degradation. The study discovered the commercial PET can be rapidly biodegraded by *T. molitor* larvae within 12–15 h by more than 70%, which is much more rapid than that reported using the rapid PET-degrading bacterium *Ideonella sakaiensis* 201-F6, published by Yoshida et al. (2016). On half-life basis, the larvae spent only 0.36% of time in comparison with strain 201-F6.

PLA biodegradation in *T. molitor* was investigated by Peng et al. (2021), who also proposed a new method for achieving both biodegradation and resource recovery at the same time. The results showed that both PLA and PLA-WB mixtures (10%, 20%, 30%, and 50% PLA, wt/wt) biodegraded in mealworms. In comparison to larvae fed PLA alone, feeding PLA-WB co-diet promoted larval development, increased survival rate (SR), and decreased cannibalization rates. The efficiency of the PLA conversion was 90.9% when PLA was the only food consumed and ranged between 81.5% and 86.9% when PLA-WB mixtures were used. When the ratio between PLA and WB was 20%, the highest yield of insect biomass was recorded.

The biodegradation and depolymerization of poly(butylene adipate-co-terephthalate) (PBAT) were confirmed by chemical and thermal modifications as well as changes in MWD (Peng et al., 2023b). Interestingly, the bran addition altered the depolymerization pattern of the ingested PBAT from broad depolymerization to limited-extent depolymerization due to competitive digestion.

In addition, *T. molitor* larvae also demonstrated biodegrading ability of vulcanized tire crumb and SBR-rubber (Aboelkheir et al., 2019). The biodegradation was characterized using TGA, FTIR-ATR, and XRD analyses, and SEM observation. Cheng et al. (2023) isolated a bacterium *Acinetobacter* sp. BIT-H3 from the gut of mealworm, that was capable of growing on vulcanized rubber as sole carbon source. Using ^{14}C-labbled graphere oxide (GO), Liu et al. (2022b) confirmed that the larvae degraded and partially mineralized GO with 0.26% $^{14}CO_2$ recovered. A common textile polymer Nylon 11 can be ingested by *T. molitor* beetles and biofragmented into MPs (Leicht and Masuda, 2022). The authors found that average 4.6% of the culturable gut bacteria in Nylon 11 fed larvae were capable of degrading 11-aminoundecanoic acid, monomer of Nylon 11 although they did not characterize if ingested Nylon-11 was biodegraded or mineralized.

To date, *T. molitor* larvae are most widely tested insect sepecies for plastic biodegradation and considered as a model plastic-degrading insect (Peng et al., 2023c).

3.1.2 *Tenebrio obscurus*

T. obscurus Fabricius 1792 larvae (or dark mealworms) are observed as native species in the USA, France, and China (Calmont and Soldati, 2008; Bai et al., 2018; Peng et al., 2019) and have unique amino acids in their biomass but are less commercially available due to longer rearing times and higher costs for incubation compared to *T. molitor*. Different from *T. molitor*, the larvae of *T. obscurus* have dark black rings on the abdomen. *T. obscurus* larvae were reported to biodegrade PS (Peng et al. 2019), LDPE, LLDPE and HDPE (Yang et al. 2021a; 2022b; Ding et al., 2024).

In a study comparing *T. obscurus* and *T. molitor* larvae from the same source, Peng et al. (2019) showed that *T. obscurus* larvae have the ability to degrade PS (Fig. 4(C)). All *T. obscurus* larvae collected from USA and China chewed and consumed PS foams. They were all more light-sensitive than *T. molitor* larvae and primarily hid in clusters beneath PS foam. The findings demonstrated that *T. obscurus* larvae could consume PS at rates that were comparable to, or even higher than, those of *T. molitor* larvae from the same source. The reduction in molecular weight of residual PS polymers following passing through *T. obscurus* larval gut was 26.0% for M_n and 59.2% for M_w compared with M_n of 107.0 kDa and M_w of 345.0 kDa of original PS foam. M_n and M_w were reduced by 11.7% and 29.8%, respectively, in the residual PS in *T. molitor* frass samples, indicating that *T. obscurus* larvae were better at depolymerizing PS than *T. molitor* larvae.

T. obscurus biodegradation of LDPE was also confirmed by comparing *T. obscurus* larvae versus *T. molitor* larvae (Yang et al., 2021a). Over the course of 36 d, both larvae were fed two LDPE foams, PE-1 and PE-2, with M_n values of 28.9 and 27.3 kDa and M_w values of 342.0 and 264.1 kDa, respectively. For *T. obscurus* larvae, PE-1 and PE-2's M_w dropped by 45.4% ± 0.4% and 34.8% ± 0.3%, respectively, while for *T. molitor* larvae, it was reduced by 43.3% ± 0.5% and 31.7% ± 0.5%. According to mass balance analysis, both *Tenebrio* spices were extensively depolymerized, and about 40% of consumed LDPE was digested as CO_2. Further tests demonstrated that *T. obscrurus* biodegraded LDPE, LLDPE and HDPE at similar rates to those by *T. molitor* (Yang et al., 2022b; Ding et al., 2024).

3.1.3 *Zophobas atratus*

The larvae of *Zophobas atratus* Fabricius 1775 (Coleoptera: Tenebrionidae) are commonly referred to as superworms. This species was initially found in Central and South America's tropical regions, but it has now been spread to other parts of the world (Tschinkel, 1981; Kim

et al., 2015; Bai et al., 2018; Rumbos and Athanassiou, 2021). *Zophobas atratus*, *Z. morio* and *Z. rugipes* Kirsch (Coleoptera: Tenebrionidae) are taxonomically classified as conspecific species (Tschinkel, 1984). Due to its high nutritional value, robust vitality, and resistance to hunger and thirst, *Z. atratus* larvae have been commercially marketed as an animal feed resource (Rumbos and Athanassiou, 2021). In comparison to *T. obscurus* and *T. molitor*, their larvae can reach lengths of 5.0–6.0 cm and be 1.5–3 times greater in size. The larvae and pupae of the same species tend to be consumed by *Z. atratus* larvae (Tschinkel, 1981). In 2010, ingestion and biodegradation of LDPE, PS, ethylene-vinyl acetate (EVA), LLDPE, and PVC materials by *Z. atratus* larvae were investigated (Miao and Zhang, 2010). The larvae did consume PS foam and all plastics tested. At the time, this study could not provide solid data to confirm biodegradation, likely due to analytical tool limits. Afterthen, the ability of biodegrading plastics by *Z. atratus* was confirmed for PS (Peng et al., 2020b; Yang et al., 2020), LDPE (Peng et al., 2020b), and PP (Yang et al., 2021b), and PUR (Luo et al., 2021).

Yang et al. (2020) and Peng et al. (2020b) tested *Z. atratus* larvae from China and USA sources with PS foams (Fig. 4(D)), and characterized egested frass with the similar methods as described during biodegradation of PS by *T. molitor* larvae (Yang et al., 2015a; Yang et al., 2018a; Peng et al., 2019). The outcomes demonstrated that PS was depolymerized and biodegraded by the larvae. The PS biodegradation was characterized for molecular weight changes using GPC analyses and formation of oxidative intermediates.

Peng et al. (2020b) also verified LDPE biodegradation using the larvae from the two different sources in USA (strain M) and China (strain G). As only source of diet, strain G larvae ingested LDPE foam at 58.7 ± 1.8 mg 100 larvae$^{-1}\cdot$d^{-1} rate and PS foam at 61.5 ± 1.6 mg 100 larvae$^{-1}\cdot$d^{-1} rate. For LDPE and PS consumption at 57.1 ± 2.5 mg and 30.3 ± 7.7 mg 100 larvae$^{-1}\cdot$d^{-1} rates, respectively, Strain M needed to supplement the diet with co-diet (cabbage or bran). FTIR and ^{1}H MNR analyses supported formation of oxidized functional groups and revealed LDPE and PS biodegradation in the two strains. According to GPC research, strain G depolymerized PS widely whereas strain M depolymerized PS to a lesser level.

Biodegradation of PP in *Z. atratus* larvae versus *T. molitor* larvae was confirmed by Yang et al. (2021b). The only diet given to *Z. atratus* and *T. molitor* larvae was PP. The GPC results showed that PP biodegradation was accomplished in *Z. atratus* and *T. molitor* larvae by a limited extent of depolymerization, *i.e.*, M_w was decreased by 20.4% ± 0.8% and 9.0% ± 0.4%, M_n was increased by 12.1% ± 0.4% and 61.5% ± 2.5%, and M_z was decreased by 33.8% ± 1.5% and 32.0% ± 1.1%, respectively.

Luo et al. (2021) confirmed biodegradation of PUR foam in *Z. atratus* larvae through mass consumption and ATR-FTIR and DSC-TGA analyses, demonstrating partial oxidation and degradation of PUR utilizing thermostability and changes in functional groups. To compare the effectiveness of ATR-FT-IR and FT-IR, Wang et al. (2022c) investigated PS and PUR biodegradation in *Z. atratus* larvae. The relative consumption rates during a 35-d period were 26.23 mg-PUR/larva and 49.24 mg-PS/larva. They discovered that PS and PUR foams in larval guts underwent comparable biodegradation and oxidation processes.

Z. atratus thus is a widely tested platic-degrading insect species due to their availability and high plastic-comsumption performance.

3.1.4 *Plesiophthalmus davidis*

Plesiophthalmus davidis Fairmaire 1878 (Coleoptera: Tenebrionidae) is a darkling beetle species indigenous to East Asia especially Republic of Korea and China and has also been observed in Israel. In the environment, the *P. davidis* larvae and adults feed on rotten wood and other lignocellulosic plant residues (Woo et al., 2020). The larvae were tested using expanded PS foam and consumed 34.27 ± 4.04 mg of PS per larva in 14 d, surviving solely on Styrofoam (Fig. 4(E)). The oxidation of the ingested PS foam was verified using FTIR and confirmed with GPC analysis. A PS-degrading gut bacterial *Serratia* sp. was isolated and characterized from the larval gut (Woo et al., 2020). This discovery has expanded the member of darkling beetles to plastic-degrading insects.

3.1.5 *Uloma* sp.

Uloma is a genus of darkling beetles (Coleoptera: Tenebrionidae) with a worldwide distribution that includes at least 150 species (Soldati et al., 2014). These species often reside beneath the bark of trees or in rotting wood, although they can also be found outside of woods and in cultivated areas. The adult beetles have size of 7 to 10 mm long. Kundungal et al. (2021) found that beeswax eating beetle larvae belonging to *Uloma* genus ingested PS foam (Fig. 4(F)). In comparison to larvae fed on PS alone, wax comb supplementation increased PS intake by nearly two times, to 37.14 mg/d per 100 larvae. The physiochemical changes of the residual PS in the frass, as determined by FTIR, ^{1}H NMR, and TGA methods, supported the biodegradation of PS. This discovery demonstrates that *Uloma* sp. has similar plastic degrading ability to *Tenebrio* sp.

3.1.6 *Tribolium castaneum*

Trbolium castaneum Herbst, 1797 (Coleoptera:

Tenebrionidae), sometimes referred to as the red flour beetle, is a widespread insect pest of stored products, especially food grains. The adults are around 3–4 mm long, of a uniform rust, brown or black color. The larvae appear similar to yellow mealworms but are much smaller in size (Fig. 4(G)). Consumption of expanded PS foam by *Tr. castaneum* beetles versus rice grain bran was reported by Fabreag and Familara (2019). They found that the beetles consumed PS foams over a 20-d period and the survival rates of the beetles did not show significant difference, suggesting that *Tr. castaneum* could degrade EPS as energy source.

Abdulhay (2020) tested the ingestion of PS, PE, and EVA foams by *Tr. castaneum* larvae. Mass reduction of plastic foams was determined over 30 d. However, no additional analytical evidences were reported.

Wang et al. (2020) confirmed that *Tr. castaneum* larvae chewed and ingested extracted PS. The examination of the gut microbiota of *Tr. castaneum* larvae fed plastic and bran revealed that *Acinetobacter* sp. was substantially linked with plastic consumption. *Acinetobacter* sp. was discovered as the only gut bacterial strain that was capable of degrading PS. They identified *Acinetobacter* AnTc-1 based on the 16S rDNA sequence and PS degrading ability through investigations utilizing GPC, ^{1}H NMR, TGA, and SEM.

Tr. castaneum larvae and adults have been considered as a candidate of plastic-degrading insect for years but further studies should be conducted to verify plastic biodegradation ability.

3.1.7 *Alphitobius diaperinus*

Lesser mealworms, larvae of *Alphitobius diaperinus* Panzer, 1797, belong to the member of family Tenebrionidae. Both adults (or litter beetles) and larvae aggressively damage and ingest expanded polystyrene (EPS) foam as we observed them in the laboratory at Stanford University (Fig. 4(H)). This species is one of the most abundant and resilient insect pests within food storage and poultry houses in the USA, and their adults damage polystyrene isolation foam (Despins et al., 1991). The lesser mealworms are also used as animal feed. The larvae are small with 11 mm long while adults are 6 mm long.

Cucini et al. (2020) reported consumption of PS foam by lesser mealworms and the change in the bacterial and fungal diversity after PS was fed. Further study indicated that the PS-fed larvae different abundant gut microbial community which contained bacteria growing on PS-film (Cucini et al., 2022). However, the biodegradation of PS by lesser mealworms has not been characterized although this species has been considered as a PS-degrading candidate.

