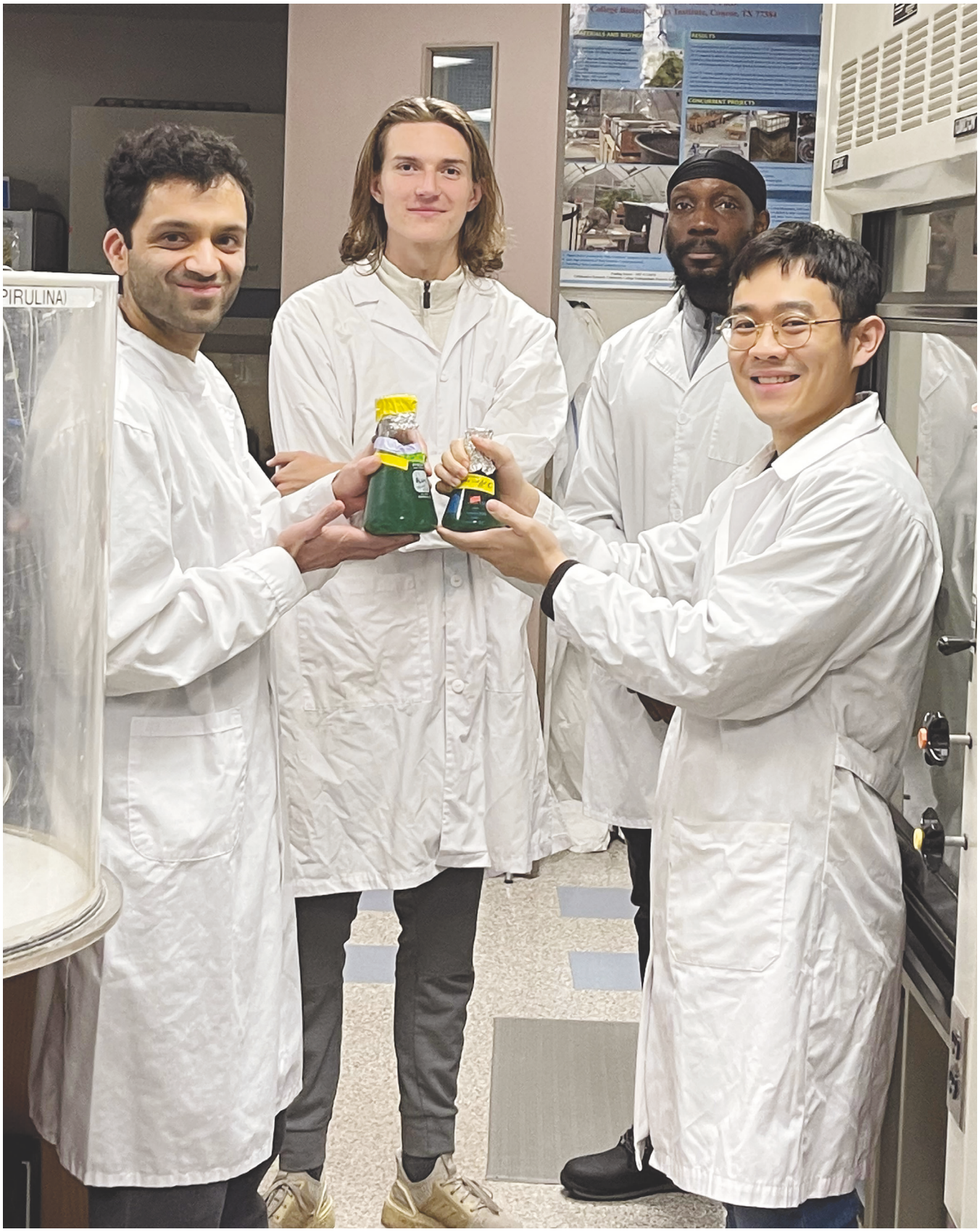

Lone Star College students prepare reagents in support of insect-mediated plastic degradation research. From left to right, they are Ranjit Inamdar, Landon Sanz, Nathanael Salako, and Thien Tran.

Potential of *Tenebrio molitor* and *Zophobas morio* in Plastic Degradation: Mechanisms, Microorganisms, and Enzymes

LANDON SANZ[1]*, THIEN TRAN[1], DANIEL KAINER[1]

[1]Department of Biotechnology, Lone Star College, TX 77384, USA

**lsanz@my.lonestar.edu*

Abstract: Plastics have become a central part of society, yet their benefits are short-lived compared to the enduring environmental impact caused by their resistance to biodegradation. Their persistence endangers natural ecosystems and all living creatures, infiltrating every part of the human food chain. Hydrolyzable plastics have functional groups that make them more susceptible to degradation, and as such, much progress has been made in understanding the factors and mechanisms that ultimately lead to their degradation. On the other hand, non-hydrolyzable polymers are devoid of functional groups, which has made elucidating their mechanism significantly more challenging, and consensus in literature can be sparse. Degradation by microorganisms has grown in popularity as a potential solution, but the rate of degradation is extremely slow in the environment. Interestingly, the larvae of *Tenebrio molitor* and *Zophobas morio* have been found to be able to degrade various resistant polymers at much higher rates than microorganisms alone. Although their ability is closely tied to their gut microbiome, their high rates of degradation are ultimately dependent upon the synergistic relationship between the host insect and gut microbiome.

Keywords: plastic, degradation, *Zophobas morio, Tenebrio molitor,* mealworm, superworm, enzymes, microorganism, mechanisms, biodegradation mechanisms, biodegradation enzymes, biodegradation, biodegradation microorganism, plastic biodegradation

Introduction

Since their widespread adoption in the mid-20th century, plastics have become integral to economic development and societal progress. They have been ubiquitous across various industries: packaging, construction, healthcare, and electronics [1]. To date, humanity has produced approximately 6,300 million metric tons of plastics [2]. This proliferation of plastics has been driven by their durability, lightweight nature, and low production costs, making them indispensable in modern life [3]. However, those characteristics make them environmentally catastrophic [4]. Plastics decompose in anywhere between a few decades to thousands of years, depending on the type of polymer, size, and environment in which they accumulate. Even then, they do not disappear; they physically break into smaller pieces known as microplastics [5–7].

To mitigate the ecological impact of plastic waste, effective recycling strategies are essential. Recycling methods are categorized into four main processes: primary, secondary, tertiary, and quaternary. They range from mechanical to energy recovery [8]. Primary recycling, or closed-loop recycling, repurposes plastic waste into equivalent products while maintaining original quality. Although this method is low-cost, it is limited by diminishing quality after several reuse cycles and is only applicable to industrial materials that have minimal contamination [9]. Secondary recycling involves cleaning, melting, and remolding plastics into lower-quality products with limited applications [10]. Tertiary recycling breaks down long hydrocarbon chains of polymers into monomers and oligomers, which can be used to synthesize new plastics, chemical feedstock, and fuels. Methods like pyrolysis, methanolysis, and glycolysis are employed in tertiary recycling but are often resource-intensive, costly, and environmentally challenging [11–14]. Quaternary recycling converts plastic waste into energy through processes like incineration. This method is primarily used to generate electricity or heat from the combustion of municipal solid waste. While it reduces landfill volumes and recovers energy, it faces criticism for its environmental impact due to emissions and ash residue requiring proper disposal [15–17].

