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Progresses in Polystyrene Biodegradation and Prospects for Solutions to Plastic Waste Pollution

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Abstract. Petroleum-based plastic pollution has been a global environmental concern for decades. The obvious contrast between the remarkable durability of the plastics and their short service time leads to the increasing accumulation of plastic wastes in the environment. A cost-effective, sustainable strategy to solve the problem should focus on source control and clean up. Polystyrene (PS) wastes, a recalcitrant plastic polymer, are among the wide spread man-made plastic pollutants. Destruction of PS wastes can be achieved using various abiotic methods such as incineration but such methods release potential air pollution and generation of hazardous by-products. Biodegradation and bioremediation has been proposed for years. Since the 1970's, the microbial biodegradation of plastics, including PS, has been evaluated with mixed and isolated cultures from different sources such as activated sludge, trash, soil, and manure. To date, PS biodegradation by these microbial cultures is still quite slow. Recently, the larvae of yellow mealworms (*Tenebrio molitor* Linnaeus) have demonstrated promising PS biodegradation performance. Mealworms have demonstrated the ability to chew and ingest PS foam as food and are capable of degrading and mineralizing PS into CO_2 via microbe-dependent activities within the gut in less than the 12-15 hrs gut retention time. These research results have revealed a potential for microbial biodegradation and bioremediation of plastic pollutants.

1. Plastic pollution status

Since 1950s, synthetic plastic products derived from petroleum have been widely used in our daily lives from electronic products, water supply and drainage pipelines, coating and wiring, to packaging and containers. In 2013, global plastic production was 299 million tons, which is a nearly 200 fold increase over the annual production of 1.5 million tons in 1950 [1]. The annual produced plastic production has been and will continue increasing in the foreseeable future [2]. According to USEPA [3], plastics wastes (PSW), which are a rapidly growing segment of municipal solid waste (MSW), are found in all major MSW categories, such as the containers and packaging category (e.g. bags, sacks, and wraps, other packaging, PET bottles, jars and HDPE natural bottles, and other containers). Compared with plastics wastes (390,000 tons) generated in 1960, the quantity of plastics wastes generation has increased relatively steadily to 31.8 million tons in 2011 [3]. With regard to percentage of MSW generation, plastics wastes generation was increased from less than 1% in 1960 to 12.7% in 2011. A recent study indicates that up to 6,300 million metric tons of plastic waste have been generated to date [4]. However, among the generated plastic wastes, less than half of it was confined to landfills or recycled. Of the remaining plastic wastes, a large proportion litters continents, oceans,

and every corner of our world to make the world a "Plastic World" [5]. In our daily life, the most widely used plastics are thermoplastics, the most common of which are: polyethylene (PE) 29.6%, polypropylene (PP) 18.9%, polyvinyl chloride (PVC) 10.4%, polyurethane (PUR) 7.4%, polystyrene (PS) 7.1%, and polyethylene terephthalate (PET) 6.9% [2] (Figure 1). These plastic materials are also major sources of microplastic pollution in ocean [2]. Of particular concern is that, several of these plastic materials, especially PS, PVC, PUR as well as polycarbonate [6]-[8], making up approximately 26% of the plastics production, are the most hazardous to environment and human health [5]. There is a growing scientific consensus demonstrating that plastic wastes are a major environmental concern of increasing global significance:
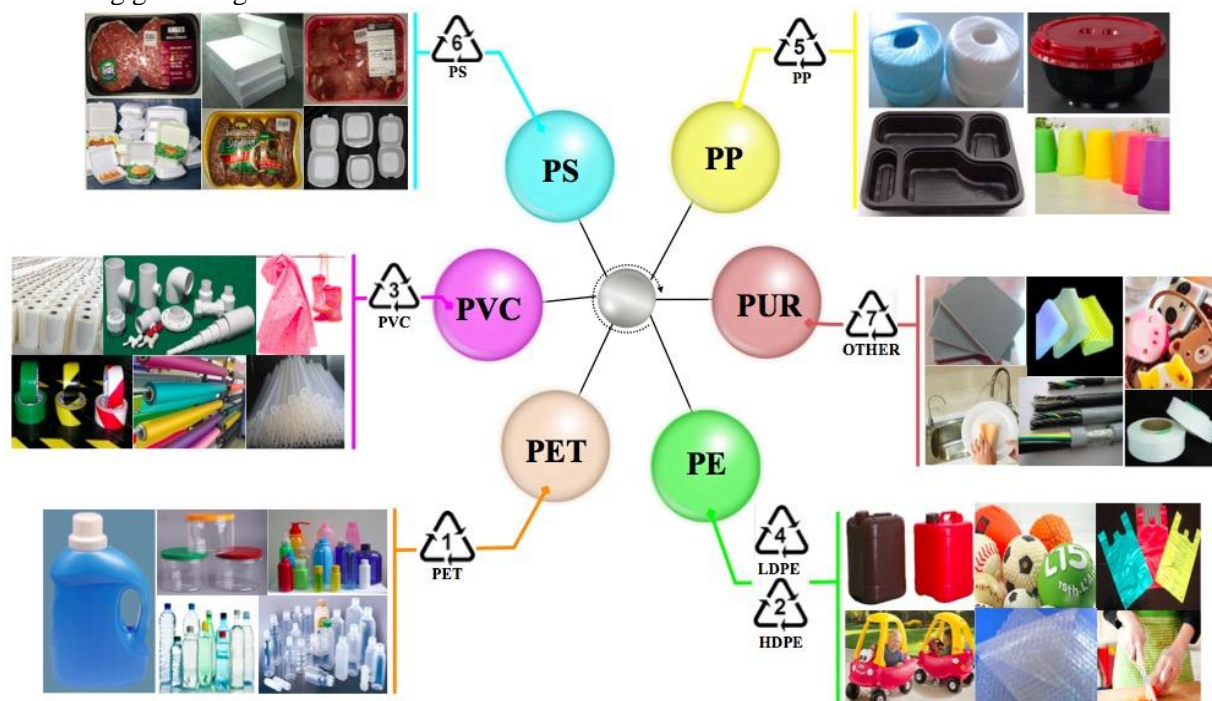