3.2 Pyralidae (Lepidoptera)

The Pyralidae family belongs to the order Lepidoptera, which has more than 150000 species with about 18000 butterflies and more than 130000 moths (Kristensen et al., 2007). Pyralidae, commonly called pyralid moths, grass moths or snout moths, is comprised of more than 6000 species described worldwide. Most pest moths in storage houses belong to this family and have lived alongside human society for thousands of years. A number of lepidopterans have been found to damage and penetrate plastic packaging materials for more than 70 years (Gerhardt and Lindgren, 1954). Recently, some numbers have shown remarkable ability to chew and degrade different polymers (Figs. 4(I) to 4(L)). The lepidopterans that have been studied for plastic biodegradation include caterpillars of the wax moth *Galleria mellonella* (Bombelli et al., 2017; Kong et al., 2019), the Indian meal moth *Plodia interpunctella* (Yang et al., 2014; Navlekar et al., 2023), *Achroia grisella* (Kundungal et al., 2019a), *Spodoptera frugiperda* (Zhang et al., 2022), and *Corcyra cephalonica* (Kesti and Thimmappa, 2019). Among above insects, three are commonly named as waxworms. They are the caterpillar larvae of wax moths, which belong to the family Pyralidae (snout moths) include two closely related species the lesser wax moth (*Achroia grisella*) and the greater wax moth (*Galleria mellonella*), and another species whose larvae share a name of the Indian mealmoth (*Plodia interpunctella*).

3.2.1 *Plodia interpunctella*

Plodia interpunctella Guenée 1845 (Lepidoptera: Pyralidae), often known as the Indian meal moth, is primarily recognized as a grain pest. The larvae of this species reach up to 12 mm and have the ability to bite through plastic and cardboard (Cline, 1978), and are frequently observed that this species ingests LDPE film (Fig. 4(I)).

Observation of *P. interpunctella* larvae chewing and penetrating LDPE film was reported as early in 1950s (Gerhardt and Lindgren, 1954). It was the first reported species that could degrade plastics based on ingestion of PE film and discoveries of PE-degrading gut microbes. Yang et al. (2014) reported *P. interpunctella* larvae chewing and eating LDPE film and isolated two gut LDPE degrading bacterial strains *i.e.*, *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 but did not report the results of characterization of biodegradation of LDPE. *P. interpunctella* larvae were considered to have capacity of biodegrading LDPE because they eat LDPE. Primary analyses of frass samples by GPC and FTIR by one of co-authors of this paper showed molecular weight reduction of the residual PE polymers and formation of oxidative groups (data not published).

Later, from midgut of *P. interpunctella* larvae, other two bacterial cultures *Enterobacter tabaci* strain Y1Mhb-3B1 and *Bacillus subtilis* subsp. *Spizizenii* NBRC 101239 B2 were also isolated; and both showed significant

degradation of PE film according to SEM observation (Mahmoud et al., 2021). Lou et al. (2022) reported that *Meyerozyma guilliermondii* and *Serratia marcescens* were isolated from the gut of the larvae of *P. interpunctella* fed on PE film and confirmed them as PE degraders by using analyses of WCA, FTIR, GPC and GC–MS. Studies by Navlekar et al. (2023) indicated that the enrichment of microbiota of *P. interpunctella* larvae fed with PE bag film showed PE degradation by ATR-FTIR analysis and significant shift of community structure. Even though it is thought to be highly possible and numerous PE-degrading bacteria were identified from the larval gut (Yang et al., 2014; Mahmoud et al., 2021; Lou et al., 2022).

Due to tiny size, *P. interpunctella* larvae are difficult to be characterzed for the fate of the ingested LDPE although a number of plastic-degradfing bacterial strains were isolated from their gut.

3.2.2 *Galleria mellonella*

The larger wax moth, often known as the honeycomb moth or *Galleria mellonella* Fabricius 1798 (Lepidoptera: Pyralidae), is a frequent pest of honey wax comb. *G. mellonella* is found all over the world. Their larvae, commonly referred to as greater waxworms or caterpillars, are commercially available at pet stores as feed for birds and small animals. *G. mellonella* has been identified to capable of rapid biodegrading LDPE (Bombelli et al., 2017; LeMoine et al., 2020) and PS (Lou et al., 2020; Wang et al., 2022b), possibly PP, PVC, PUR (Zhu et al., 2022; Nyamjav et al., 2023b), and PLA (Shah et al. 2023).

Bombelli et al. (2017) first reported the rapid biodegradation of LDPE film by *G. mellonella*. Subsequently, several investigations have demonstrated LDPE biodegradation by *G. mellonella* larvae and the generation of intermediates (Vasileva et al., 2018; Kundungal et al., 2019b; Cassone et al., 2022). Recent seminal works revealed the modality deployed by *G. mellonella* larvae to degrade PE: the larvae produce enzymes belonging to the phenol oxidase family. They are defined hexamerins. To date, four of these proteins have been found in the saliva of the larvae. Three out of four produce oxidation and breaking down of PE, after a few hours' exposure, as revealed by spectroscopy analyses as well as HT-GPC. PE does not need to be pre-treated, and the saliva/enzymes work at room temperature in an aqueous solution. Gas chromatography mass spectrometry (GC-MS) revealed the formation of small oxidized molecules, such as ketones, acids *etc*. (Sanluis-Verdes et al., 2022; Spínola-Amilibia et al., 2023). Whereas the details of the enzymatic reaction are still unknown, this discovery opens up a new way of exploring the plastic degrading insects in a holistic way, inclusive of any contribution from the insect and the symbiotic microbes in the gut.

G. mellonella larvae from many sources in the world have shown an ability to not only consume PE film but also PS (Fig. 4(J)) and other plastics. Biodegradation of PS in *G. mellonella* larvae was also confirmed (Lou et al., 2020). Peydaei et al. (2020; 2021) further tested the chemical compositions of different plastics (PS, PE, and PP films or foams) masticated and ingested by *G. mellonella*. In the frass samples, the FTIR spectrum revealed the development of functional carbonyl groups of oxidized polyolefin metabolic intermediates, indicating biodegradation. Burd et al. (2023) tested the larvae from a source in Brazil for biodegradation of LDPE and PS foam plates. They analyzed the plastic plates after contacted with the larvae with FTIR and contact angle to show possible oxidation and surface modification but did not report analysis of the frass as established methods described in Section 2.0. The larvae consumed both LDPE and PS plates and damaged the surface. Complete digestion of PS MPs in *G. mellonella* larvae was confirmed by forced injections of PS MPs (< 75 μm) (Wang et al., 2022b).

Zhu et al. (2022) tested a total of nine waste electrical and electronic equipment (WEEE) plastics and pristine plastics as feed for larvae of *G. mellonella* versus *T. molitor*. *G. mellonella* larvae favored pristine plastics to the two WEEE plastics, *i.e.*, rigid PUR and PS, with the following decreasing preference: PUR > phenol–formaldehyde resin > PE > PP > PS ≈ PVC. When the larvae were fed multiple plastics, the larvae randomly ingested all the plastics. *G. mellonella* larvae appeared to have higher consumption rates of the WEEE plastics than *T. molitor* larvae did.

Nyamjav et al. (2023b) reported that one *G. mellonella* larva consumed 0.22 ± 0.02 mg of PP film per day over 12 d period. The biodegradation of PP was not characterized but they found that an isolated *Bacillus cereus* sp. and the enrichment of gut microbiota biodegraded PP. Shah et al. (2023) analyzed metaboliites of *G. mellonella* fed on molasses supplemented with PLA blocks. The results indicated the larvae could degrade PLA but PLA feeding caused several metabolitc distubances .

G. mellonella larvae are widely studied plastic-degrading insect specie, being next to *T. molitor* and the first insect with identified PE-degrading enzymes (Peydaei et al., 2020). However, their PE degrading compacity apperears insect source-dependent, *e.g.*, several high school students in USA reported to the authors that *G. mellonella* larvae did not consume LDPE films. Their observations are different from the results observed in Spain, Republic of Korea, China and Canada. It remains unknown whether there is source dependent plastic-eating behavior among the larvae of this species.

3.2.3 *Achroia grisella*

Achroia grisella Fabricius 1794 (Lepidoptera: Pyralidae),

or lesser waxworm, is a small moth with worldwide distribution. They reside in bee colonies as nest parasites and feed on honey wax comb. Adults of *A. grisella* are about 13 mm long. The larvae appear smaller than greater waxworms. This species has been increasly studied for plastic degradation since its larvae are also commercially available.

Chalup et al. (2018) reported that *A. grisella* larvae consuming "silo-bags", a material commonly used in the country for hive management. Kundungal et al. (2019a) studied the biodegradation of HDPE film by *A. grisella* larvae (Fig. 4(K)) and found that they were able to complete their life cycle when fed with HDPE film. The egested frass of the lesser waxworm fed on waxcomb (WC), HDPE, and HDPE-WC were studied by analyzing the changes in physiochemical properties through FTIR and ^{1}H NMR techniques in addition to weight loss percentage of PE and survival rates of the tested lesser waxworms. The post-degradation studies of WC and PE showed 90.5% ± 1.2% and 43.3% ± 1.6% weight loss, respectively, by a group of 100 lesser waxworms. Over an 8-d period, PE consumption increased with an ingestion of 1.83 mg of PE per day per larva. Supplementing the HDPE feed of lesser waxworms with WC facilitated enhanced PE degradation showing 69.6% ± 3.2% weight loss. Twenty-eight day survival rates for lesser waxworms fed on WC, PE, and PE-WC were 91.3% ± 1.01%, 74.6% ± 2.9%, and 86% ± 1.4%, respectively. Residual polymers in egested frass were examined utilizing FTIR and ^{1}H NMR, confirming oxidization and biodegradation of HDPE. The authors did not test biodegradation of LDPE which is less resistant to biodegradation than HDPE.

Biodegradation of LDPE by *A. grisella* larvae were tested by feeding with honeycomb wax (HCW), LDPE plus HCW) and LDPE only (Ali et al., 2023). After 10 d of incubation, *Achroia grisella* decreased HCW, HCW-LDPE, and LDPE weight by 97.8% ± 1.8%, 77.1% ± 1.7%, and 59.1% ± 1.5%, respectively, with an average LDPE degradation rate of 2.96 mg/d per larvae. A significant reduction of *Achroia grisella* larvae activity during feeding on LDPE compared to HCW and HCW-LDPE by 65.5% and 30.5%, respectively. However, the activity of HCW-feeding larvae was significantly higher than HCW-LDPE-feeding larvae by 26.8%. The average SR of the investigated larvae fed on HCW, HCW-LDPE, and LDPE was 95.3% ± 1.5%, 92.6% ± 1.2%, and 81.3% ± 1.5%, respectively. The conversion of consumed LDPE to CO2 was estimated to be up to 97%. GPC analysis showed that antibiotic suppression negatively impact LDPE depolymerization.

3.2.4 *Corcyra cephalonica*

Corcyra cephalonica Stainton 1866 larvae, commonly known as rice moths (Lepidoptera: Pyralidae) grow into a small moth that is known as a major pest around the world due to its caterpillars' proclivity for feeding on dry plantstuffs such as seeds, including cereals (*e.g.*, rice), as well as flour and dried fruits. The larvae of *C. cephalonica* are also capable of damaging PE and PVC films (Fig. 4(L)), as reported by Cline (1978).

Rice moth larvae were tested for consumption of LDPE film in the absence and presence antibiotics (Kesti and Thimmappa, 2019). Over a 20-d period, 50 larvae consumed 25% and 21% of 1.0 g added LDPE. Based on suvirval rates, the larvae could live with LDPE as energy source or biodegrade LDPE. But No analytical data on the characterization of LDPE biodegradation was provided. Further study is needed to characterize the fate of ingested LDPE.

3.3 Other insects

Other than Families Tenebrionidae and Pyralidae, the following orders of insects may be considered as potential candidates of plastic-degraders or as a source of undiscovered plastic-degrading gut microbes as below.

3.3.1 Other families in order Coleoptera

The order Coleoptera (beetles) is the most diverse of all insect orders and is represented by about 40000 described species, that collectively constitute almost 40% of reported insect diversity (Stork, 2018). Many additional species of this order could potentially have the ability for plastic biodegradation. Riudavets et al. (2007) discovered that three store pests *i.e.*, the lesser grain borer or *Rhyzopertha dominica* (Coleoptera: Bostrychidae), rice weevil or *Sitophilus oryzae* (Coleoptera: Curculionidae), and cigar beetle or *Lasioderma serricorne* (Coleoptera: Bostrychidae) chewed and ingested PE plastic films. The members belong to family Scarabaeidae, commonly named as white grubs (Rush and Hoffard, 1989), fed on herbaceous plant roots and other soil organic matter with lignocellulose could also be plastic-degrading candidates. Gallitelli et al. (2022) reported that the larvae of *Protaetia cuprea* Fabricius 1775, also known as the copper chafer in the family Scarabaeidae penertrated into EPS box and generated PS fragments but the authors assumed that the larvae only chewed without ingesting the EPS fragments. To date, there have been no explicit studies of biodegradation of plastics by above insects and these species hold promise for future research efforts.

3.3.2 Other families in order Lepidoptera

Similarly, other insect species in the order Lepidoptera may also be discovered with the ability to biodegrade plastics. For instance, the larvae of *Hofmannophila pseudospretella* (or the Brown House moth) in Family Oecophoridae was known to eat PE, PS, and nylon films

(Whalley, 1965) but their plastic-degrading ability has yet been studied or verified.

More recently, Zhang et al. (2022) isolated PVC-degrading bacteria *Enterococcus*, *Klebsiella* and other species from the larvae of *Spodoptera frugiperda* J.E. Smith 1797, belonging to Noctuidae family (Lepidoptera: Noctuidae). They reported that the *Klebsiella* strain EMBL-1 removed PVC by 19.57% with significant reduction in M_n and M_w by 12.4% and 15.0%. This insect species is commonly known as fall armyworm, and populations are native to tropical regions of the western hemisphere. This moth is a strong flier, and is able to migrate long distances. It is an agricultural pest and has spread to West and Central Africa in 2016 and China in 2018. This species now threatens Africa, Europe, and Asia and has a very wide host range of over 80 plants, but prefers grasses, corns, sorghum, Bermudagrass, and crabgrass. The larvae of *S. frugiperda* survived (70%) by feeding on PVC film for 5 d versus 25% in starvation control, 33% with PVC under antibiotics suppression, and 100% in the larvae fed with corn leaves, indicating that the larvae could receive energy from PVC biodegradation. The PVC fragments retrieved from excreted feces had significant surface damage, according to SEM examination. Biodegradation by the larvae requires further characterization of mass reduction of ingested PVC and Cl balance after PVC ingestion.