Despite these varied recycling strategies, 79% of plastic waste ends up in landfills or the environment, 12% is incinerated, and only 9% is recycled. This low recycling rate is due to several factors, including contamination of recycling streams, economic challenges, and the technical limitations of current recycling methods. Consequently, innovative strategies are urgently needed to enhance plastic recycling rates. The production of plastics continues to grow at an alarming rate of 8.4% annually [2]. In 2022 alone, an estimated 400 million metric tons of plastic were added to the global total [18].

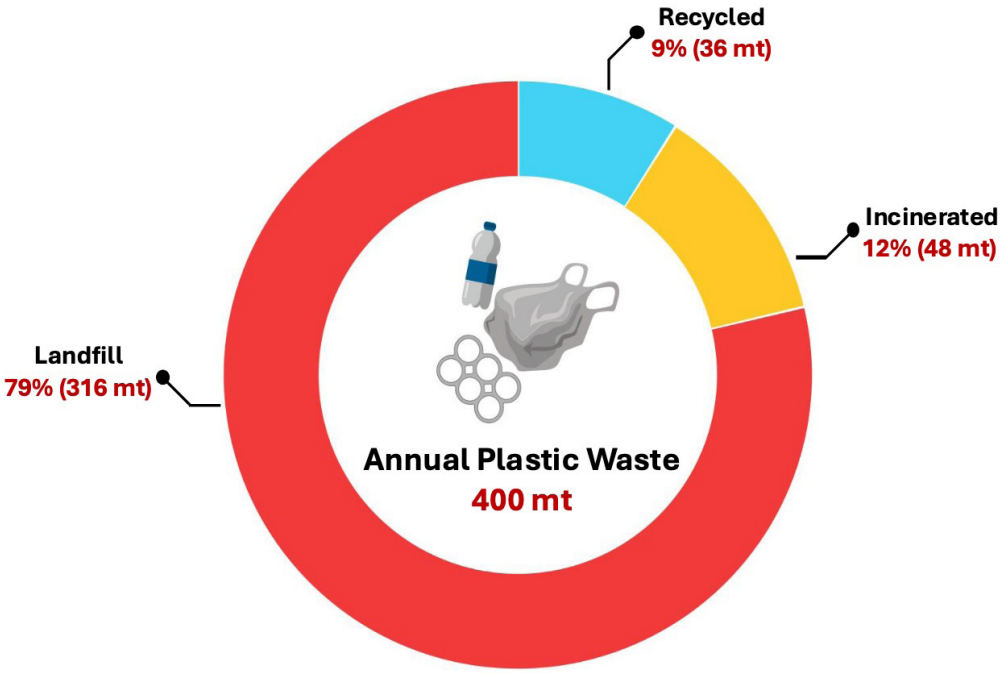

Fig. 1. Fate of Annual Plastic Waste

In this quest, the potential of biological degradation by organisms has shown promise. Various species, ranging from microbes to larger organisms, have demonstrated the ability to break down plastic materials [5,10,19,20]. For example, *Ideonella sakaiensis* can break down polyethylene terephthalate (PET) into components for new polymer synthesis [21]. Other studies have found similar results in two highly active enzymes isolated from the bacterium *Thermobifida fusca*, and LC-cutinase. Slight enzyme modifications resulted in a weight loss of 19.2 mg (42% of the original total) in PET films over 50 hours [22].

Despite the promising biodegradation avenues being explored, there are still concerns that current microbial degradation approaches are too slow. Additionally, most studies that have close to elucidating mechanisms behind biodegradation have been done on hydrolyzable plastics, namely PET. Non-hydrolyzable plastics are much less susceptible to more typical biodegradation pathways because they lack functional groups. This makes them comparatively much more difficult to degrade, and as such, very few enzymes have been identified. Given that non-hydrolyzable plastics constitute the majority of global plastic production, ongoing research into their biodegradation is essential [1,2].

Interestingly, a few organisms capable of degrading plastics have come to light and shown much higher rates of degradation. *Tenebrio molitor* and *Zophobas morio (=Zophobas atratus)* are commonly known as the mealworm and superworm, respectively. Their degradation capabilities ultimately depend on their gut microbiome, as the use of antibiotics halts degradation abilities in most cases. However, isolates of the gut microbiome alone show slower rates of degradation, suggesting that the gut itself is an important contributor.

The process of plastic degradation in the two species includes physical chewing, microbe colonization, enzyme secretions, bioemulsifying agents, assimilation into biomass, and subsequent metabolism. This complex system has been termed as a "bioreactor" [23]. The co-dependent synergistic effects of the gut microbiome and the host itself offer an interesting perspective on the possible mechanisms behind the biodegradation of more recalcitrant polymers. This review aims to provide comprehensive yet accessible information on all relevant aspects, enabling independent researchers to explore their hypotheses regardless of their prior experience in this field. It does so by offering an overview of plastic degradation, *T. molitor, Z. morio*, mechanisms, key potential enzymes and microorganisms, techniques to evaluate biodegradation, factors affecting consumption, and responses of *T. molitor and Z. morio* to plastic. By synthesizing current research and identifying gaps, this review advances the field while also serving as a valuable resource for researchers across various disciplines.

Methods

To gather relevant literature for this review, a comprehensive search was conducted using ScienceDirect, Google Scholar, and Scopus. No date restrictions were applied to capture the full development of the literature, but experimental data on degradation were primarily drawn from articles published between 2013 and 2024. Articles were included regardless of cost of access. Only articles originally in English were included. Abstracts were screened for relevance, prioritizing studies with experimental data on *T. molitor and Z. morio* related to plastic degradation. Reviews and theoretical papers were excluded unless they provided significant insights into degradation mechanisms, plastic pollution, alternative uses, techniques, or ecology. Limitations include inconsistent experimental conditions, language bias, and potential publication bias. The following keywords were employed either individually or in combination with "*Tenebrio molitor*" and/or "*Zophobas morio*": "plastic degradation," "biodegradation," "microbial degradation," "polymer biodegradation," "plastic waste," "plastic waste management," "polystyrene," "PS," "polyethylene," "PE," "non-hydrolyzable plastics," "hydrolyzable plastics," "polymer," "microplastic," "microplastic pollution," "plastic consumption," "plastic degradation rates," "depolymerization," "depolymerization mechanisms," "enzymatic degradation," "oxidative degradation," "biofilm formation," "biodeterioration," "functional groups," "molecular weight," "carbon mass balance," "mineralization," "biodegradation pathways," "bioremediation," "circular economy," "mealworm," "superworm," "insect degradation," "gut microbiome," "gut bacteria," "microbial communities," "microbial colonization," "microbial enzymes," "enzymes," "hydrolase," "cutinase," "lipase," "alkane hydroxylase," "monooxygenase," "cytochrome P450," "enzyme activity," "reactive oxygen species," "oxidative cleavage," "bioemulsification," "emulsifying agents," "microbial adherence," "microbial isolation," "plastics in the gut microbiome," "nitrogen fixation," "bioreactor," "chewing plastic," "carbon sources," "isotopic analysis," "biomass assimilation," "hydrocarbon chains," "synthetic microorganisms," "biodegradation efficiency," "temperature," "nutritional supplements," "review," "biodegrading organisms."