Figure 1. The most widely used plastics on the market

- Soil contamination by plastic wastes causes severe environmental pollution, and could lead to the decline of crop production. The plastics wastes in landfill occupy a great area of land (Landfill disposal of 10,000 tons plastic wastes occupy 0.067 hm^2 land), and also inevitably release large amounts of chemicals including oligomers, catalyst remnants, polymerization solvents, and a wide range of plastic additives [7]. These hazardous chemicals could leech from the plastic wastes and impact the soil quality [9], as well as neighboring groundwater.
- Water contamination by plastic wastes has been a major concern because they harms large ocean animals, deteriorate fishing industry, and damage aquatic environment. A recent report indicates that approximately 0.48-1.27 million tons of plastic waste enters ocean annually and the introduction of plastic into the ocean is increasing at an astonishing rate, with an estimated doubling time of 10 years [10], [11]. Studies have shown that the plastics wastes occupied 60-80% of all marine debris are widespread in the marine environment, at the sea surface, on shorelines and on the seabed [12]. Abiotic degradation, by wind, rain and sun, could cause harmful materials and chemicals to leech from plastic wastes, causing a secondary pollution problem within surface water, groundwater, and marine water bodies.
- Air pollution is another serious environmental concern. Referring to the disposal and management of solid wastes, PSW is co-incinerated with MSW. The combustion of PSW could result in the emission of air pollutants such as volatile organic compounds (VOCs), heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs), dioxins, NO_x, SO_x, etc. if off gases are not managed properly. At worst, some carcinogenic substances such as PAHs, nitro-PAHs, dioxins, etc., could be released as airborne pollutants during the

incineration of plastic wastes containing PVC, PET, PS and PE [13].

● Plastic pollution has the potential to poison animals and pose a serious threat to human health. According to a hazard-ranking model based on the United Nations' Globally Harmonized System of Classification and Labeling of Chemicals, the chemical ingredients of more than 50% of plastics are hazardous [7]. These harmful chemicals leeched from the plastic wastes or in the form of small or micro plastic debris are more likely to infiltrate food webs [14], and potentially impact ecologically important species including mussels, salt-marsh grasses and corals [14], [15]. Humans and mussels that ingested the chemicals from plastics and small or micro plastic debris could accumulate in the body, and harm the cells and other tissues [14], [16].