3.3.3 The order Diptera

The larvae of member of flies, that are insects of the order Diptera, could have plastic-degrading candidates. Black soldier fly (BSF), *Hermetia illucens* Linnaeus, 1758 (Diptera: Stratiomyidae), is a common and widespread fly. The larvae of BSF are able to grow on a wide variety of organic substrates including manures and wastes and a feed source for animals. De Flippis et al. (2023) tested BSF larvae by feeding particular PE-(0.4–1.0 mm) and PS-(0.4–0.8 mm) agar gel (5:1, w/w). The survival rates of the larvae fed with PE- and PS-agar were similar to that fed with agar only without weight increase. Surface damage of both PE and PS particles was observed on SEM observation.^{1}H MNR analyses showed appearance of oxidative groups in the residual PE and PS MPs. This insect species could degrade both PE and PS and further characterization on the degradation rate and depolymerization process is needed.

3.3.4 The order Blattodea

Blattodea includes ~4400 species of cockroaches in almost 500 genera and about 3000 species of termites from ~300 genera (Zhang, 2011). Cockroaches can chew, ingest, and penetrate plastic packaging materials, and this behavior is observed worldwide. Gerhardt and Lindgren (1954) reported that German cockroaches, or *Blattella germanica* Linnaeus 1767, can chew and penetrate PE film. The team members of the authors and collaborators have observed that American cockroaches (or *Periplaneta americana*) living in southern regions of USA aggressively chewed and consumed PS coffee cups, and egested PS-containing frass and the Dubia roaches, or *Blaptica dubia* Seville 1838 (Blattodea: Blaberidae) chewed and ingested PS foam. However, to date, the biodegradation of plastics by any cockroach species has not yet been published although the biodegradation of plastics by cockroaches is under investigation.

Termites also are suggested as a potential plastic-degrading candidate because they primarily attack wood and plant materials containing cellulose, hemicellulose, and lignocellulose for food (Kumar et al., 2022). Some researcher suggest that transferring knowledge from research on lignocellulosic degradation by termites and their gut symbionts to that on synthetic polymers has become a new research hotspot and technological development direction to solve the environmental bottleneck caused by synthetic plastic polymers (Al-Tohamy et al., 2023). López‐Naranjo et al. (2013) found wood-plastic composites with 40% (wt/wt) wood and recycle HDPE were degraded by higher termites (*Nasutitermes nigriceps*) using FTIR and DSC analyese and SEM observation. Yang et al. (2021c) found that abnormal leakage occurred on buried HDPE pipes due to multiple of holes and cracks. The damage was due to biological degradation by termites which had nest near the HDPE pipes according analyese of ATR-FTIR, XRD and DSC. However, to date, no report on plastic degradation directly by the termites has been published.

3.3.5 The order Orthoptera

Orthoptera is an order of insects that comprises the grasshoppers, locusts, and crickets, including the closely related bush crickets, or katydids, and wētā. The order is subdivided into two suborders: Caelifera including grasshoppers, locusts, and close relatives; and Ensifera, including crickets and close relatives. More than 20000 species are distributed worldwide. Chewing and ingesting plastic foams, especially PUR foam by crickets has been observed and reported. The cricket *Gryllodes sigillatus* Walker 1869 has been shown to readily ingest and tolerate fluorescent labeled MPs mixed into their feed (Fudlosid et al., 2022). Ritchie et al. (2023) combined tissue dissection, organic material digestion, sample filtering and automated imaging techniques to show how fluorescently labeled MPs provided to insects (*e.g.*, in their diet) in a laboratory setting can be isolated, identified and quantified. As a proof of concept, they fed *G. sigillatus* a diet of 2.5% (w/w) fluorescently labeled plastics and isolated and quantified plastic particles within the gut and frass. The crickets pulverized the ingested fluorescent PE particles (~100 μm) into smaller even NPs with ~1000-fold reduction (Ritchie et al., 2024). However, the evidence of biodegradation PS and

PUR remains unclear.

4 Characterization of biodegradation process

4.1 Physiologic response to plastic biodegradation

During plastic degradation, the physiology of the insects has been investigated as indication of the health of the animals. Plastics do not contain necessary elements and nutrients other than C and H needed for protein synthesis, cell growth and larval development. If plastics serve as only diet source, the insects or their larvae live under nutrition-deficient conditions. The parameters monitored include 1) survival rate (SR%) or mortality rate, weight change, larval size change as well as larval pupation rate; 2) plastics or/and diet consumption rate; 3) composition of insect and/or larval biomass (proteins, fats, water *etc*.). Items 1) and 2) are commonly monitored by investigators.

Plastic-degrading insects can biodegrade or digest ingested plastics as carbon and energy source for life activity. But response of survival rate to feeding plastics depends on insect species and polymers fed.

The larvae belonging to darkling beetle family can live with plastics as sole diet for short to middle term (3–5 weeks) with high survivability or low mortality, The SR of *T. molitor* larvae fed PS or LDPE foams as sole diet showed the similar SR to that fed with normal diet (wheat bran) (Yang et al., 2015a; Brandon et al., 2018; Yang et al., 2018a, 2018b). However, for long-term, SR deteriorates due to lack of nutrients needed when plastics is supplied as sole diet. For a medium term (8 weeks), significant difference of SRs were observed depends on biodegradability of plastics. The SRs of *T. molitor* larvae fed respective diets were bran, 96.3%; PLA, 78%, PS, 76.7%; and PVC, 69.2% versus unfed control, 47.3% (Peng et al., 2023a) with biodegradation rate of PLA > PS > PVC. A long-term results indicated that after 98 d, the SR of *T. molitor* larvae fed with PS was 11.5% similar to 11.8% of unfed larvae (starvation) versus 81.5% with wheat bran (Yang et al., 2018b). For short-term tests, *T. obscurus* and *Z. atratus*, both belong to Tenebrionidae, show similar trend (Peng et al., 2019, 2020b).

The larvae of pyralid moths, *e.g.*, *G. mellonella* larvae response to feeding plastics in different ways from darkling beetles. Lou et al. (2020) found the larvae fed with PS showed lower SRs than those fed with normal die beeswax. Burd et al. (2023) also reported that the survival rates of *G. mellonella* larvae fed plastics PS or LDPE were 60% and 82%, respectively much lower than 92% of the control fed beeswax even for short-term 7.25 d.

Due to nutrition deficiency of plastics, the plastic-degrading insect larvae, regardless belonging to darkling beetles (*T. molitor*, *T.obscurus*, *Z. atratus*, and *Uloma*) or to pyralid moths (*G. mellonella*, and *Achrola grivella*), fed plastics (PS, LDPE, HDPE, or PVC) as sole diet sufferred weight loss but kept the weight well above unfed larvae even during short-term (< 4–5 weeks) because they obtained carbon and energy source from digestion of ingested plastics (Brandon et al., 2018; Yang et al., 2018a, 2018b, Kong et al., 2019; Kundungal et al., 2019a, 2019b, 2021; Peng et al., 2019, 2020a, 2020b). For example, according to Bożek et al. (2017), *T. molitor* larvae fed PS, PVC, and PLA experienced a reduction in plastic mass of 9%, 12%, and 3%, respectively, while those fed plastics experienced a loss of weight of 18%, 15%, and 19%, while those fed WB only experienced a 45% increase in their weight. For *G. mellonella* larvae, the physiologic response also dependes on the source of the larvae. Réjasse et al. (2022) tested *G. mellonella* larvae reared in their laboratory in France found that the larvae fed with only LDPE lost weight (1.3-fold) after 3 d and 100% died. The unfed control died after 3 d while the larvae fed with pollen-beeswax and beeswax only increased weight by 10 and 1.3 times, respectively after 16 d. Similarly, the SR of *A. grisella* larvae fed with HCW > LDPE plus HCW (1:1, w/w) >LDPE only (Ali et al., 2023).

Results indicated that co-feeding normal diet which provides necessary nutrients and minerals could help or enhance plastic biodegradation. *T. molitor* larvae fed with normal feed wheat bran (WB) enhanced PS, LDPE, PP and PVC consumption rates significantly (Brandon et al. 2018; Yang et al., 2018a; 2018b; Peng et al., 2020a; 2020b; Yang et al., 2021b). For example, when the larvae were co-fed soy protein or WB, PS consumption rates were higher than those when they were fed only with PS. From an initial feed of 1.8 g PS foam, when supplementing the PS-fed mealworms with 0.9 g WB and 0.9 g protein, the 28-d PS consumption (%) was 24.5% for PS fed only, 42.8% for PS co-fed WB, and 84.5% for PS + WB + protein (Yang et al., 2018a). The enhancement created by a co-diet is because plastics contain only carbon and hydrogen elements, and although plastic can provide energy and a carbon source to the larvae, it does not provide necessary nutrients for enzyme synthesis and larval development. The same observation was also reported during biodegradation of PS, LDPE, and PP by *Z. atratus* and *T. obscurus* larvae with co-diets (Peng et al., 2019; 2020a; 2020b; Yang et al., 2021b). However, the consumption of LDPE and PS was not enhanced and even decreased when *G. mellonella* larvae were fed plastics plus beeswax (Kong et al., 2019; Lou et al., 2020).

When PS or PVC was used as sole diet, the development of *T. molitor* larvae to pupae was negatively impacted (Yang et al., 2018a; 2018b; Peng et al., 2020b; Yang et al., 2021d). When PS was fed along with wheat

bran (WB), *T. molitor* larvae completed all stages of their lives (larvae, pupae, beetles, and egg). This is because the nutritional supplementation during plastic decomposition also permits *T. molitor* mating and reproduction, and hence selective breeding (Yang et al., 2018a). After mating and laying eggs, a second generation of mealworms emerged. Larvae of the second generation fed PS and WB resembled those of the first generation. After the new generation larvae were reared with PS foam and WB for three months, they appeared to have a higher affinity for PS foam and maintained the capacity to consume and degrade PS was maintained, and perhaps enhanced, compared with M_w and M_n of residual PS in frass collected from the first and second generation larvae, the PS depolymerization extent by the second generation appeared higher than that of the first generation. The juvenile mealworms of the second generation grew up and evolved into pupae and beetles.

The plastic-degradation ability is also influenced by larval age. Zhong et al. (2023) tested micro-PS degradation and oxidation after ingestion by *T. molitor* larvae in 3 month-old (~80 mg each) and younger larvae in 2 month-old (~20 mg each). The mature larvae biodegraded PS MPs effectively while younger larvae showed less plastic depolymerization and weaker modification in functional groups of PS.

Przemieniecki et al. (2022) studied effect of different polymer diets *i.e.*, cardboard (CEL), PS foam, oxo-degradable PE (~1% starch additive), PE regranulate, and PS polymers versus oatmeal (OAT) fed for 45 d, on the development of *T. molitor* larvae, their microbiome, the biochemical activity of their digestive system, and their feed-metabolizing capacity. The OAT had high counts of pupae and adults while the CEL and PE-oxo had lowest counts. The larvae developed best on LDPE regranulate, Lignocellulose-based CEL diet was the worest for larval delopment.The larvae fed PS diet had high protein content.

4.2 Depolymerization patterns

Biodegradation of plastics such as PS, PE, PVC, PP, *etc*., involves the decomposition or breaking down of polymer chains (depolymerization) followed by biodegradation and mineralization of intermediates to CO_2 and H_2O (Yang et al., 2015a; 2015b; Yang et al., 2021a; 2021b; Peng et al., 2020a). GPC analysis was frequently employed to describe this depolymerization, characterizing the changes in molecular weight and MWD of polymers in terms of the M_n, M_w, and M_z (Wu and Criddle, 2021).

During biodegradation of plastics by insects (Yang et al., 2015a; 2018a; 2018b; 2021a; 2021b; Brandon et al., 2018; Peng et al., 2019; 2020a; 2020b; 2022b), two depolymerization patterns, *i.e.*, broad depolymerization (*i.e.*, a decrease in both M_n and M_w) and limited extent depolymerization (*i.e.*, an increase in M_n or in both M_n and M_w) are observed during biodegradation of plastics based on the results of analysis of residual plastic polymers versus virgin plastics, depends on polymer type, molecular weight, polymer physical and chemical structure, insect species and insect source, *etc*. To date, the GPC results of biodegradation of commercial PS foams with M_w between 120 to 300 kDa by *T. molitor and T. obscurus* have showed only broad depolymerization patterns (Yang et al., 2015a; 2018a, 2018b; Brandon et al., 2018; Peng et al., 2019). Biodegradation of high purity narrow molecular weight PS by *T. molitor* indicated that biodegradation of PS with M_w from 6.7 to 612 kDa showed broad depolymerization but biodegradation of PS with extremely high molecular weight (1346 kDa) was performed *via* limited extent depolymerization patterns (Peng et al., 2022b) (Figs. 5(A) and 5(B)). Biodegradation of commercial LDPE by *T. molitor* occurred with both broad and limited extent depolymerization patterns (Brandon et al., 2018; Yang et al., 2021d). Further studies indicated that the depolymerization patterns were affected by PE polymer type and molecular weight. *T. molitor* and *T. obscurus* larvae degraded commercial LDPE microplastics (M_w 110.5 kDa) *via* broad depolymerisation but LLDPE (M_w 222.5 kDa) and HDPE MPs (M_w 182 kDa) *via* limited extent depolymerisation (Yang et al., 2022b). Biodegradation of LDPE MPs with M_w of 0.84, 6.4, and 102 kDa and HDPE with low molecular weight (M_w 5.2 kDa) was performed *via* broad depolymerization while degraded HDPE with M_w 105 and 132.7 kDa *via* limited depolymerisation (Yang et al., 2022b). The biodegradfation of commercial PET polymers (M_w 29.43 kDa) and high purity PET polymers with respective M_w of 1.10 and 27.0 kDa was performed *via* broad depolymerization while PET with 63.5 kDa *via* limited depolymerization (He et al., 2024).