Results and Discussion

Understanding the Plastics: PS and PE

Of the vast different types of plastics, polystyrene (PS) and polyethylene (PE) are some of the most resistant to degradation and will be the focus of this review. PS consists of a linear chain of carbon atoms with a phenyl group attached to every second carbon atom. PE consists of a linear chain of carbon atoms with two hydrogen atoms attached to each carbon. These polymers are less susceptible to degradation for several reasons. Most importantly, they lack hydrolyzable groups such as carbonyl, amide, and carbon-alkene bonds. Hydrolysable bonds are common targets for enzymatic attack by the likes of hydrolase, cutinase, lipase, etc [24, 25]. Without the ability to attack said functional groups, initial degradation is largely limited to terminal depolymerization. The ends of the hydrocarbon chains are oxidatively cleaved into shorter chains (oligomers, dimers, and monomers) via enzymes that have high redox potential, resulting in broad or limited-extent depolymerization, depending on the molecular weight [25–29]. Additionally, the absence of hydrolyzable groups also increases hydrophobicity, making it difficult for enzymes and microorganisms to interact with the plastic surfaces effectively [30, 31].

High molecular weights (MW) also contribute to resistance, as they are often too large to penetrate microbial cell walls where they would be further degraded by intracellular enzymes [24, 32]. Additionally, polymers consist of regions with tightly packed molecular chains, known as crystalline regions, and regions with loosely and randomly packed molecular chains, known as amorphous regions. Amorphous regions are more prone to enzymatic attack [33]. Therefore, polymers with high crystallinity, which have more tightly packed chains, are more resistant to biodegradation, also known as the chain-flexibility hypothesis [34].

Finally, additives are often incorporated into polymers during manufacturing to enhance their properties and tailor their uses, frequently increasing stability. One of the most common types of additives are stabilizers [35], including antioxidants, which inhibit or delay oxidative degradation [36]. Additives have also faced scrutiny due to their documented human health risks [37] and the generally apparent lackluster control of their usage [38]. Compounding this issue is the fact that additives are not covalently bonded to the polymers, making them prone to leaching into the environment over time [39, 40]. The spread of harmful additives is exacerbated by the increasing prevalence of microplastics (MPs), defined as plastic particles under 5mm [38–40]. MPs can be categorized into primary MPs, directly released into the environment through industrial activities, and secondary MPs, formed from the gradual degradation of larger plastic pieces [41, 42]. Secondary MPs are the most abundant, and the effect on marine life is well-documented. Some studies on commercially sold marine life documented that up to 100% of the analyzed fish contained MPs, most notably in Southeast Asia [43–48]. The widespread presence of MPs thus facilitates the broader distribution of toxic additives, heightening environmental and health concerns and warranting more research into how plastic pollution can be controlled.

Mechanism of Biodegradation

A distinction must be made between the terms "consumption" and "biodegradation." Consumption, in the context of these studies, refers to the act of an insect ingesting a feed, the feed traveling through the digestive system and then being egested. It is measured by the difference in the mass of the feed before and after insect inoculation. Consumption does not indicate whether the feed's chemical structure has been modified and broken down by biological means; this is part of biodegradation. Biodegradation encompasses many complex processes that are necessary to understand. The general mechanisms of biodegradation are becoming increasingly understood, with new insights consistently emerging through the study of host insects.

Biodegradation through insects can be broken down into a few key steps: (1) Biodeterioration of the surface and structure of the polymer, increasing its susceptibility to enzyme attack and facilitating microbial attachment and colonization. First, environmental factors such as UV radiation, heat, moisture, pH, salinity, and atmospheric pressure modify the crystallinity, molecular masses, hydrophobicity, and functional groups of the polymer [25, 49]. Next, physical chewing by insects increases surface area, roughness, porosity, and pore size, facilitating microbial colonization [49, 50]. (2) Microbes adhere to the polymer surface and create biofilms, which are effective strategies for supporting growth on hydrophobic surfaces [50–52]. (3) The host secretes emulsifying agents that are able to coat and separate, thus creating more surface area on hydrophobic plastic particles for microbes to colonize further. These secretions are independent of the gut microbiome [53]. (4) The surrounding colonizing microbes and host secrete extracellular enzymes such as alkane hydroxylase, alkane monooxygenase, cytochrome P450, monooxygenase, flavin-binding monooxygenase, aromatic ring hydroxylase, lipases, depolymerases, esterases, proteinase K, cutinase, urease, and dehydrase, which depolymerize polymers into small fragments such as oligomers, dimers, and monomers [26, 54–58]. (5) The newly formed cleaved fragments then permeate through the microbial membrane into the cell. (6) Intracellular enzymes further oxidize and break down these fragments into fatty acids. (7) Fatty acids are then assimilated into microbial biomass or oxidized to go through metabolic pathways, forming mineralized end products. Mineralization typically results in H_2O under aerobic conditions, CH_4 under anaerobic, and CO_2 under either [57–59].

Techniques to Measure and Confirm Degradation of Plastics

Fourier-transformed Infrared Spectroscopy (FTIR) tracks chemical changes on the polymer surface and the formation of functional groups that result from biodegradation, thus characterizing modifications in the polymer structure. Typically, any peaks associated with a digested plastic but not with the undigested plastic feedstock will be evidence of biodegradation. However, FTIR results can be skewed by additives that may have been released during degradation, so special care must be taken during analysis to control for this, typically

with pre-treatment. Researchers have found that PS digested by *T. molitor* exhibits weaker peaks in regions characteristic of benzene rings, suggesting ring cleavage. Additionally, studies have found a broadening of peaks associated with hydrogen bonds of carboxylic acid and hydroxyl groups, which are not typically present in PS [60–62]. This indicates oxidation and chemical changes consistent with biodegradation.

Proton nuclear magnetic resonance (^{1}H NMR) determines molecular structure with respect to hydrogen atoms within molecules. It is used to characterize degradation by identifying new peaks in digested PE associated with alkene bonds and new peaks in digested PS associated with oxygen incorporation [62]. Similarly, new alkene bonds have also been identified in digested polyvinyl chloride (PVC) [63]. None of these peaks are found in their respective natural chemical structures, indicating a chemical change and, thus, degradation.

^{13}C Cross-polarization/magic angle nuclear magnetic resonance (CP/MAS NMR) enhances the signals of low-sensitivity nuclei in solid-state samples. It has identified phenyl derivatives and possible indicators of fragments produced during the depolymerization of PS [23]. Another study used CP/MAS NMR to detect weaker intensities for the resonance signals assigned to the PS carbons in the frass spectrum, indicating a decline in the PS content in the frass [64].