2. Plastic wastes control and management strategies

Except for the risks and hazards to the human health and the environment safety, the improper management and disposal of plastic wastes impact the aesthetic of the environment, badly affect the beauty of urban and remote environments. The generation of plastic wastes can be mainly classified into two categories (Figure 2): (1) preconsumer or industrial plastic wastes; and (2) postconsumer plastic wastes. Preconsumer or industrial plastic wastes referred to the discarded plastics, which are produced during the plastic production and product fabrication processes. These kinds of plastic wastes can be directly recycled and re-used by the processing and manufacturing departments.
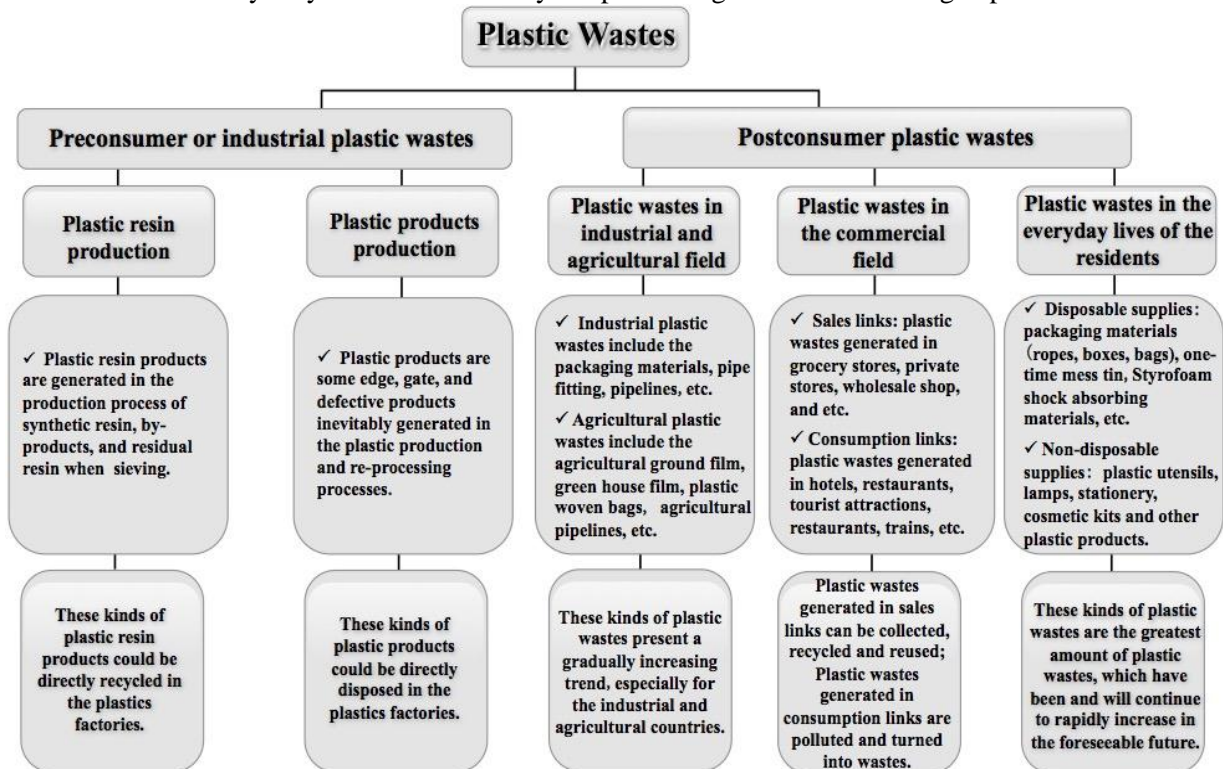

Figure 2. Sources of plastic wastes and strategies for the management of plastic wastes

Sustainable plastic wastes management solutions should apply methods or technologies that meet the requirements for efficient recycling of resources without generation of harmful substances (toxic and hazardous by-products or end-products) to humans or the environment. As we described elsewhere [2], the overall strategy for controlling plastics pollution should primarily focus on source reduction, increased use of biodegradable materials, improved reuse, recycle and recovery of plastic via improved separation efficiency from waste streams, and subsequently the development of cost-effective clean-up and bioremediation technologies. We will use biodegradation of polystyrene (PS) as example to evaluate the feasibility of biodegradation.

3. Biodegradation of polystyrene wastes

Industrial production of PS began around 1930 [17]. Today, PS products are widely used in our daily lives. The application of PS products includes use of expanded PS (EPS), trade name Styrofoam, in building insulation and packing, extruded PS (XPS) in containers (coffee cups and food trays), and high density PS materials as liquid containers. Most PS are used as foam products (Figure 1). With the rapid development of economy and society, the demand for PS materials and products has been continually increasing.   By 2013, annual global production of PS was around 21 million tons, accounting for about 7.1% of the total global plastic production [18].

3.1. Feasibility of Biodegradation of PS

The term of "biodegradation" represents the predominance of biological activity in the degradation of plastic polymers into lower molecular weight compounds and mineralization into CO_2 and water [19]. The plastic polymers are too large to pass through cellular membranes. During biodegradation, the polymers are first converted into small fragments, then further degraded and mineralized within microbial cells [20]-[23]. Attempts to investigate direct biodegradation of PS has been performed since 1970s. PS products have been considered extremely durable and recalcitrant against microbial attack.