In general, when *T. molitor* and *T. obscurus* larvae degrade the same type of plastics, the polymers with extremelyhighMWisdegraded*via*limitedextentdepolymerization while the polymers with low MW or short chains trends to broad depolymerization.

PS and LDPE foams were biodegraded by *Z. atratus* larvae using both broad depolymerization and limited extent depolymerization (Peng et al., 2020b; Yang et al., 2020), depends on the larval sources. For examples, the larvae from a source in China performed broad depolymerisation of PS with M_n 107 and M_w 345 kDa (Fig. 5(C)); while the larvae from a source in the USA performed limited extent depolymerisation of PS with M_n 69.6 and M_w 168 kDa (Fig. 5(D). Both *T. molitor* and *Z. atratus* displayed limited extent depolymerization patterns during biodegradation of commercial PP foams (Yang et al., 2021b). The limited extent depolymerization was also observed during microbial biodegradation of PUR by a landfill microbial culture (Gaytán et al., 2020), biodegradation of PS in *G. mellonella larvae* (Lou et al.,

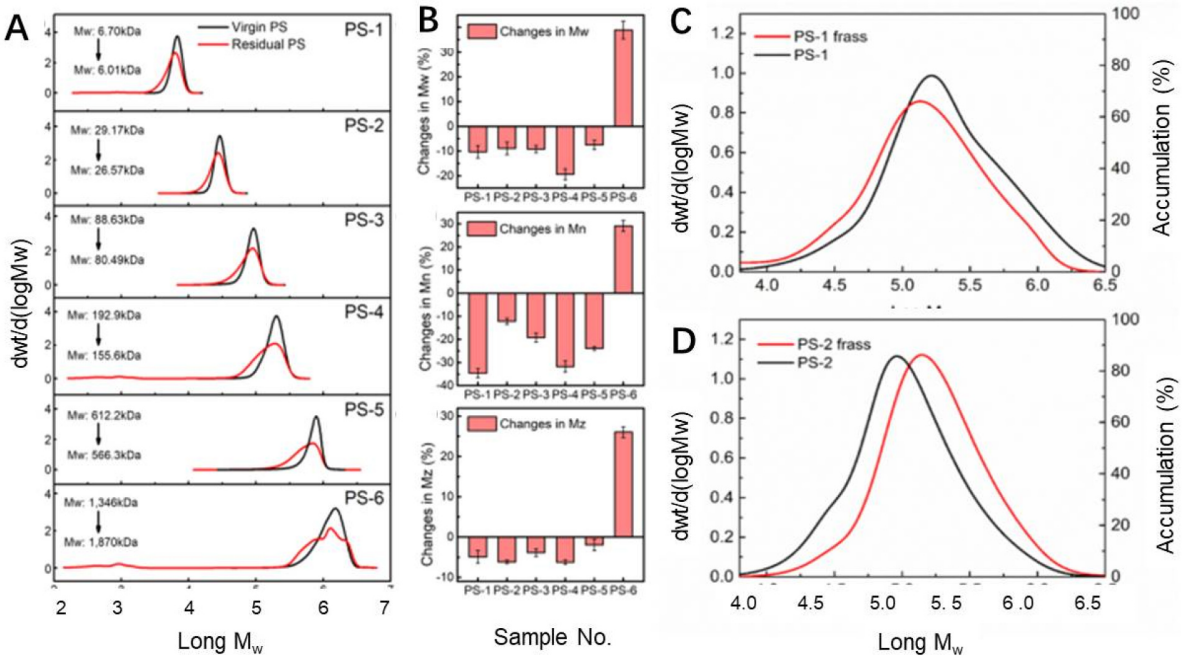

Fig. 5 Depolymerization of PS by insect larvae. (A) Changes in molecular weight distribution of PS polymers after biodegradation by *T. molitor* larvae. (B) The changes of M_n, M_w, and M_z of different PS polymers after biodegradation by *T. molitor* larvae. The molecular weights of the high purity PS polymers (kDa) are PS-1, 6.7; PS-2, 29.2; PS-3, 88.6; PS-4, 192.9; PS-5, 612.2; PS-6, 1346. (C) Change in molecular weight distribution of PS (initial M_n 107 kDa; M_w 345 kDa) after biodegradation by *Z. atratus* larvae from a source in China, showing broad depolymerisation pattern. (D) Change in molecular weight distribution of PS (initial M_n 69.6 kDa; M_w 168 kDa) after biodegradation by *Z. atratus* from a source in the USA, showing limited extent depolymerisation pattern. Data adapted from Peng et al. (2020a; 2022b).

2020), and PS foam biodegradation by land snails *Achatina fulica* (Song et al., 2020). During biodegradation or enzymatic degradation, the limited extent of depolymerization is most likely due to the selective decomposition of polymer chains with lower molecular weights at a faster rate than large molecular portions.

The depolymerization pattern is impacted by polymer types and also clearly impacted by molecular weight and physical properties (*e.g.*, branching, crystallinity, *etc*.) of polymers. The broad depolymerisation pattern indicates that the polymers are relatively easy to be biodegraded while the limited extent depolymerisation reveals that the polymers are persistent to biodegradation or the insects have limited ability to digest the polymers. In addition, crosslink reactions during plastic degradation could also result in the increase in molecular weight, which has been observed, especially during UV oxidation of plastics (Lucas et al., 2008). However, it is unknown if it occurred during the biodegradation in insects.

4.3 Impacts of polymer physical properties

For the same type of polymers, molecular weight has significant impact on biodegradability. The higher molecular weight the polymer is more persistent to degradation as reported previously during photo degradation, microbial degradation and enzymatic degradation (Min et al., 2020; Inderthal et al., 2021). Other physical and chemical structures and characteristics of polymers besides molecular weight influence degradation and biodegradation, with the most significant influencing elements being physical structure, mechanical property, surface hydrophobicity, crystallinity degree, and branching degree (Shah et al., 2008; Restrepo-Flórez et al., 2014; Min et al., 2020; Wei et al., 2020; Inderthal et al., 2021).

PE can be categorized into LDPE, LLDPE, and HDPE based on chain length and branching with different crystallinity degrees. Using *T. obscurus* and *T. molitor* larvae, Yang et al. (2022b) examined the effect of physicochemical properties on biodegradation utilizing high-purity PE MPs, which included LDPE, HDPE, and LLDPE with varying M_w and crystallinity degrees. Biodegradation rates were found to be highly dependent on polymer type or physical structure, with LDPE > LLDPE > HDPE (with respective M_w of 222.5, 110.5, and 182 kDa), and the PE MPs with reduced M_w showing a larger extent of depolymerization. According to the results of a dominance analysis performed by the authors, a lower degree of branching structure and a higher degree of crystallinity have a detrimental effect on depolymerization and biodegradation. The results confirm

that physical and chemical properties of PE significantly affect the biodegradation capability of PE in both *T. molitor* and *T. obscurus* larvae. Similar effects could also occur during biodegradation by other insects' larvae.

Peng et al. (2022b) tested effect of PS molecular weight on biodegradation. With high purity PS MPs with respective M_w of 6.70, 29.17, 88.63, 192.9, 612.2, and 1346 kDa, *T. molitor* larvae showed decreasing mass reductions of 74.1%, 64.1%, 64.4%, 73.5%, 60.6%, and 39.7% over 24 d. As mentioned previously, all PS samples were biodegraded *via* broad depolymerization pattern except that with ultra-high WM (1346 kDa), which was biodegraded *via* limited extent depolymerization (Peng et al., 2022b). This indicated that the biodegradation efficiency or mass reduction was negatively impacted by ultra-high molecular weight. Peng et al. (2023e) further demonstrated that the removal and biodegradation of PE MPs negatively influenced the physiologic performance, growth, and homeostasis of *Tenebrio molitor*.Biodegradation of high purity PET with low, medium, and high molecular weights by *T. molitor*, *i.e.*, M_w values of 1.10, 27.10, and 63.50 kDa with crystallinity 53.66%, 33.43%, and 4.25%, respectively, showed a mass reduction of > 95%, 86%, and 74% via broad depolymerization (He et al., 2024). Similar patterns of impact of MW on depolymerization/degradation were also observed during PP biodegradation by *T. molitor* using five high purity MPs with respective 0.83, 6.20, 50.40, 108.0, and 575.0 kDa. MW *i.e.*, the high MW had negatively altered depolymerization patterns, gut microbiome and host gen transcriptome, and thus PP biodegradation.

4.4 Metabolic pathways of plastic degradation

The metabolic pathways of biodegradation of plastics has been investigated via several methods *i.e.*, 1) analysis of gut contents using GC, GC-TOF-MS, HPLC *etc*. to determine intermediates; 2) analysis of composition of frass or excrement using Py- GCMS for intermediates or/and their pyrosized fragments; 3) metabolite analysis of gut contents; and 4) transcript analysis of upregulated genes of gut microbes or insect host or both to estimate enzymes involved.

Tsochatzis et al. (2020, 2021) identified the metabolic intermediates of PS degradation in *T. molitor* obtained in Denmark using GC-MS and GC-TOF-MS. Styrene, PS oligomers (dimers, trimers), and several fatty acids were identified as depolymerized products. Different diets (4:1 PS/bran, 20:1 PS/bran) were fed to larvae in this study, and the larvae's growth and development were compared in the presence and absence of water (Tsochatzis et al., 2022). They confirmed that biodegradation and metabolism of PS caused the increase in stress levels.

Based on microbiome in PS-fed *Z. atratus* larvae, Sun et al. (2022a) proposed pathways and enzymes of bacterial PS and styrene degradation without contribution by the host gut.

Zhong et al. (2022) used transcriptomic responses of PS and PE feeding on *T. molitor* larvae to study metabolic pathways and found that the fatty acid degradation pathways for the digestion of PS and PE degradation intermediates. According to the KEGG mapping results, the DEGs involved and validated by RT-PCR in this process were medium chain specific acyl-CoA (MCAD), peroxisomal acyl-CoA oxidase, 3-ketoacyl-CoA thiolase, and cytochrome P450 6A1. The weaker suppression of MCAD than the peroxisomal acyl-CoA oxidase, which oxidizes the CoA esters of 2-branched fatty acids, indicated that the production of medium-chain fatty acids was greater than that of branched fatty acids. Moreover, PS degradation was significantly enriched in isoniazid metabolism.

Researchers have proposed different metabolic pathways of plastics in *G. mellonella*. For PS biodegradation, an *in vivo* potential metabolic pathway, or the styrene oxide–phenyl acetaldehyde and 4-methylphenol–4hydroxybenzaldehyde–4-hydroxybenzoate pathways were proposed (Wang et al., 2022b), suggesting that the host digestive enzymes are responsible for PS biodegradation. LeMoine et al. (2020) proposed a hypothesized model for LDPE degradation in *G. mellonella*, adapted from the model of biodegradation by bacterial *Geobacillus thermodenitrificans* (Feng et al., 2007). The model describes mechanism of the biodegradation processes through RNA sequencing of the gut tissues of *G. mellonella*, *i.e.*, after the initial processing of LDPE by the gut microbes, the larvae secret key enzymes to further break down the alkane chains into aldehydes and then carboxylates; these fatty acids are then stored or degraded *via* β-oxidation and produce energy *via.* the Krebs cycle. Beyond these proposed scenarios, it has been known that saliva enzymes produced by the *G. mellonella* oxidize and break down PE, causing the formation of small oxidized compounds, as revealed by GC-MS-MS (Sanluis-Verdes et al., 2022; Spínola-Amilibia et al., 2023). This is the first proved step of any proposed metabolic chain of plastic degradation by insects.

4.5 Biofragmentation

Results on biofragmentation of plastic particles by non-plastic degrading insects (*e.g.*, mosquito) and macroinvertebrates (*e.g.*, Antarctic krill) found that biofragmentation of ingested plastics resulted in generation of nano-sized particles (Gopinath et al., 2022; Dawson et al., 2018). Mosquito (*Aedes aegypti*) larvae exposed to PS MPs (2–15 μm) transferred NPs *via* pupae to adults. The fluorescently labeled NPs were observed in the salivary glands of adult mosquitos (Gopinath et al., 2022). Laser light scattering sizer can measure particular size with a

measurement range of 0.01 to 3500 μm and Py-GCMC analysis is commonly used to quantify the content of plastic particles in organs, blood and animal tissues (Peng et al., 2023c, 2023d).

Formation and accumulation of smaller sized polymer residues such as MPs and even NPs have been a concern of biodegradation of plastics in insects since fragmentation occurs as the invertebrates, especially the insects, chew, ingest, and digest plastics. Peng et al. (2022a) examined the particle size distribution of residual plastic particles in the frass of *Z. atratus* fed with PS and LDPE foams using a laser particle sizer (0.01–3500 μm). The findings showed that the residual PS and PE particles' size-frequency distributions were comparable, with respective mean values of 174.3 μm and 185.8 μm based on total polymer volume. The particle percentage on a number basis reached its peak at approximately 6 μm (6.3 μm for PS particles and 5.7 μm for PE particles). All particles were MPs (> 4 μm) or no NPs (1 to 100 nm) were detected in the egested frass. This indicated that NPs may not accumulate in *Z. atratus* larvae's frass. The absence of NPs in frass of LDPE and PS-fed *T. molitor* larvae has also been confirmed.