Thermogravimetric analysis (TGA) can provide evidence of degradation through composition analysis. The composition changes are measured by changes in the mass loss of plastic over a range of temperatures. Studies using this technique find plastics that were digested by *T. molitor* or *Z. morio* have different weight loss rates than undigested plastic, specifically, the thermal stability decreases. For example, in one study using both insects, feedstock PS had a 95.5% weight loss rate at a range of 366.7- 431.4°C, while digested PS had weight loss rates of 71.4% and 87.4% at the same range for *Z. morio* and *T. molitor*, respectively [65]. This technique has also been used with polyurethane (PU) [66], PVC [63], and low-density polyethylene (LDPE) [27].

Gel permeation chromatography (GPC) is one of the most widely used measures of biodegradation. It separates components of a mixture by size and allows for the number-average molecular weight (Mn), weight-average molecular weight (Mw), and size-average molecular weight (Mz), which describe the distribution of the size of polymers. Typically, a reduction in molecular weights (Mn, Mw, Mz) in digested polymers compared to pre-digestion will be evidence of degradation via broad depolymerization (BD). However, after digestion, polymers that naturally have very high MW will express a lesser reduction or even an increase in Mn, Mw, and Mz; this classifies limited-extent depolymerization (LD). LD most likely occurs because low MW chains are depolymerized at a faster rate than high MW chains, leaving a greater proportion of high MW chains post-digestion, overall increasing the Mn, Mw, and Mz. However, depolymerization still occurs; thus, an LD pattern is still evidence of degradation when the overall molecular weight distribution (MWD) shifts towards a low-MW direction [28]. A study observed this pattern when PS with varying MWs was fed to *T. molitor*. PS with low to high MW (<~600kDa) showed consistent BD patterns, while the ultrahigh MW PS (1346 kDa) showed LD patterns [29].

Mass balance tests are a strong estimative way to quantify degradation rates by measuring the difference between inputs and outputs. Inputs include the feed, while output is the weight of the frass prior to extraction, the weight of the residual polymers in the frass recovered through solvent extraction, and the change in weight of the surviving insects. If the output no longer matches the input, biodegradation is evident. Capturing and quantifying gasses during the process allows for the estimation of mineralization, i.e., the amount of plastic (organic material) that was converted into gasses, presumably CO_2 (inorganic material) [25]. This test has commonly shown increased plastic degradation efficiency over time. For example, just 18.84% of PU fed to *T. molitor* was converted to CO_2 in the first five days of incubation; this number rose to 29.80% by day 20 [67]. Another study saw the amount of PS being converted to CO_2 rise from 20.7% to 47.7% over 15 days [23].

Carbon-13 (^{13}C) isotopic tests are used to quantify biodegradation by feeding ^{13}C-enriched plastic is fed to insects, who incorporate it into their biomass and form metabolic products. As plastic is degraded, the insect releases $^{13}CO_2$. By trapping and measuring the amount of $^{13}CO_2$ released, biodegradation and mineralization can be quantified. Isotopic tests can also be used to see if plastic was assimilated into the insect biomass by measuring the ^{13}C values in fatty acids [23]. One study measured an increase in $^{13}CO_2$production from *T. molitor* fed ^{13}C-enriched PS compared to those fed non-^{13}C-enriched PS, indicating mineralization. They also found higher ^{13}C values in the fatty acids of *T. molitor* fed ^{13}C-enriched plastic, indicating that a limited fraction of the ^{13}C was assimilated into the mealworm biomass [26]. These increases in ^{13}C indicated biodegradation of the PS.

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) are additional techniques used to evaluate the surfaces of polymers before and after inoculation. These methods are often applied in experiments that involve isolating bacteria and incubating them with a polymer. Initial signs of degradation observed include changes in color, holes, surface roughening, cracks, and biofilm formation. However, these techniques should be used in tandem with other strategies, as changes in the surface could only be an indicator of biodeterioration rather than biodegradation. Nonetheless, studies that have detected surface changes using these techniques often confirm biodegradation through additional methods, establishing a strong correlation between surface alterations and actual degradation [50, 52, 68, 69].

Finally, differential scanning calorimetry (DSC), X-ray photoelectron spectroscopy (XPS), X-ray diffusion diffraction (XRD), the "clear-zone" test, and mass spectrometry (MS)-based detection methods are additional techniques that can provide evidence of biodegradation. They were less commonly used in the reviewed studies, so a description will not be provided. However, they have been reviewed in excellent depth elsewhere [25, 58, 70].

Natural Diet, Environment, Life Cycle, and Behaviour: *Zophobas morio*

Z. morio belongs to the beetle family of Tenebrionidae, with origins tracing back to tropical regions of Central and South America [71]. Rearing *Z. morio* efficiently depends on humidity and temperature, requiring 22-27°C [72] and 50-60% humidity [73]. While wheat bran with raw vegetables as supplements for nutrition and water is the most commonly used diet for rearing *Z. morio* [74, 75], the exact nutritional requirements are not agreed upon. Studies have successfully used various organic wastes such as vegetable waste, garden waste, chicken feed, horse manure, and cattle manure [76]. Avian feed and high-calcium cricket feed have also seen success [48]. By-products from beer brewing, potato processing, and bioethanol production have also been somewhat viable feeds [77, 78]. However, less-optimal substrates increase larval periods and mortality [79]. For example, high-starch diets that contained cookie remains had a survival rate of 0% [78]. A notably interesting behavior of *Z. morio* is that pupation is dependent on 4-6 days of isolation; crowded larvae will completely fail to begin metamorphosis [74]. Additionally, crowding increases rates of cannibalism and dehydration [80, 81].

Natural Diet, Environment, Life Cycle, and Behaviour: *Tenebrio molitor*

T. molitor, like *Z. morio*, belongs to the beetle family of Tenebrionidae and is said to originate in Europe [82]. For both adult and young *T. molitor* larvae, a humidity of 50%-75% and a temperature of 25°C are optimal parameters for maximal survival rate [83]. Notably, extreme humidity conditions (12% and 98%) at normal temperatures (25°C) showed no detrimental effect on *T. molitor* survival, indicating that temperature is a more influential factor [84, 85]. One study reported the greatest growth rate at 31°C but did not control humidity nor mention ending survival rates [64].

Substantial interest in using *T. molitor* as an alternate protein source has resulted in numerous studies on the most effective type of substrate. The most common and reliable substrate used is wheat bran [86–90], often combined with a source of moisture such as vegetables [91, 92]. A source of moisture maximized growth [93] by lowering developmental time and increasing survival rate [91]. Adding an additional protein source, such as casein, lactalbumin, or yeast (at concentrations of 5-10%), further enhances growth, survival rates, and weight gain [86, 94, 95]. Crucially, a range between 50% [96] and 80% [95] of carbohydrates has yielded the best biomass growth, longevity, and reproductive capacity results. Similarly to *Z. morio, T. molitor* has been successfully reared on food by-products and waste, such as brewery spent grains [97], orange albedo [98], watermelon rind [99], malt residual pellets [100], and a host of other vegetables, cereals, oats, legume, and beverage by-products that have been reviewed more in-depth elsewhere [101].

Alternate Uses: *Tenebrio molitor* and *Zophobas morio*

Z. morio and *T. molitor* have been studied extensively, particularly in the context of circular economies, due to their flexible substrate-rearing requirements and rich nutritional profiles [102-104]. While exact measurements of nutritional value tend to vary depending on study conditions, both species are extremely dense in nutrition. An extensive review [105] found protein concentrations to be 47% and 54% for *Z. morio* and *T. molitor*, respectively, and 18:1n9 (a common fatty acid present within all species) concentrations of 39% and 41% for *Z. morio* and *T. molitor*, respectively. In addition to fat and protein, both species are rich in calcium, zinc, copper, magnesium, iron, aluminum, and manganese [106, 107]. This nutrition is why both species have been

considered a possible protein source for animal feed and human consumption. One study [108] found that a small supplementation of *Z. morio* (2%) for fishmeal in the diet of young pigs had no negative effect on growth and increased fat digestibility, which is likely because *Z. morio* has high concentrations of monounsaturated and low polyunsaturated fatty acids [109]. Beyond nutrition, both *Z. morio* and *T. molitor* are a viable source of chitin, which can be extracted and used in various applications, including medical and pharmaceutical products [110–112]. Additionally, both *Z. morio* and *T. molitor* are suspected to be sources of antimicrobial peptides, as studies have found improved immunity in animal livestock after supplementing minor amounts of feed with *Z. morio* and *T. molitor* [108, 113]. Despite limited research on this, it is documented that insects can synthesize antimicrobial peptides [114]; thus, further research into these two species is warranted.

Polystyrene and Polyethylene Biodegradation by *Tenebrio molitor* and *Zophobas morio*

While publications on insects and their ability to degrade plastic date back to the 1950s [115, 116], research on the subject was relatively quiet until it was propelled into the spotlight of bioremediation when Yang et al. published their team's findings on the surprising effectiveness of *T. molitor* in degrading PS [23]. *T. molitor* was able to degrade PS within hours, with a consumption rate of 12 mg/100 worms/day, resulting in a 31% total mass loss over 30 days. Egested frass from *T. molitor* was analyzed, revealing that the long-chain structure of PS molecules had been depolymerized, producing lower molecular weight fragments. Carbon mass balance tests were conducted to test the efficiency of carbon usage, which indicated that the carbon content of the egested frass decreased from 73.6% to 49.2%. This surprising finding suggested that *T. molitor* adapted to utilize the carbon source better, resulting in increased degradation efficiency over time. Another study found the same increase in degradation efficiency and hypothesized that it is likely due to an increase in microbial abilities [60]. Furthermore, ^{13}C-labeled PS was used to confirm that the carbon from PS was being mineralized to $^{13}CO_2$, with significant ^{13}C enrichment observed in the CO_2 released by the mealworms compared to the control. These tests demonstrated that the carbon was being broken down and utilized by *T. molitor* rather than simply passing through their digestive systems.

This ability is not restricted to specific strains or the environments of which they are from. A study found that *T. molitor* from 12 different sources from around the world displayed similar results of consumption and degradation and were confirmed by the same techniques as used before [117]. Yang et al. then tested the effect of antibiotics on *T. molitor*. The result was a loss of ability to degrade PS, indicating that PS biodegradation depended on the gut bacteria. This was validated through an insignificant change in average molecular weights compared to undigested PS and a lack of $^{13}CO_2$ production after being fed ^{13}C-labeled PS [56].

To test the viability of bacteria outside the gut, gut suspensions from *T. molitor* fed PS for two weeks were prepared and incubated with PS pieces. The resulting culture was spread on LB agar, and isolated colonies were obtained. These colonies were then spread on a CFBAM plate with added PS films. After 28 days of incubation, the film's surface exhibited deterioration with pits and cavities, while the uninoculated control film remained smooth. After 60 days of incubation, decreases in the average molecular weight of the PS films inoculated by the bacteria confirmed PS degradation. Yet, the weight loss was just 3.89 mg (7.4% of the original total), indicating that factors inherent to the mealworm itself significantly impact degradation rates. Yang et al. theorized the physicochemical treatments by *T. molitor*, such as chewing, ingesting, and host secretion of enzymes, are likely critical for the effective depolymerization of PS.

A later study further differentiated the role of the host body and gut microbiome in degradation [53]. When incubated with PS, respiration activity in the gut microbiome cultures of *T. molitor* increased when supplemented with gut supernatant from PS or bran-fed *T. molitor*, but not with supernatant from antibiotic-treated *T. molitor*. This suggests the gut microbiome secretes factors that enhance PS degradation. The supernatant also exhibited emulsification activity that effectively coated and separated hydrophobic particles, creating more surface area on the hydrophobic plastic particles for microbes to adhere to, which is one of the initial steps in microbial degradation [22, 110, 111]. Emulsification was observed in all diets, including antibiotics, which suggested that it is a factor of *T. molitor* itself, independent of the gut microbiome, and could be a reason why the isolated gut microbiome is less effective at degradation. The supernatant was fractioned by molecular weights and assessed for two qualities: increased respiration activity when used as a supplement and increased surface coating. The two groups most efficient at both (<30 kDa and 30-100 kDa, respectively)

were combined and used as supplements. This combination led to the greatest increase in respiration activity seen. The author noted that because unfractionated supernatant had lower respiration activity, supernatant >100 kDa may contain inhibitory agents [53]. The independence of microbial secretions and host secretions was further proven when the only antibiotic-treated supernatant that had increased activity was 30-100 kDa. These findings indicate that although isolated gut microbiomes have lower degradation rates, there is potential to enhance them.

Once PS susceptibility to *T. molitor* was established, Brandon et al. investigated the potential biodegradability of PE [62]. They found that PE and PS consumption rates (mg/100 worms/day)/(32-day PS consumption %) were comparable at 23.1 mg/48.3% and 16.9 mg/31.6%, respectively. It was also evident that *T. molitor* could adapt and more efficiently utilize PE throughout the experiment, as previously shown with PS [23]. Biodegradation of PE was proven through the same techniques used previously with PS, showing decreased average molecular weights and new functional groups. Specifically, ^{1}H NMR showed new peaks associated with alkene bonds in PE groups, and FTIR provided evidence of oxygen incorporation through the presence of alcohol groups and C-O bonds. These new functional groups align well with the theory that oxidation is the first step in PE depolymerization [118-120]. Mass balance tests showed that a higher percentage of carbon from PE was assimilated into the biomass compared to PS.