After 48 h of force-feeding with PS MPs (< 75 μm) into their gut, it was reported that the PS MPs completely disappeared from the guts of PS-degrading *G. mellonella* larvae. This resulted in no NP accumulation and complete digestion of the PS MPs (Wang et al., 2022b). The results suggested that NPs might not accumulated in the intestinal tract of insects or invertebrates capable of biodegrading plastics. Enzymatic reactions take place on polymer surfaces, and since NPs have a larger specific surface area than MPs, their rates would be exponentially increased for polymers with NP size. As a result, the probability of NP accumulation in larval guts would be extremely low.

The findings of these studies showed that the ingested LDPE and PS foams were fragmented into MPs but not into NPs in the plastic-degrading insects, such as *Z. atratus*, *T. molitor*, and *G. mellonella* larvae, and the results suggested that NPs could not accumulate in the frass or excrement of these insects. The generation of NPs in intestinal tract and the NP penetration into larval tissues and glands were observed after *T. m*olitor ingested PS MPs. However, The level of NPs in the glands continuously decreased and enventually disappeared under detection limits (0.77 ng/larva), suggesting that plastic-degrading *T. molitor* could remove or degrade the PS NPs in their tissues and glands. However, more research on the generation and fate of NPs during plastic degradation by insects is needed, which can be conducted with Pyralidae and Tenebrionidae (Coleoptera and Lepidoptera, respectively) insect larvae, as well as various polymers like PP, PET, PVC, and PUR.

4.6 Oxidative stress response

Exposure to micro- and nano-sized plastic particles causes oxidative stress responses and ecotoxicity to macroinvertebrates have been widely (Wang et al., 2021). Zielińska et al. (2021) fed *T. molitor* and *Z. atratus* larvae with Styrofoam for 30 d and conducted cytotoxicity and oxidative stress assays using insect or issue homogenates. They concluded that the larval biomass did not have any increased cytotoxity. The biomass of *T. molitor* larvae increased protein content and decreased fat and carbohydrate content, while *Z. atratus* larvae did not exhibit any significant changes.

Negative impacts of PS ingestion on *T. molitor* larvae was reported by Dunn (2021). The resulrs showed that all larvae exposed to PS demonstrated some degree of vacuolization, with vacuoles visible within the epithelial cells lining the midgut. Mild-moderate vacuolization was noted in the larvae fed with bran plus PS but the larvae fed with PS alone demonstrated severe vacuolization, indicating that intestinal inflammation and damage occurred after the larvae ingested PS.Vacuoles are degradative organelles, enclosed compartments filled with water, inorganic and organic molecules, enzymes, and cellular waste. Formation of vacuoles in PS-fed larvae might be due to degradation of cell surface proteins. The larvae exposed to PS alone also demonstrated decreased levels of catalase activity, a marker of oxidative stress.

Peng et al. (2023c) investigated the response of ingestion of plastic diets (PLA, PS and PVC MPs) during biodegradation of the three polymers. When MPs were fed as sole diet, the enzymatic activities of antioxidation increased with sequence of PVC > PS > PLA > wheat bran (normal diet). The oxidative stress depends on biodegradability of the plastics tested *i.e.*, PVC > PS > PLA, When the three polymers were fed with co-diet wheat bran, the anti-oxidative activities decreased. The PLA with bran group showed almost the same activities with bran-fed groups. This indicated that co-diet feeding can significantly reduce ROS and thus ecotoxicity caused by MPs. Similar observation was reported that exposure to PLA induced oxidative stress and reduced the ceramide levels in molasses-supplemented PLA-fed larvae of *G. mellonella* (Shah et al., 2023), indicating that the larvae could ingest PLA but this process caused some metabolic stress.

Updated research results reveal that the elevation of reactive oxygen species is not only oxidative stress response but also be the results of enhanced metabolic activities in the insect intestine for biodegradation of plastics because strong oxidative reactions are needed to break C–C bonds of plastics. Chen et al. (2023) demonstrated that ROS was generated in the gut of *Z. atratus* larvae under different feeding trails with PS as a sign of the oxidative decomposition of ingested PS. When antioxidative reagents vitamin C or GSH was added in the diet, ROS levels decreased, PS depolymerisation behaved poorly and O/C ratio in residual PS decreased. The combinatorial effect of ROS and extracellular oxidases of

gut microbes indicated vital roles in effective the oxidative depolymerization of PS in the gut of *Z. atratus* larvae.

4.7 Fate of additives

To date, little research has been done on the impact and fate of plastic additives during biodegradation of plastics by insects. Commercial plastic products contain various additives. For example, PE products contain antiblock compounds, antioxidants, and slip agents (Hahladakis et al., 2018); PS foams contain flame retardant; and PVC products contain plasticizers. The impacts and fate of the additives on biodegradation in insects remain unknown except for hexabromocyclododecane (HBCD). To date, only one report has been published on the fate of flame retardant HBCD in PS degraded by *T. molitor* larvae as well as larvae-fed shrimps (Brandon et al., 2020). Little or no bioaccumulation of HBCD occurred in PS-degrading mealworms, as it was instead likely egested as frass. Also, no evidence of toxicity was observed when *Litopenaeus vannamei* (Pacific white leg shrimp) was fed mealworm biomass from the larvae reared with PS containing HBCD. Further studies should address whether the ingested plastics and chemical additives (*e.g.*, plasticizers, flame retardant, stabilizers *etc*.) within plastic-degrading insects leads to toxicity and bioaccumulation in plastic biodegradation systems.

4.8 Kinetics and modeling

Modeling and kinetic analyses of biodegradation of plastics *in vitro* or in the environment has been investigated intensively, mainly on degradation kinetics (Leejarkpai et al., 2011), degradation rates (Chamas et al., 2020; Min et al., 2020; Baldera-Moreno et al., 2022), and half-life (Lott et al., 2021). Molecular structure and physical properties were utilized to establish a ranking of plastic polymers' biodegradability (Min et al., 2020). The plastic polymers are ranked from high biodegradability such as PLA, PHB, *etc.*, to resistant to biodegradation *e.g.*, PET, PVC, PS, PE, PP, *etc*.

To simulate the size-reduction process during the digestive biofragmentation of the ingested plastic particles, Peng et al. (2023c) developed a digestive biofragmentation model for the size distribution of residual plastic particles on the basis of polymer volume based on the following assumptions: 1) The ingested plastic products enter the intestine through physical chewing and grinding via the mouthparts and intestinal peristalsis, and thus the size of the plastic particles is less than the mouthparts; 2) The biodegradation by the gut microbiome and digestive enzymes reduces the size of the plastic particles; 3) The physical grinding continues in the intestinal tract; 4) Both biodegradation and grinding result in the biofragmentation of ingested plastic particles; 5) The biofragmentation process is the function of the surface area of the particles; and 6) The biofragmentation and biodegradation kinetics follow a first-order reaction. The maximum initial size of the ingested plastics is assumed to be corresponded to the size of the mouthparts of the larvae. According to the above assumptions, the size-reduction rate of the ingested plastics is a function of the size and specific surface area of particles, the grinding activity, and the biodegradation rate of the polymers within the intestinal microenvironment (Eqs. (6) and (7)):

$$\frac{\mathrm{d}y_i}{\mathrm{d}t} = -\left(k_1 \times A_i^{\alpha} + k_2 \times \frac{1}{A_i^{\beta}}\right) \times y_i, \tag{6}$$

$$A_i = \frac{1}{R_i}, \tag{7}$$

where $\frac{\mathrm{d}y_i}{\mathrm{d}t}$ is the volume reduction rate of the plastic particles with particle size i at time t (h); k_1 is the biodegradation rate constant of the plastics; k_2 is the grinding rate constant of the plastics; A_i is the instant specific surface area of the plastic particles with particle size i (nm^2) ; y_i is the quantity of the plastic particles; R_i is the diameter of the plastic particles with particle size i (nm); α and β are the correction coefficients. The modle was validated with the measurement of residual LDPE and PS particles eggested in the frass of *T. molitor* larvae fed LDPE and PS foam (Peng et al., 2023d). The simulation results were consistent with the analytical results; *i.e.*, the ingested polymers were removed by more than 70% after digestion in the larval gut, and small-sized particles were removed or biodegraded at an ultrafast speed. Based on these findings, the authors hypothesized that nano-sized plastic particles were unlikely to accumulate in the intestines of macroinvertebrates *e.g.*, *T. molitor* larvae that have high plastic-degrading capacities.

5 Biodegradation mechanisms

Biodegradation of complicated or persistent organic materials such as lignocellulose in termite guts has been believed to be accomplished through symbiotic digestion with a diverse community of bacterial, archaeal, and eukaryotic gut symbionts (Brune, 2014). Plastic-derading insects, *e.g.*, *T. molitor* larvae do have ability of degrading lignocellulose in wheat straws, rice straws, and corn straws with significantly reduction of cellulose by 53%, 52.7%, and 37.0%, hemicellulose by 58.9%, 55.5%, and 24.1% and lignin by 41.3%, 49.8%, and 31.1%, respectively (Yang et al., 2019a; 2019b). Updated findings suggest that synergistic interactions between gut microbes and the digestive system of insects (digestive enzymes, enzymes, and factors, *etc*.) are responsible for the biodegradation of plastics in insects. Research on gut microbiome related to biodegradation of plastics has been

conducted in several areas as described below.

5.1 Contribution of the insect tissues/cells

Recent studies on PET biodegradation by *T. molitor* indicated that metagenome sequencing, transcriptomic, and metabolic analyses indicated symbiotic biodegradation of PET by the host and gut microbiota (He et al., 2024). After PET was fed, the host's genes encoding degradation enzymes were upregulated, including genes encoding oxidizing, hydrolyzing, and non-specific CYP450 enzymes. Gut bacterial genes for biodegrading intermediates and nitrogen fixation also upregulated. The multiple-functional metabolic pathways for PET biodegradation ensured rapid biodegradation resulting in a half-life of PET less than 4 h with less negative impact by PET MW and crystallinity. Studies on *G. mellonella* pointed toward the contribution of insect-produced hexamerins as first step in the degradation reaction (Sanluis-Verdes et al., 2022; Spínola-Amilibia et al., 2023). In the physiology of the animal, it is plausible that the first step in a degradation reaction of an exogenous component is situated within the first part of the digestive apparatus, that is the mouth and the saliva therein. Whether the enzymes are produced in the gut of the animal or elsewhere is still unknown. After the first oxidation, the broken down materials pass into the gut of the animal, where they might be further degraded by the action of the insect's microbiota.

5.2 Contribution of gut microbiota and host

Suppression of gut microbiota with antibiotics has been used to examine the contribution of gut microbes to biodegrade or metabolize certain organic matters and substrates. Feeding *T. molitor* larvae with antibiotics, such as gentamicin, nystatin, and ampicillin, can provide information of the role that gut microbiota play in digestive processes (Genta et al., 2006). The function of gut microbes in the degradation of plastic has been evaluated primarily by comparing changes in molecular weights of ingested plastic in the presence of antibiotic suppression of gut microbes to those in the absence of antibiotic treatment (Yang et al., 2015a; Wu and Criddle, 2021). Gentamicin is a commonly used single antibiotic (Yang et al., 2015a; 2018a; 2021b; Peng et al., 2019; 2020a). However, multiple or mixed antibiotics have been used frequently in order to achieve broader inhibition of gut microbes (Yang et al., 2020; Wu and Criddle, 2021).

Gentamicin was utilized in an antibiotic suppression experiment that significantly reduced the gut flora of *T. obscurus* and *T. molitor* larvae, but it did not prevent LDPE depolymerization because M_w, M_n, and M_z continued to decline. This demonstrated that, in contrast to PS degradation in *T. molitor* (Yang et al., 2021d), LDPE biodegradation in *T. obscurus* was less influenced by gut microbes or independent of gut microorganisms, indicating that LDPE depolymerization is accomplished by intestinal digestive enzymes although LDPE-degrading bacteria were isolated from the gut (Yin et al., 2020). Dunn (2021) tested effects of four antibiotics on PS comsumption and physiology of *T. molitor* lavae under 6 diet conditions *i.e.*, PS only, bran only, bran + PS, bran + PS plus penicillin (for Gram positive bacteria), bran + PS plus ciprofloxacin (for Gram negative bacteria), bran + PS plus ampicillin (Gram positive and some Gram negative bacteria), and bran + PS plus clindamycin (for anaerobic bacteria). The results showed that the larvae fed with antibiotics consumed less PS than that without antibiotics.

Recent studies have shown that inhibiting the gut microbes in *T. molitor* larvae with gentamicin or mixed antibiotics effectively impacted PS, PP, and PVC depolymerization (Yang et al., 2018a, 2021b; Peng et al., 2020b). However, LDPE and HDPE depolymerization continued to occur to a smaller extent or with an alteration in the depolymerization pattern (Yang et al., 2018a; Yang et al., 2021d). Thus, PS, PVC, and PP degradation appear to be relatively gut microbe dependent, whereas LDPE and HDPE degradation appears to be gut microbe independent. Suppressing gut microbes with gentamicin reduced PS and PVC depolymerization in *T. obscurus* larvae (Peng et al., 2019; Peng et al., 2020a); however, gentamicin had no effect on LDPE depolymerization (Yang et al., 2021d). For *Z. atratus* larvae, antibiotic suppression inhibited PS, LDPE, and PP depolymerisation (Peng et al., 2020b; Yang et al., 2021b). In general, the response to antibiotic suppression appears similar among the Tenebrionidae tested, *i.e.*, gut-microbiota dependent except for with PE in *T. molitor* and *T. obscurus*. For PET degradation, under antibiotic suppression of gut microbes, the PET was still depolymerized by *T. molitor* larvae (He et al., 2024), indicating that the host digestive enzymes could degrade PET independently.

Both *Z. atratus* strains successfully depolymerized LDPE to a limited extent for the tested materials. The results of antibiotic suppression studies showed that either PS or LDPE depolymerization as considerably reduced when antibiotics were utilized as a suppressant. This suggested that the ability of *Z. stratus* to depolymerize/biodegrade PS and LDPE relatively depended on gut microbes (Peng et al., 2020b; Yang et al., 2020).

The contribution of gut microbiota to plastic degradation, especially in Lepidoptera family is questioned by several investigators. Kong et al. (2019) investigated the long-chain hydrocarbon wax metabolism in *G. mellonella* and found that *G. mellonella* tested lacked intestinal microbiota for decomposition of long-chain fatty acids produced from wax metabolism, and concluded that the wax was metabolized and mineralized

mainly independent of gut microbes. They suggested that similar mechanisms could occur during LDPE degradation. This is not surprising altogether: in fact, no specific microbiome has ever been identified in the tubular/linear gut of Lepidoptera or Coleoptera, a general sign of no specialized digestive functions associated with the insect intestine (Engel and Moran, 2013). On this line, Serrano-Antón et al. (2023) did not identify any specific bacteria in the gut of *G. mellonella* larvae which could possibly be related to PE degradation. Wang et al. (2022b) found that low-MW PS microbeads (25 μm) with M_n 540 and M_w 550 Da and PS MPs (200 mesh) with M_n 95.6 kDa and M_w 217 kDa were completed digested in *G. mellonella* larvae within 24 h under antibiotic (kanamycin, gentamycin, tetracycline, ampicillin, polymyxin B, vancomycin, and neomycin, 1.0 mg/mL each, at 10 μL/individual for twice) suppression condition. The results supported that *G. mellonella* can degrade PS gut microbe-independently. On the other hand, Cassone et al. (2020) reported that *G. mellonella* larvae fed on PE excreted glycol, but those excretions were reduced by antibiotic treatment. The gut microbial abundance increased during the early phases of feeding on LDPE (24–72 h). They concluded that that during short-term exposure, the intestinal microbiome of *G. mellonella* is intricately associated with PE biodegradation *in vivo*.

For *A. grisella* larvae, when the larvae were fed with HCW containing streptomycin/gentamicin/rifampicin with a ratio of 1.5/1/3 against Gram-positive and Gam-negative bacteria, GPC analysis indicated that LDPE depolymerization was negatively impacted (Ali et al., 2023). The gut microbiota contribute to LDPE depoly-menrization significantly.

Even more, the bioinformatic analysis revealed a lack of both consistency and intrinsic to the omics tools and to the paucity of the available databases for the study of insect-associated microorganisms. The consequence of this landscape is dangerously inaccuracies in the data, with the presence of false positives contaminating the outcome of the informatic analyses. This scenario calls for adopting extra-care in the interpretation of the results, together with the necessary verification of the identified bacterial species in their potential capacity to degrade the material of choice (Serrano-Antón et al., 2023).

In summary, biodegradation of plastics in plastic-degrading insects has been hypothesized as symbiotic reactions and the contribution of gut microbiota dependes on insect family. The biodegradfation 1) performed by gut microbes under special insect intestine; 2) conducted by the enzymes secreted by both gut microbes and host; and, 3) mainly performed by host digestive enzymes and could be accelerated in the presence of plastic-degrading microbes.

5.3 Plastic-degrading gut microbiome

Insect gut microbiomes have been characterized for understanding the dynamics, community shift, and predominance of microbes during the biodegradation of plastics. To collect gut microbial samples, two sampling methods have been reported: 1) rinsing of extracted gut tissue and preserving it in phosphate buffered water (pH 7.0) for the extraction of DNA (Brandon et al., 2018), and 2) the collection of whole intestinal tissue for the extraction of DNA (Yang et al., 2018b). The latter method not only includes insect DNA but also collects all the microorganisms, including those attached to the gut wall. Once the DNA is extracted, amplified with appropriate primers and then sequenced, the raw sequencing data are processed.

Several parameters have been used to describe shifts in gut microbial community structure including the number of species in the community, ecological metrics of richness, and the dominance and evenness of species quantified by the Shannon and Simpson indices. Additional statistics often used to characterize gut microbial communities include principal coordinate analysis (PCoA), microbial community composition analysis, hierarchical cluster analysis, PERMANOVA and differential abundance analysis. Differentially expressed genes (DEG) is commonly analyzed to investigate the change in the gut microbiome or shaft of community after the insects received different diets.

Gut microbiome studies of *T. obscurus, T. molitor*, and *Z. atratus* larvae have revealed highly diverse communities that vary with source origins, past diet composition, rearing conditions, and diet (Brandon et al., 2018; Yang et al., 2018b; Peng et al., 2019, 2020a; Sun et al., 2022a; Navlekar et al., 2023). After the PS and LDPE plastics feeding, the gut microbiome of *T. molitor* and *Z. atratus* larvae shifted to a new community that can efficiently biodegrade PS and LDPE (Brandon et al., 2018; Yang et al., 2021d; Sun et al., 2022a). However, the diversity index does not provide significant results. Brandon et al. (2018) analyzed microbiota of *T. molitor* larvae fed PS, LDPE, and WB and the inverse Simpson index (a measure of community alpha diversity) did not significantly differ among diets. Przemieniecki et al. (2022) also reported formation of specific microbial communities after *T. molitor* larvae fed with cardboard, PS foam, oxo-degradable PE (~1% starch additive), PE regranulate, and PS polymers versus oatmeal.

Principal coordinate analyses (PCoA) using genera and species level to understand gut microbiome differences among diets (*e.g.*, fed and unfed plastics, WB, or plastics plus WB) can be analyzed using the Bray-Curtis dissimilarity index. The results of PCoA indicated that significant changes in microbiome were observed after the insect larvae received PVC, PP, PLA, and PUR (Luo et al., 2021; Peng et al., 2020a, 2021; Yang et al., 2021b). For example, Peng et al. (2022a) investigated microbiomes of *Z. atratus* fed respective diets of WB, PS, and LDPE for 28 d. PCoA of a total 11 samples generated

three clusters. The microbial structures in PS group, LDPE group, and WB group clusters were separated from each other due to the differences among the gut microbial community structures developed. However, compared to the WB group, the microbial communities in the PS group and LDPE group showed significant homology in terms of taxonomic similarity, indicating that the structure of the gut microbial community is similar.

DEG or differential abundance analysis of gut microorganisms at species or genus level is a useful tool to understand the community change in detail and to find the microbes associated with plastics degradation. Brandon et al. (2018) reported that the gut microbiome of *T. molitor* larvae fed with PS and PE revealed *Citrobacter* sp. and *Kosakonia* sp. OTUs strongly associated with both LDPE and PS. Sun et al. (2022a) identified bacterial genera *Pseudomonas*, *Rhodacoccus*, and *Corynebacterium* associated with PS degradation in *Z. atratus* larvae.

It is generally assumed that the insect gut acts as an important habitat for plastic-degrading microorganisms and key enzymes that are involved in plastic biodegradation. Some studies implied that when intestinal microorganisms of plastic-ingesting insects such as *T. molitor* are inhibited by antibiotics, the ability of *T. molitor* to discern long-chain polymers of petroleum-based plastics is suppressed to a certain extent, which indicates that the gut microorganisms of *T. molitor* play a decisive role in the degradation of plastics (Brandon et al., 2018; Urbanek et al., 2020). In addition, different feeding conditions were found to improve the composition of bacterial communities of insect gut relative abundance of bacteria genera contributing to plastic degradation, which further determines metabolic pathways and modes of degradation of plastic in the insect intestine (Brandon et al., 2018, 2020; Peng et al., 2019; LeMoine et al., 2020; Lou et al., 2020; Wang et al., 2022c).

Ding et al. (2023) investigated the response of the gut microbial taxa and their functional groups of *T. molitor* and *T. obscurus* fed LDPE-only and LDPE plus co-diet (4:1 LDPE/co-diet), normal feed bran or corn flour as conventional diet, and crop residue rice straw and corn straw. They found that for the larvae of both species, the presence of crop residues increased LDPE consumption and depolymerization. The group of the larvae fed the co-diet showed more complex correlations of the gut microbiome than the larvae fed the LDPE diet alone and differences were also found in the active metabolic pathways for LDPE degradation in *T. molitor* and *T. obscurus*. In particular, they confirmed that nitrogen fixation and biodegradation were identified as key active processes. The gut microbiome exhibited a high level of adaptability to HDPE, LLDPE and LDPE, altering the width of the gut microbial ecological niche and community diversity depends on PE types (Ding et al., 2024).

The composition of the gut microbial population in *G. mellonella* was dramatically changed by the mastication of PS, PE, and PP plastics, while the microbial community of the salivary glands did not appear to be affected. The PE diet promoted the growth of *Enterobacter* and *Desulfovibrio vulgaris*, while the PS and PP diets led to a greater abundance of *Enterococcus* (Peydaei et al., 2021). Cucini et al. (2020) investigated gut bacterial and fungal diversity of PS-fed *Alphitobius diaperinus* larvae and observed that significant shifts of both gut bacterial and fungal communities as PS fed, similar to observations reported in other darkling beetles.

However, studies on population-level changes in the microbiome of plastic-fed insects are severely constrained by the paucity of available omics databases and by intrinsic limitations of current bioinformatic tools, which suggests that this type of data should be interpreted with great care (Serrano-Antón et al., 2023).

5.4 Nitrogen fixation

The results of analysis of gut microbiome of plastic-derading insects indicated that gut microbial community in *T. molitor* larvae had ability to upregulate nitrogen-faxing activities under N-deficient plastic diet. Yang et al. (2023b) found after fed with PS foam, acetylene reduction activities of larvae from six sources in China ranged from 12.3 to 32.9 nmole ethylene/h/gut which was much higher than those fed normal diet bran and also significantly reduced when the larvae fed with antibiotic gentamycin. Analyses of $\delta^{15}N$ values of biomass indicated that the larvae fed PS as only diet had lower $\delta^{15}N$ than those fed bran and much lower than those received antibiotic gentamicin (Yang et al., 2023b), indicating N-fixation did help the larvae for nitrogen sources because N-fixation trends to decrease $\delta^{15}N$ value in biomass.

Nitrogen fixation function of *T. molitor* and *T. obscurus* larvae fed with LDPE and various co-diets (wheat bran, wheat bran, corn flour, corn straw and rice straw) depends on the protein contents of co-diet (Ding et al., 2023). Upregulation of gut-microbial genes related to N-fixation was also observed in *T. molitor* larvae fed PET (He et al., 2023). However, it is likely that the N-fixation actitiviy in the insect gut can supply certain N source for survival but not enough for development or growth. To date, the research on other plastic-degrading insects has lack of reports. It remains unknown whether the nitrogen fixation function is species-, genus- or family-specific or ubiquitous among the known plastic-degrading insects.

5.5 Enzymes and bioreagents

Research has attempted to identify enzymes involved in biodegradation of plastics, which has focuses on *G. mellonella* and *T. molitor*. For the insects with gut microbe-dependent performance, the enzymes secreted by

both gut microbes and the host are considered. For *G. mellonella*, the enzymes by host were a main focus (Kong et al., 2019).

Peydaei et al. (2020) tested exposure of PE film to salivary gland proteome and found that salivary glands assisted in PE degradation in *G. mellonella* larvae. Three enzymes, belonging to the phenoloxidase family, capable to degrade LDPE were discovered in the saliva of the wax worm (Sanluis-Verdes et al., 2022; Spínola-Amilibia et al., 2023). The *G. mellonella* saliva revealed to be a high concentrated cocktail of four phenoloxidases, renamed Demetra, Ceres, Cora and Cibeles. HT-GPC of saliva-treated LDPE showed degradation of polyethylene with formation of small oxidised compounds within a few hours from exposure. LDPE degradation activity of three recombinant enzymes out of the four so far identified was confirmed using both spectroscopy and GC-MS analyses. Cibeles did not show any plastic degrading activity. Cryo-Electron Microscopy (Cryo-EM) and crystallography of the saliva revealed structural features undescribed within the phenoloxidase superfamily: the canonical catalytic site, well described in the hemocyanin, perhaps the most studied of this family, is not conserved, while several superficial metal binding sites have been recognized within each enzymes (Spínola-Amilibia et al., 2023). To date, Sanluis-Verdes et al. (2022) and Spínola-Amilibia et al. (2023) reported for first time that three enzymes, belonging to the phenol oxidase family, were identified within the saliva of *G. mellonella* that can reproduce the same effect. Future research will be required to dissect the modality of action of these enzymatic activities and to quantify their effect on plastics.

Przemieniecki et al. (2020) compared the effects and changes in the gut microbiome and enzymatic profile of *T. molitor* fed with different diets, *i.e.*, cellulose, LDPE, and PS. Results found that diets could modify the activity of hydrolytic enzymes secreted by the digestive system. In addition, the abundance of diazotrophic bacteria increased during the utilization of PS, suggesting that *T. molitor* larvae synthesized proteins from ingested atmospheric nitrogen by gut microbiota.

Peng et al. (2022a) collected gut contents of PS and LDPE-fed *Z. atratus* larvae for RT-qPCR analysis to identify upregulated bacterial genes responded to PS and LDPE feeding. Functional enzymes of gut microbiota including serine-hydrolase and aryl esterase revealed upregulation in both PE-fed and PS-fed larvae. They are possibly associated with plastic degradation,

On the basis of analysis of the gut microbiota structures, metabolic pathways, and enzymatic profiles of PS- and corn straw-fed larvae, Mamtimin et al. (2023) found that the high similarity of gut microbiomes adapted to biodegradation of PS and corn straw-fed *T. molitor* larvae indicated the plastics-degrading ability of the *T. molitor* larvae originated through an ancient mechanism that degrades the natural lignocellulose. Thus, it is hypothesized that plastic-degrading enzymes could be screened from the enzymes with oxidizing or hydrolyzing lignocelluloses according to the metagenomic sequencing of microbiome of plastic-feeding mealworms frass.