The success of *T. molitor* in degrading PS and PE prompted researchers to explore whether other insect species might possess similar or even greater capabilities. This led to the discovery that *Z. atratus (=Z. morio)* can also degrade and mineralize PS [64]. *Z. atratus* showed a consumption rate of 58 mg/100 worms/day, a rate over 3 times greater than that of *T. molitor*, and consumed 65% of the total PS over 28 days. This difference could be due to physical differences between the two species. *Z. atratus* are larger and have a mandibulate mouthpart that enables them to chew plastics better, which is the first step in insect biodegradation mechanisms. Analysis of the egested frass revealed lower weight-average molecular weights than that of undigested PS, once again suggesting the depolymerization of long chains of PS. Thermal characterization through TG-FTIR showed the egested frass had weaker styrene peaks than undigested PS, indicating a reduced presence of styrene. New peaks attributed to aromatic carbons of phenyl derivatives were also observed. *Z. atratus* was able to mineralize PS at rates similar to *T. molitor*. Finally, antibiotic treatment suppressed PS-degradation, once again suggesting the role of the gut bacteria.

Changes in the Gut Microbiome of *Tenebrio molitor* and *Zophobas morio*

For the sake of brevity, it should be assumed that all discussed changes in the gut microbiome of *T. molitor* and *Z. morio*, as well as the microorganisms involved, have been demonstrated to be associated with the depolymerization and/or mineralization of plastic. Each referenced study has adequately proven these processes through one or more established techniques, of which most were mentioned above, and controlled for relevant variables.

Although the specifics of each step in the proposed mechanism are not well known, the importance of the gut microbiome has been well established. When *T. molitor* and *Z. morio* are fed with plastic, massive shifts in their microbiota occur. The diversity of the microbial community exposed to a specific plastic depends on many factors and does not always respond the same way to the same plastic. For example, when *T. molitor* was fed PS with low MW, the phylum Firmicutes had a relative abundance of 83.15%. However, in groups fed PS with medium and high MW, the abundance of Firmicutes dropped to 20.13% and 21.84%, respectively. At the family level, Streptococcaceae was the most abundant in the low MW PS group at 63.33%, while Enterobacteriaceae dominated in the medium MW PS group at 79.84% [29].

Diversity in the microbiome can also be influenced by the structural complexity of a polymer. For instance, the gut microbiome of *T. molitor* on a natural bran-fed diet had 180 operational taxonomic units (OTUs). When fed polypropene (PP), which has a relatively simple chemical structure, the number of OTUs dropped to 102. In contrast, in the PU and ethylene vinyl acetate groups, which are more structurally complex plastics, the OTUs increased to 186 and 188, respectively. Despite the survival rate decreasing as plastic complexity increased, all three groups consumed nearly the same amount of plastic [121].

Availability of nutrition also alters the microbial communities and typically increases degradation rates by providing an energy source for microorganism growth and synthesis of enzymes [60, 61, 122]. Recent interest in nitrogen fixation has emerged because plastics are completely nitrogen-deficient. Despite this, both *T. molitor* and *Z. morio* have been able to survive surprisingly long on plastic alone. Some of the bacteria found in these insects have nitrogen-fixation potential, which could provide the necessary nitrogen for their survival. This is relevant because many bacteria associated with plastic degradation, such as *Klebsiella* sp., *Mixta* sp., *Kluyvera* sp., and *Citrobacter* sp., are also known to have nitrogen-fixation potential and may actually be less significant in the degradation process [123, 124]. Instead, they could be microorganisms that grow under nutritional stress, creating a more competitive environment that inhibits the growth of bacteria actually capable of degrading plastics [122–124]. However, nitrogen fixation and the ability to biodegrade polymers are not mutually exclusive. For instance, *Klebsiella grimontii* MA76 was recently isolated and showed plastic-degrading potential despite the species being diazotrophic. The same study found that three different strains of *Acinetobacter septicus* had different growth rates on PS plates, highlighting the variability of microbial behavior even among strains of the same species [69]. These observations illustrate the necessity of researching microorganisms responsible for degradation at the strain level and that isolation is a powerful and essential technique.

While the functions of microbial communities are complex, and the effectiveness of biodegradation can vary among strains of the same species, many studies consistently arrive at similar conclusions across various taxonomic ranks and testing settings [13, 119, 125, 126]. One study that highlighted this was when *T. molitor* from 12 different sources worldwide were analyzed. Despite the original bacterial communities being diverse among all sources, feeding them PS resulted in similar patterns of bacterial abundance across all sources, suggesting that the ability to digest and degrade PS is genetic. Notably, the Enterobacteriaceae family abundances nearly doubled when fed PS only compared to a normal diet [117].

Among the many microorganisms that are associated with plastic degradation, the abundance and growth of the Enterobacteriaceae family when exposed to plastic has been quite consistent, regardless of the type of plastic or the species of the host. Brandon et al. [62] found *Citrobacter* sp. and *Kosakonia* sp. in *T. molitor* to be strongly associated with both PE and PS diets and are part of the Enterobacteriaceae family. Wang et al. [127] found that *Cronobacter, Lactococcus,* unclassified *Enterobacteriaceae, Lactobacillus*, and *Citrobacter* were significantly increased in abundance in PE-fed *Z. morio*. Luo et al. again found Citrobacter sp. to be associated with PE-fed *Z. morio*, along with *Dysgonomonas* sp. and *Sphingobacterium* sp. in the PS-fed group, and *Mangrovibacter* sp. in the PU-fed group. Tang et al. [128] isolated *Klebsiella pneumoniae* (part of the Enterobacteriaceae family) and *Aeromonas* sp. from PS-fed *Z. morio* and *T. molitor*, respectively, by growing them on PS plates.

Shan Jiang et al. [126] found that the relative abundance of Enterobacteriaceae increased in all *T. molitor*, *Z. morio*, and *Galleria mellonella* (another plastic-degrading insect) when fed PS. When Bo-Yu Peng et al. [63] fed PVC to *T. molitor*, the gut microbiome shifted from a diverse one to one dominated by families Streptococcaceae, Spiroplasmataceae, Clostridiaceae, and Enterobacteriaceae. In a subsequent study, Bo-Yu Peng et al. [129] tested polylactic acid with *T. molitor* and found similar shifts in the microbiome to families of Streptococcaceae, Spiroplasmataceae, Clostridiaceae, Lactobacillaceae, and Enterobacteriaceae. This trend continued in a study by Yumeng Wang et al. [65], when the relative abundance of unclassified Enterobacteriaceae sharply rose in *Z. morio* when fed PS and PU. While the abundance of *Klebsiella* did not significantly change across different diets, it remained relatively high. In another study, *Klebsiella* sp. and *Sierra marcescens* from *T. molitor* were associated with PS degradation [53], but these bacteria failed to degrade PS when isolated, indicating a dependence on the host [53].

Interestingly, Woo et al. [130] isolated *Serratia* sp. WSW from *Plesiophthalmus davidis* (another plastic-degrading insect) onto a PS plate. Biofilms formed, resulting in a six-fold increase in bacteria, and C-O bonds covered the newly formed cavities, indicating biodegradation. The molecular weights were unchanged, but the MWD shifted toward a low-MW direction, indicating an LD pattern more attributed to *Z. morio*. *Serratia marcescens, Klebsiella oxytoca*, and *Pseudomonas aeruginosa* were isolated and found to be able to degrade PS films [131]. When fed PS, *Pseudomonas* sp. and *Lactococcus* were dominant in *Z. morio*, while *Spiroplasma* was dominant in PE-fed *T. molitor* [127]. *Pseudomonas* sp. is able to degrade PVC, PU, and PE

[132], so it was studied more in-depth, and the strain *Pseudomonas aeruginosa* DSM 50071 was isolated. The strain degraded PS, diminished much of the polymer's hydrophobicity, and formed carbonyl groups. The gene expression of serine hydrolase was upregulated during PS degradation, and when the strain was treated with a serine hydrolase inhibitor, bacterial growth on the PS was diminished, weight reduction of the PS was halved, and no carbonyl groups formed in comparison to the control group [133]. This study showed that PS degradation depended on a well-known hydrolase to be active, suggesting that it likely has a role in the depolymerization of PS into monomers.