Brandon et al. (2021) provided evidence that *T. molitor* larvae secreted emulsifying factor(s) with molecular weight of 30–100 kDa that mediate plastic bioavailability. The insect gut microbes also secreted factor(s) less than 30 kDa that enhanced respiration on PS *in vitro* gut microbial culture. With the gut fluid of *T. molitor* larvae containing these factors in PS enrichment, eight bacterial strains associated with the biodegradation of PS were identified including *Serratia marcescens*, *Klebsiella aerogenes,* and *Citrobacter freundii*. The results demonstrated that both the larva itself and gut microbiota result in accelerated biodegradation of PS in *T. molitor*.

5.6 Symbiotic reaction concepts

For years, most researchers consider that microorganisms play a vital role in the biodegradation of plastics, including environmental microbes (*in vitro*) and gut microbes of insects (*in vivo*) (Yang et al., 2022a) However, the contribution of the intestinal microbiota to the biodegradation of plastics is complicated or controversial, and depends on the insect biology, polymer types, and gut microbial structure. For example, several studies have implicated that biodegradation and depolymerization of LDPE in *T. molitor* and *G. mellonella* are less dependent or independent of intestinal microorganisms (Kong et al., 2019; Yang et al., 2021d) but depolymerization of PS, PP, and PVC in *T. molitor* and PS in *G. mellonella* is highly dependent on gut microbiomes (Yang et al., 2015a; Yang et al., 2018a; 2021b; Lou et al., 2020; Peng et al., 2020a). As described above, PE- and PS-degrading gut organisms were isolated from both *T. molitor* and *G. mellonella* larvae. Studies found that unlike the PS biodegradation fully relies on the presence of the intestinal microorganisms in *T. molitor* (Brandon et al., 2021), the degradation of different PE plastics by plastic degrading insects subjected to the inhibition of antibiotics does not completely stop depolymerization of PE in *T. molitor* (Yang et al., 2021d). In addition, bacterial consortia isolated from *T. molitor* and *P. interpunctella* showed higher LDPE degradation performance (Yin et al., 2020; Lou et al., 2022).

From the updated studies, we conclude that plastic degradation is more likely related to the synergetic effect of intestinal micro-ecosystems (such as intestinal digestive enzymes, intestinal microbial extracellular enzymes, *etc.*) (Kong et al., 2019; LeMoine et al., 2020; Przemieniecki et al., 2020, 2022). Based on research into microbiomes, antibiotic suppression tests, transcription and isolation of plastic-degrading gut microbes, together with the discovery of insect-produced plastic degrading enzymes, the biodegradation of plastics by insects has been considered as a symbiotic system by the insect host

and gut microbes. A conceptual model is proposed in Fig. 6, which is modified mainly on the basis of the previous works (Yang et al., 2015a; Yang et al., 2018b; Brandon et al., 2021; Sanluis-Verdes et al., 2022; Peng et al., 2023a; 2023b; 2023c; Spínola-Amilibia et al., 2023; He et al., 2024). This conceptual model not only covers the biodegradation mechanisms in *T. molitor*, *T. obscurus*, *Z. atratus* and other Tenebrionidae members but also works for *G. mellonella* and other Pyralidae. The model is described as below.

1) Plastic materials (foam, film, fragments, particles, *etc*.) in contact with the mouth of the larvae is exposed to the animal saliva, containing enzymes capable of oxidizing it. The formed small oxidized compound, together with leftover, undegraded plastic fragments are then ingested.

2) The ingested plastic materials enter the intestine through physical chewing and grinding *via* the mouth-parts and intestinal peristalsis. These activities reduce the size of the plastics and increase the contact surface area of the particles exposure to saliva enzymes, gut microbes, digestive enzymes secreted by both microbes and insect host, and bioemulsifying reagents.

3) Oxygen and nitrogen in air enter the intestine and then oxygen serves as electron acceptor for aerobic and facultative microbes as well as oxidative enzymes while N_2 is utilized as N source by N-faxing microbes under N deficient conditions (*e.g.*, plastics as sole diet).

4) The ingested plastics particles were further mixed with digestive reagents (*e.g.*, bioemulsifying compounds), digestive enzymes (oxidative and hydrolytical enzymes) and gut microbes and are depolymerized into smaller oxidized and/or hydrolysed fragments and intermediates, and also undergo size reduction by physical grinding in the intestinal tract.

5) The biodegraded intermediates are further biodegraded into various intermediates and some of them are mineralized into CO_2 and H_2O, and part of the intermediates are assimilated into biomass of the host and gut microbes.

6) The residues of undigested plastic polymers and biodegraded intermediates are egested as excrement (or frass).

6 Isolation of plastics-degrading gut microorganisms

6.1 Isolates from Pyralidae family

6.1.1 *Plodia interpunctella*

Plastics-eating and degrading insects have been attractive as a source of plastics-degrading microorganisms. Yang et al. (2014) first isolated LDPE-degrading *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 from the gut of *Plodia interpunctella* larvae and characterized their PE degradation capacity. These bacterial cultures of YP1 and YT1 (10^8 cells/mL) could efficiently degrade about 10.7% ± 0.2% and 6.1% ± 0.3% of the 100 mg PE films, respectively, over an incubation period of 60 d.

Mahmoud et al. (2021) isolated three bacterial strains

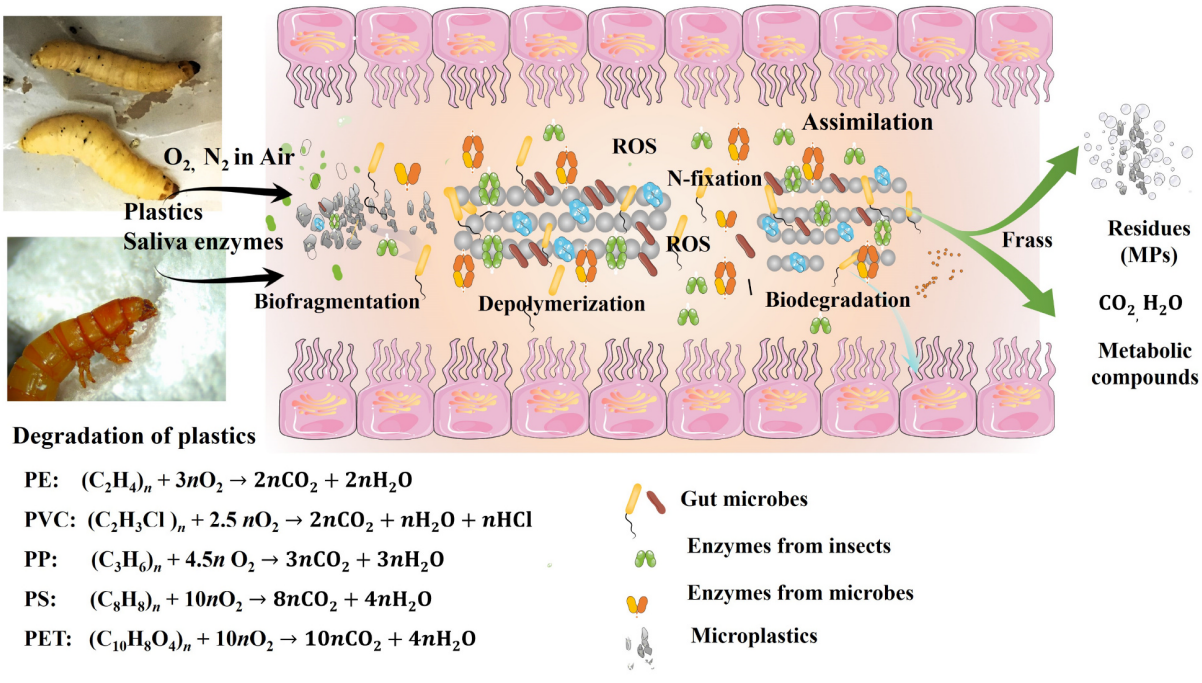

Fig. 6 Conceptual model for the biodegradation of plastics in insect intestinal tracts and future research directions and perspectives for plastic-ingesting insects.

from the midgut of *Plodia interpunctella* and identified them through 16S rRNA gene sequencing as *E. tabaci* strain YIM Hb-3(B1) and *B. subtilis* subsp. *Spizizenii* strain NBRC 101239(B2), respectively. SEM analysis revealed the damage on the surface of PE pieces after 60 d of incubation with these strains. The biodegradation was verified by formation of carbonyl groups using FTIR spectra.

Lou et al. (2022) isolated PE-degrading yeast strain *Meyerozyma guilliermondii* ZJC1 (MgZJC1) and Gram-negative bacterial strain *S. marcescens* ZJC2 (SmZJC2) from *Plodia interpunctella* larvae. Similarly, the consortium of these two strains had higher LDPE-degrading ability than single cultures and 15.9% mass reduction was achieved after 60-d incubation. These discoveries revealed that the gut microbiota consisting of multiple plastic-degrading bacteria and/or consortia could work much effectively for plastic-degradation.

6.1.2 *Galleria mellonella*

Although Kong et al. (2019) found that larvae of *G. mellonella* did not harbour beeswax-degraders, which indicated microbiota-independence for the wax degradation and possibly LDPE degradation, isolation of PS-degrading microorganisms from *G. mellonella* larvae has been quite successful, indicating that plastics (PE and PS) degrading microorganisms were widely present in *G. mellonella* larvae.

Ren et al. (2019) isolated PE degrading strain *Enterobacte*r sp. D1 from the gut of *G. mellonella* larvae. FTIR and liquid chromatography-tandem mass spectrometry (LC-MS) analyses demonstrated that the oxidation reaction took place on the PE film surface after being exposed to bacterial strain D1 treatment.

A PE-degrading fungus, *Aspergillus flavus* PEDX3, was isolated from the digestive tract of *G. mellonella* larvae by Zhang et al. (2020). This strain had the ability to degrade LDPE microplastic particles and has genes of two PE-degrading enzymes *i.e.* two laccase-like multicopper oxidases (LMCOs) genes, AFLA_006190 and AFLA_053930. Jiang et al. (2021) isolated a PS degrading bacterium, *Massilia* sp. FS1903, from the gut of *G. mellonella* larvae fed with PS foam. After 30 d of incubation with 0.15 g PS, 80 mL MSM, at 30 °C and PS with M_n 64.4 and M_w 144.4 kDa, this strain reduced the weight of the PS films significantly by 12.97% ± 1.05% (w/w). Two bacterial strains LDPE-DB1 and LDPE-DB2 molecularly identified as *Citrobacter freundii* and *Bacillus* sp., respectively, were isolated from A. grisella larvae and identified as zldpe-degraders (Ali et al., 2023).

Nyamjav et al. (2023b) isolated a bacterial strain *Bacillus cereus* from the gut of the *G. mellonella* larvae and confirmed its capability of PP surface microbial degradation using SEM, FTIR and X-ray spectra. HT-GPC analysis further confirmed that *B. cereus* biodegrades PP through broad depolymerization, suggesting that *B. cereus* possesses a complete set of enzymes required to initiate the oxidation of the carbon chain of PP.

6.2 Isolates from Tenebrionidae

6.2.1 *Tenebrio molitor*

Yang et al. (2015b) isolated bacterial *Exiguobacterium* sp. strain YT2 from *T. molitor* larvae. *In vitro* suspension culture of strain YT2 (10^8 cells/mL) degraded 7.4% ± 0.4% of the PS pieces (2500 mg/L) over a 60 d incubation period. The biodegradation of PS was characterized *via* established methods *i.e.*, SEM and AFM observation, analysis with XPS, micro-ATR/FTIR, and GPC, *etc*.

The two unknown aerobic bacterial strains, TM1 and ZM1, were isolated from *T. molitor* and *Z. atratus*, respectively, using yeast extracts on agar plates by Tang et al. (2017). These strains developed on PS plates, which were made by incorporating PS emulsion in chloroform into an agar basal medium.

Two Gram-negative bacterial species, *Mixta tenebrionis* sp. nov. and *Myroides albus* sp. nov. were isolated from the gut of the plastic-eating *T. molitor* larvae and *Z. atratus*, respectively (Xia et al., 2020a, 2020b). However, researchers did not further determine the plastic biodegradability by either of these strains using any physiochemical analytical methods.

A bacterial consortium of two PE-degrading bacterial strains, *Acinetobacter* sp. NyZ450 and *Bacillus* sp. NyZ451 was isolated from the gut of PE-feeding *T. molitor* larvae. Interestingly, each strain had a poor ability when working independently (Yin et al., 2020). The consortium was capable of growing with PE as sole carbon source and removed about 18% of PE mulching film (w/w) over 30 d. The molecular weights of PE mulching films was reduced by 14%, 24%, and 21% for M_n, M_w, and M_z, respectively, with formation of tight biofilm on the surface of PE mulching film.