Enzymatic Activities and Roles

With a strong foundation of knowledge regarding which microorganisms are most associated with plastic degradation, studies have shifted focus toward identifying enzymes involved in the biodegradation mechanism. Przemieniecki et al. conducted one of the earlier studies in this area, using a combination of metagenomic analysis and enzymatic activity tests with *T. molitor* [122]. The study first measured microbial abundances, and the results were consistent with previous studies [62, 117, 134]. Planctomycetes and Nitrospirae increases were specific to PS diets, and *Pantoea* was specific to PE diets. Enzymatic testing revealed that while the types of enzymes were similar, the digestive tract produced them much more actively than the microorganisms. This finding could be one explanation as to why isolated microorganisms have such slower degradation rates. Alkaline phosphatase and acid phosphatase activity had a strong correlation with PS, while C8 ester lipase activity was elevated in PE diets [122]. Luo et al. theorized that the increased lipase and proteinase activities in PE-fed *Z. morio* were a result of nutritional deficiencies [135]. Przemieniecki et al. mentioned that high levels of depolymerizing enzymes tend to be correlated with high levels of phosphatase activity; this idea merges the correlations of elevated degradation rates, lipases, and phosphatases. A transcriptomic analysis on *T. molitor* showed that hydrolases were the most upregulated in both PE and PS groups. In LDPE, the upregulated hydrolases acted on ester bonds, sugars, and C-N bonds, overall expanding fatty acid metabolic processes. This aligns with another study that saw significant changes in fatty acid profiles and metabolic activities, supporting the theory that assimilation into the biomass and, ultimately, mineralization depends on the decomposition of fatty acids [119, 136]. Furthermore, the consumption of both expanded PS and LDPE in *T. molitor* enriched 42 KEGG pathways that indicated fatty acid degradation is involved in the breakdown of PS and PE [137].

After biodeterioration, microbial adherence, and the secretion of bioemulsifying agents, the depolymerization of hydrocarbon chains begins. As established, the method for depolymerization depends on the type of plastic. Non-hydrolyzable plastics, such as PS and PE, are more difficult to degrade, and their exact mechanisms are still unknown. PS depolymerization most likely starts with either an attack by functional enzymes at the β-carbon (main chain cleavage) or the aromatic ring (side-chain cleavage). The main chain is more likely to be cleaved since the alkane chain is weaker than the aromatic alkene bonds. Enzymes possibly responsible for this include alkane hydroxylase, alkane monooxygenase, cytochrome P450, monooxygenase, flavin-binding monooxygenase, or aromatic ring hydroxylase [54, 125, 138]. Cytochrome P450 is a likely candidate because of its ability to participate in monooxygenase, peroxidase, and peroxygenase reactions [54]. Additionally, cytochrome P450 contributes to the production of reactive oxygen species (ROS) [139]. ROS generation increases in the gut when *Z. morio* is fed PS and degraded. However, if ROS generation is prevented, there is a significant decrease in PS depolymerization, proving the significance of ROS [140].

The initial oxidation of PS and PE is most likely by cytochrome P450 on an alkane, forming a primary alcohol. The primary alcohol is further oxidized into an aldehyde and subsequently converted into a fatty acid [120]. Cytochrome P450 can regioselectively oxidize subterminal carbons, forming secondary alcohols, which are then again oxidized into ketones. Subsequent ketones can be converted to esters by the addition of an oxygen atom by Baeyer-Villiger monooxygenase, which are then finally cleaved by esterase to form an alkanol and fatty acid [54, 120, 141]. Finally, these fatty acids are subsequently stored in the host or undergo β-oxidation for the citric acid cycle to produce metabolic products [142]. A metatranscriptomic analysis ties all the aforementioned concepts together [143]. It revealed similar findings as previous studies [122, 144]; xenobiotics, aromatic compounds, and fatty acid degradation pathways were enriched in *T. molitor*-fed PS or corn straw. Monooxygenase, superoxide, dehydrogenase, and cytochrome P450 were all shown to be involved in PS degradation. Additionally, the analysis revealed an upregulated gene, *lac640*, in both PS and

corn straw groups. When overexpressed in *E. coli*, this gene exhibited PS and lignin degradation abilities. These comprehensive findings that involve many of the suspected mechanisms behind PS degradation show that research is on the correct path. The successful PS degradation abilities in the overexpressed gene are a step towards harnessing and manipulating these biochemical mechanisms for bioremediation strategies.

Factors that Affect PS Consumption Rates

While each species exhibits varying degrading capabilities on a host of different types of polymers, such as PS, PE, PVC, PU, PLA, and PP [56, 62, 63, 129, 135, 145], PS has seen the most attention. This focus is likely due to the early preliminary successes and consistency in results. In the following years, Yang et al. investigated factors that may affect PS consumption rates, such as added nutrition, temperature, and multiple common types of PS waste [61]. *T. molitor*-fed PS with added bran or soy protein had consumption rates (mg/100 worms/day)/(32-day PS consumption %) of 44.1 mg/67.6% and 49.1 mg/76.8%, respectively, significantly higher than the 22.2 mg/39.1% rate observed when fed PS alone. These results were further amplified with a higher bran-to-PS ratio (16:1) and higher temperatures (25°C and 30°C), resulting in 84% and 78.5% PS consumption rates, respectively. Another study [60] found that the consumption rate of PS alone was nearly identical at 24.3 mg/41.5%. Yet, there was a lower rate in the 1:1 bran:PS co-diet group at 33.23 mg/56.8%, compared to the previous study [61] that reported ~64% consumption in their 1.3:1 bran:PS ratio diet. The difference can likely be attributed to the group sizes tested (130 vs. 410 worms), as crowding increases stress and lowers consumption rates [146]. Further studies consistently reveal the same positive correlation between the addition of nutrition and increased PS consumption rates; increasing the nutrition:PS ratios and adding protein amplify this even more [117, 147]. However, this has mainly been done with standard diets such as bran and soybeans.