Another PVC-degrading bacterial consortium EF1 was enriched from *T. molitor* larvae, consisting of bacterial strains belonging to ten genera (*Stenotrophomonas* (about 78%), *Enterococcus* (about 15%), *Acinetobacter* (about 2%), *Xylella*, *Pseudochrobactrum*, *Ochrobactrum*, *Lactobacillus*, *Streptococcus*, *Bacillus*, and others (about 4%) in ten families, including Xanthomonadaceae (~80%), Enterococcaceae (~18%), Moraxellaceae (~2%), Brucellaceae, Aeromonadaceae, Streptococcaceae, Lactobacillaceae, Rhizobiaceae, Bacillaceae, and Yersiniaceae) of four phyla (Proteobacteria (~82%), Firmicutes (~18%), Actinobacteria, and Spirochaetes) (Xu et al., 2023). This consortium reduced 6.13% of PVC mass after 30 d incubation with release of Cl^-. GPC analysis indicated reduction of M_w from 63.71 to 52.86 kDa and M_n from 34.75 to 24.83 kDa. The biodegradation of PVC was also

supported by SEM and AFM observation, change in WCA, FTIR and TGA analyses, and GC-MS for intermediates. This study indicated that PVC biodegradation can be performed by gut microbes in *T. molitor* larvae, supporting the conclusion of gut microbe-dependence by *in vivo* antibiotic suppression test (Peng et al., 2020a).

By supplementing bio emulsifying factor(s) secreted by *T. molitor*, Brandon et al. (2021) isolated eight bacterial strains from gut microbial enrichment culture, *i.e.*, *S. marcescens*, *K. aerogenes*, *Stenotrophomonas maltophilia*, *Enterococcus faecalis*, *P. aeruginosa*, *C. freundii, and Enterobacter asburiae*. They were observed to form colonies and grow on PS sheets as their sole carbon source.

Zhang et al. (2023) isolated a HDPE-degrading *Bacillus* strain PELW2042 from the gut of *T. molitor*. A significantly decreased by 23.31% ± 1.25% and 30.07% ± 1.37% for M_w (121.7 kDa) and M_n (50.7 kDa), respectively were observed by *Bacillus* strain PELW 2042, the molecular during 42-day inoculation, indicating that the strain PELW2042 has a high ability to degrade HDPE.

In the study by Ort et al. (2023), they stated that *Lactococcus* was the only bacteria that could be associated with the PUR diet through the *T. molitor* digestive tract.

6.2.2 *Zophobas atratus*

Kim et al. (2020) reported isolation of PS-degrading bacterial strain *Pseudomonas aeruginosa* strain DSM 50071 from the gut of *Z. atratus* larvae fed on PS. PS biodegradation was confirmed by chemical structural changes using XPS, FTIR, and NMR. Using RT-qPCR analyses and enzyme inhibitor treatment assays, they further pointed to serine hydrolase (SH), a candidate enzyme, as being a participant in the plastic biodegradation process. It was then also reported that *P. aeruginosa* strain was capable of biodegrading PS, polyphenylene sulfide (PPS), PE, and PP (Lee et al., 2020). Tan et al. (2021) reported an isolated PS-degrading bacterium *Bacillus megaterium* from *Z. atratus* laevae.

Nyamjav et al. (2023a) isolated a strain *Citrobacter koseri* from the gut of *Z. atratus* larvae fed with PVC. The gut microbiota composed of diverse microbial species showed equal oxidation of PVC compared to *C. koseri*, verifying that *C. koseri* and the culturable microbiota from the gut of superworms present similar potential to utilize pure PVC film as a carbon source.

6.2.3 Other species in Tenebrionidae

Acinetobacter sp. strain AnTc-1 was isolated from *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae, which chewed and ate extruded PS foam (Wang et al., 2020). *Acinetobacter* sp. was strongly correlated with ingesting PS, as evidenced by a comparison of the gut microbiomes of *T. castaneum* larvae fed wheat bran versus PS. PS biodegradation by strain AnTc-1was characterized using GPC, ^{1}H NMR, TGA, and SEM observation. After *in vitro* incubation with strain AnTc-1 22 for 60 d, the PS mass weight was reduced by 12.14%, with 13% and 25% decrease in M_w and M_n, respectively.

A number of bacterial species have also been isolated from other plastic-eating insects. Woo et al. (2020) isolated *Serratia* sp. WSW (KCTC 82146) from the gut flora of *Plesiophthalmus davidis* larvae. *Serratia* sp. strain WSW introduced both C–O and C=O bonds to PS film, while its degradation was less prominent *via* the gut flora.

6.3 Isolates from other families

Zhang et al. (2022) isolated a PVC-degrading bacterial strain *Klebsiella* sp. EMBL from the intestine of the larvae of an insect pest, *Spodoptera frugiperda*. This strain has the ability to depolymerize PVC and use it as its only energy source. Using transcriptomic, genomic, proteomic, and metabolomic analyses, the authors hypothesized that dehalogenases, catalase-peroxidase, enolase, oxygenase, and aldehyde dehydrogenase were among the proteins and genes potentially involved in PVC degradation.

Plastic-degrading microorganisms have been isolated from termites. Two bacterial strains degrading PE carry bag were isolated from primitive termites (dry wood termities), which belonged to *Lysinibacillus* and *Bacillus* sp. (Thamil, 2016). Over 30 d incubation with 1 g PE bag in 200 mL medium, the respective bacterial culture reduced plastic mass by 24.7% and 27.8%.

7 Future perspectives

7.1 Expansion of members of plastics-degrading insects

To date, only seven species in Tenebrionidae and four species in Pyralidae have been identified as plastics-degrading insects, although these two families have 20000 and 6000 species around world, respectively. As described in Section 3.3, insects in other orders could also be candidates of plastic-degraders. We expect more new members of plastics-degrading insects will be added to the list of plastics degraders in the future.

The feasibility tests will be expanded to test the biodegradability and limitations of biodegradation of these polymers in other insects and macroinvertebrates. To date, it is also known that macroinvertebrates such as the land snail *Achatina fulica* can also depolymerize and fragment PS and PET (Song et al., 2020). The search for

plastic-degrading aminals will also be expanded into other macroinvertebrates. Additionally, feasibility tests using other untested polymers (*e.g.*, nylon, polycarbonate, *etc.*) with a variety of physical and chemical properties will be conducted.

7.2 Isolation and characterization of gut plastics-degraders

The screening of microbial strains that are capable of degrading plastics will continue using gut microbiota of plastics-degrading insects since they are considered highly efficient bioreactors for plastics-degradation and are a reservoir of plastics-degraders. Gut-microbes of plastics-degrading insects such as *T. molitor*, *T. obscurus*, *Z. atratus* and *G. mellonella* will continue to be the sources for the isolation and selection of microbes with novel, highly efficient plastics-degraders in an *in vitro* environment. New sources of other insects or invertebrates are likely to be added in the source list of plastic-degrading microorganisms in the future.

7.3 Eco-toxicology of plastic degrading insects

To date, research on ecotoxicology of micro- and nano-plastics has been mainly focused on insects and macroinvertebrates with non- or poorly plastic-degrading abilities. Recently, toxicology associated with the biodegradation of plastics has been emphasized (Sanchez-Hernandez, 2021) because biofragmentation of plastics into smaller-sized particles is observed (Song et al., 2020; Peng et al., 2023a, 2023b, 2023c, 2023e). This knowledge gap is critical to understanding the capacity and factors limiting or impacting the enzymatic activities related to plastic degradation and larval development. Other than the additive HBCD, which has no effect on mealworms (Brandon et al., 2020), the influence of other additives in plastics on degradation, physiology of the host insects, and the fate of these materials have yet been investigated and should be addressed in future studies.

7.4 Modeling and kinetics

Peng et al. (2023b) developed a digestive biofragmentation model for biodegradation and size reduction of ingested plastics, which was validated using tests with *T. molitor* larvae fed with LDPE and PS foam. Further validation is needed by testing different insect species and various plastic polymers. To date, a little has been done for the kinetic models developed for plastic degradation in insects. Future work could develop comprehensive and multidisciplinary models involving chemical-physical properties of polymers, secretion of digestive enzymes by the host, microbial enzymes, microbial growth kinetics, and absorption rates of degraded products under movement in intestine.

7.5 Metabolic pathways

To date, research on metabolic pathways of insect plastic biodegradation has been limited. Currently, only limited results with the primary proposed pathways have been reported on biodegradation of PS, PE, and PVC in *G. mellonella* and *T. molitor*. Because of the complexities of insects and their gut microbiota, rearing condition, and varieties of physical-chemical properties of polymers, the biodegradability and metabolisms of major plastic polymers remain basically unknown.

7.6 Gut microbiome and microbial ecology

Future research should be conducted on molecular and reaction mechanisms among diverse insects and their gut microbiome's ability to degrade petroleum-based plastics in order to construct biomimetic insect intestinal microecological environments and regulatory strategies thereof. Future research should focus on revealing the microbial populations responding to degradation of plastics in insect intestine, interspecific interaction mechanisms of key microorganisms, and synergistic reactions of enzymes secreted by the host and gut microbiota. The impact of the response of the host to the plastic diets on gut microbiota and *verse visa* should also be explored.

7.7 Selection and engineering of key enzymes and microbes

Further work will focus on identification of ezymens especially for biodegradation of plastics (Bertocchini and Arias, 2023). The search for key enzymes reached a milestone recently with the discovery of *G. mellonella* produced phenoloxidases found in the larva's saliva, and capable of degrading LDPE. This is the first step in this quest using insects to degrade plastics. Recently, research to identify key enzymes and regulatory mechanisms related to plastic degradation in insect and with their gut microbiota has been initiated by investigating 16S RNA genes and transcripts of insect guts. Future studies will identify more key enzymes *via* gene sequence analysis and gene upregulation by the gut plastics-degrading microbes and insect hosts. The selected genes could be cloned into expression vectors *e.g.*, *E. coli* Rosetta (DE3) for enzyme production and characterization. Engineering the genes to develop highly efficient microbial cultures and production of plastics-degrading enzymes should be explored to attract more researchers.

7.8 Applications

Additional applications of the discoveries of insect plastic biodegradation could be accomplished and potentially include: 1) natural attenuation on the basis of

understanding mechanisms of enzymatic degradation by the larval enzymes, together with microbial degradation in relation to microbes, genes, evolution, and environmental ecology and microbial ecology, as well as plastic modification and regulation to enhance biodegradability and; 2) bioremediation/bioaugmentation *via* isolation and development of highly efficient microbial cultures through conventional and engineered approaches; 3) design and synthesis of biocatalysts *e.g.*, enzymes, bioimpulsifiers based on the understanding mechanisms of enzymatic degradation, of plastics; 4) resource recovery from plastic wastes or used plastics *via* development of processes including pre-treatment (*e.g.*, thermal pyrolysis) and microbial/enzymatic processes to produce organic acids and bioproducts; 5) modification or redesign of the composition and polymer structures of new plastic products based on the discoveries of enzymatic degradation mechanisms; as well as 6) development of nutriution additives and/or probiotics to prevent human heathy from harmness by ingestion of microplastics.

8 Conclusions

Insect-mediated biodegradation of plastics, mainly by Tenebrionidae and Pyralidae, is a radical innovation breakthrough for plastic degradation. Rapid biodegradation of plastics has been observed for major petroleum-based polymers *i.e.*, PE, PP, PVC, PS, PET and PUR. The estimated time span of half-life of plastics within insecxt intestine can be achierved on basis of hours.

The factors influencing and controlling insect physiology and survivability and significantly impacting on plastics biodegradation rates and extent include physical and chemical properties of the polymers (molecular weights, branching, crystalline, surface hydrophobicity, *etc*.) and nutrition conditions.

Research on the the gut microbiome, metabolome, proteome and transcriptome has indicated that biodegradation of plastics in plastic-degrading insects are mainly symbiotic reactions and performed by the synergistic enzymatic reactions contributed by the host and gut microbiota.

The plastics-degrading capacities of insects, depends insect species, are closely associated with their biodegrading abilities for original or natural diets (*e.g.*, lignocellulose, soil organic matter, wax) in the environments.

Biodegradation of ingested plastics resulted in biofragmentation of plastics into smaller particles and the insects and their gut microbes showed increased oxidative stress response and sign of ecotoxicities, depending on biodegradability of polymers.

Future studies on biodegradation of plastics by insects will undoubtedly reveal of additional insect plastic degraders; and investigations on microbiome, metabolite, gene transcripts, and enzymes will fill scientific gaps and lead to development of new methods, approaches and bioproducts for waste plastic management, resource recovery and bioremediation of plastic pollutants as well as sustabable polymers.

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Abbreviations

AFM	Atomic force microscopy
ABC	ATP binding cassette
ATR-FTIR	Attenuated total reflection flourier transformed infrared spectroscopy
CM	Chlorinated methane
DEG	Differentially expressed genes
DSC	Differential scanning calorimetry
DMF	Dimethylformamide
EPS	Expanded polystyrene
EVA	Ethylene-vinyl acetate
GO	Graphere oxide
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography mass spectrometry
GC-TOF-MS	Gas chromatography–time-of-flight mass spectrometry
GPC	Gel permeation chromatography

HCW	Honeycomb wax
HDPE	High-density polyethylene
HFIP	1,1,1,3,3-hexafluoroisopropanol
^{1}H MNR	Proton nuclear magnetic resonance
HT-GPC	High temperature-gel permeation chromatography
KEGG	Kyoto encyclopaedia of genes and genomes
LB	Luria-Bertaini medium
LCFBM	Liquid carbon free basal medium
LD	Limited-extent depolymerization
LDPE	Low density polyethylene
LLDPE	Liner low density polyethylene
M_n	Number-average molecular weight
MPs	Microplastics
M_w	Weight-average molecular weight
MWD	Molecular weight distribution
M_z	Size-average molecular weight
MW	Molecular weight
OAT	Oatmeal
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PBAT	Poly(butylene adipate-co-terephthalate)
PDI	Polydispersity index
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactic acid
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
Py-GCMS	Pyrolyzer-gas chromatography mass spectrometry
ROS	Reactive oxygen species
RT-PCR	Real-time polymerase chain reaction
SDS	Sodium dodecyl sulfate (SDS)
SEM	Scanning electron microscope
TCB	1,2,4-trichlorobenzene
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
WB	Wheat bran
WCA	Wet contact angle
XPS	X-ray-photoelectron spectroscopy
SR	Survival rate

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