One interesting study [148] focused on three different factors: bedding, pre-treatment of PS, and supplemental nutrition. The bedding was made from either inedible beads or oats. Pre-treatment involved soaking expanded PS cups in either lemon-lime soda, lemon juice, or tomato paste. Supplemental nutrition was either spinach, protein powder paste, cucumber, or lemon slices. PS consumption was consistently higher on beaded bedding, which aligns well with previous observations that *T. molitor* strongly prefers nutritious, particularly protein-dense, substrates. Oat bedding is a high protein substrate, suggesting that *T. molitor* consumed it first rather than the PS. This assumption is consistent with a previous study [61] that found extremely high nutrition:PS ratios having lower consumption rates, as the insects interact less with PS and more with the nutritious substrate. The ratio between the number of *T. molitor*/total mass of insects and the masses of nutrition and PS will be an important factor to consider for future optimization of consumption rates. Pre-treatment PS foam also consistently resulted in greater consumption. The author theorized that the acidity of the pre-treatment weakened the cup's harder shell [148]. Other studies [61, 131] also report higher rates of PS consumption among softer, less dense plastics, as species' success is heavily attributed to the mechanical action of chewing [122, 149, 150]. Finally, outside of cucumber, which had no effect on consumption rates in just the oats bed, all supplemental nutrients increased the consumption rate [148]. This is likely due to the added moisture, of which the benefits in both species are well documented [62, 64]. One study proved the benefits of moisture applied to PS consumption too, where adding water to diets increased PS consumption and survival rate; the author suggested that the water facilitates the growth of gut microbiota, leading to the increased degradation [147].

How *Tenebrio molitor* and *Zophobas morio* Are Affected by Plastic Consumption

Plastic consumption and degradation of the worms have been documented heavily in reports from other studies. However, for future applications, it is vital to understand the effect plastic consumption has on insects. Studies vary, but generally, insects' survival rates greatly depend on incubation times. Thirty-day experiments are the most common among studies, and survival rates of *T. molitor*-fed PS stay consistent at around ~85-90%. This tends to be higher than in starvation groups, indicating that the carbon in polymers is of use [56, 61, 151]. However, survival rates during longer incubation times have large drops. One study investigating survival rates found just 4-12% survival rates among *T. molitor*-fed PS after 91 days; another saw a similar 11.5 ± 4.9% survival rate after 98 days [117, 151]. Starvation groups see similar survival rates over longer periods, but groups with supplemented nutrition are much less affected, with survival rates hovering around 80%. These results are relatively consistent with PE-fed groups [152]. In *Z. morio*, survival rates differ among polymers more, with PS-fed groups at a 100% survival rate while PE-fed groups at an 81.67% survival rate [135].

The plastic waste affects the growth of the *T. molitor* because it slows down their metabolism and provides insufficient nutrients like B vitamins, proteins, trace metals, and nitrogen [153]. Furthermore, when mealworms are fed with PS, the protein content of the worms is highest while the fat and carbohydrate content are reduced compared to worms on a conventional diet, which contributes to their weight loss because lipids were consumed due to the low nutrition available [143, 154]. Comparing the two worms, *T. molitor* had some decrease in mass, and they were physically lethargic on a plastic diet. On the other hand, *Z. morio* had an insignificant decrease in weight and similar behavior on a plastic diet versus their normal diet [154]. Indeed, comparing the health outcomes of *T. molitor* and *Z. morio* is challenging due to the variability in study designs, including differences in rearing conditions and the types of polymers tested. The influence of these factors on the results highlights the need for standardization in research methodologies to ensure consistent and comparable outcomes.

Technological Applications and Future Prospects

The studies reviewed highlight the potential of *T. molitor* and *Z. morio* for plastic degradation, but their practical applicability is limited. Generally, these insects use polymers for sustenance rather than growth, leading to mass loss and lower survival rates on polymer-only diets. This makes rearing and subsequently reproducing worms solely on plastic impractical and thereby unlikely for integration into a circular economy focused on plastic degradation. For instance, at a consumption rate of 0.58 mg of PE per *Z. morio* per day found by Yang et al., it would take over 7 tons of *Z. morio* to degrade 1 ton of PE in a month [64]. This scenario is complicated not only by *Z. morio*'s cannibalistic behavior in crowded conditions but also by the significant energy requirements that would pose technological and economic challenges. Although supplementing nutrients might improve survival rates and pre-treatments may improve consumption rates, the added costs and logistical barriers would likely be even more prohibitive for large-scale operations. Such a system may be more practical for small-scale, home-based applications for plastic-conscious individuals and families. While *T. molitor* and *Z. morio* are popular alternative protein sources, non-excreted additives and MPs would likely prevent their integration into human food chains. Their use as feed for non-human food chain animals (e.g., zoo animals) could be more likely considered, but this could raise animal welfare concerns. Nonetheless, if such toxicological concerns could be answered, their use as a feed source would hold economic value.

A promising approach is to understand and apply the degradation mechanisms of these insects to existing waste management technologies. As mentioned, factors of both the host and gut microbiome have shown to be synergistically responsible for more efficient degradation. If researchers can replicate these relationships *in vitro*, microbial-enzymatic solutions could be applied to landfills to potentially reduce their volume. However, isolated microbial communities have shown to be only a fraction as efficient as the insects, highlighting the need for further research. To optimize the comparability and reproducibility of future results, standards need to be established and followed for all aspects of experiments, such as rearing conditions, insect characteristics, co-dieting, polymer pre-treatment, and polymer characterization.

With standards set, studies should consider determining which microbial genera are directly responsible for degradation. While sequencing insect gut tissue alone is possible, the microorganisms that are efficient plastic degraders outside versus inside the gut are likely different. As such, isolating and incubating microbes on polymer films provides a more reliable method for identifying key microorganisms. Once responsible microbes are identified, attention should focus on enzymes, using metagenomics to predict and annotate genes and enzymes based on comparisons with known enzymes. Novel genes, not detectable by metagenomics, can be identified through proteomics techniques such as mass spectrometry, liquid chromatography, and associated tandem systems. Additionally, transcriptomics and metabolomics can provide deeper insights. A combined approach of these techniques has been used to identify a variety of potential PS-degrading enzymes [142]. Often, consortia of microbes are more efficient than isolated ones, likely because the different metabolic pathways and enzymes from various species and strains complement each other; how they do so should be studied. In the future, with this knowledge, synthetic microorganisms could be designed by modifying enzymes and constructing metabolic pathways. Further understanding of mechanisms could support certain modifications of polymer compositions and structures. Finally, there should be an investigation into the differences in enzymatic activity, metabolic pathways, and depolymerization patterns using insect secretions as a supplement versus not. These secretions show promise and warrant further study.

Conclusion

The danger plastic pollution poses to all aspects of life, and the environment should not be understated. Combined with constant growth in plastic production, innovative and cost-effective recycling solutions need to be developed. Current methods have been well-researched yet sparsely implemented due to high costs, technical limitations, and the production of secondary pollution. Microbial degradation, while promising, faces challenges due to slow degradation rates. *T. molitor* and *Z. morio* have shown the ability to not only consume but degrade a variety of polymers. The environment, co-diet, and type of polymers are just some things that affect consumption rates. Their degradation relies on gut microbial communities and is enhanced by host factors, suggesting a complex interaction between mechanical factors, microbes, enzymes, and metabolic pathways. Replicating and utilizing such mechanisms could lead to engineering approaches that optimize degradation rates or establish their use in tandem with established chemical recycling processes.

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