In 1980s styrene, a monomer used for synthesis of polystyrene [24], was assumed as an intermediate of PS breaking down product. Late professor D. Grbić-Galić at Stanford University and others conducted comprehensive research on its biodegradation. In earlier studies [25]-[27], mixed microbial cultures and *Pseudomonas* were found to grow on styrene as sole carbon source. Based on previous research, a strain of *Pseudomonas fluorescents*, which was capable of growing on styrene as the sole carbon source, was isolated from enrichment cultures (styrene, phenyl acetic and *o*-hydroxyl phenyl acetic acids) [28]. Styrene-degrading bacterium, *Xanthobacter* sp. strain 124X, and 14 strains of aerobic bacteria and two strains of fungi were isolated from a sewage culture with the styrene as the carbon source [28], [29]. Additionally, a novel flavin adenine dinucleotide-requiring styrene monooxygenase activity was further detected (oxidized styrene to styrene oxide (phenyloxirane)) [30]. The metabolic pathways of bacteria for styrene biodegradation under anaerobic pathways was also investigated under strictly anaerobic conditions [31] and a pure culture of *Enterohacfer cloacae* DG-6 (ATCC 35929) performed an almost stoichiometric conversion of styrene to CO_2 and low concentrations of aromatic and alicyclic compounds. To date, however, it has not been confirmed that styrene is the intermediate produced during PS biodegradation.

Since the early 1970s, researchers have studied the biodegradation of PS and other plastic wastes by microorganisms. Guillet et al. (1974) tested biodegradation of two photodegraded polystyrene-5% vinyl ketone copolymers which were laboratory-synthesized using ^{14}C styrene. The polymers were pretreated by a UV accelerator, resulting in decreasing viscosity molecular weight from 795,300 and 450,000 to 32,000 and 10,000. After pretreatment, bio-oxidation was observed based on the release of [14]CO_2 with added activated sludge. Approximate 0.7% of total ^{14}C was released as [14]CO_2 after 75 days. The [14]CO_2 release rate from undegraded polymers was very low and then appeared level off [32]. Kaplan et al. [33] investigated the PS biodegradation capabilities of 17 species of fungi in axenic cultures, five groups of soil invertebrates, and a variety of mixed microbial communities including sludge, soils, manures, trash, and decaying plastics. After 35 days, only 0 to 0.24% PS appeared to be biodegraded by the fungi in axenic cultures. The PS biodegradation mixed microbial communities (including silt loam, cow manure, activated sludge, decaying plastics, etc.) ranged from 0.04 to 0.57% after 11 weeks. They concluded that the PS biodegradation by the fungi in axenic cultures and mixed microbial communities was performed extremely slowly. Sielicki et al (1978) studied the biodegradation of [*β*-^{14}C] polystyrene in the soil and liquid enrichment cultures by measuring [14]CO_2 released. Biodegradation of polystyrene in soil was varied from 1.5 to 3.0% over a 4 month period based on the release of [14]CO_2 [34]. The PS removal was still limited but appeared a 15 to 30 times of those reported by Kaplan et al. [33]. Mor and Sivan also reported the isolation of biofilm-producing strain (C208) of the actinomycete *Rhodococcus ruber* which was could degrade PS [35]. The strain C208 was incubated with PS flakes as sole carbon source, resulting in 0.5% and 0.8% of weight reduction in PS flakes within 4 and 8 weeks. Atiq et al. (2010) reported that *Paenibacillus urinalis*

strain NA26, *Bacillus* sp NB6, and *Psueudomons aeruginosa* NB26 were isolated from buried PS film in soil after 8 months [36]. These bacterial strains colonized and used EPS as a sole carbon source. Some intermediate-like compounds (1-phenyl-1, 2 ethandiol and 2-phenylethanol) were detected in the medium using HPLC but no significant surface changes were observed using FTIR analysis. The PS biodegrading ability of these strains has not yet been convinced.

In addition, Nakamiya et al. (1997) showed that hydroquinone peroxidase of a lignin decolorizing bacterium, *Azotobacter beijerinckii* HM21 degraded polystyrene in a dichloromethane-water system [37]. However, no follow up studies of biodegradation of PS by strain HM21 have been reported.

Previous studies on PS biodegradation, as seen above, have shown promising signs although the degradation is limited (Table 1). More research is still needed to understand if any microorganisms capable of rapid-degrading PS are present in environment and their living conditions.

Table 1. Reported microbial cultures degrading polystyrene

Authors	Culture source	PS removal	Test Period
Sielicki et al. [34]	The soil and liquid enrichment cultures	1.5%−3.0%	4 months
	The fungi in axenic cultures	0%−0.24%	35 days
Kaplan et al. [33]	Mixed microbial communities	0.04%−0.57%	5-11 weeks
Mor & Sivan [35]	*Rhodococcus ruber* C208	0.5% or 0.8% weigh reduction	4 or 8 weeks
Atiq et al. [36]	*Paenibacillus urinalis* NA26 *Bacillus* sp NB6 *Psueudomons aeruginosa* NB26	No weight reduction reported but found intermediates	8 weeks
Yang et al. [38]	*Exiguobacterium* sp. strain YT2 from mealworms	7.4 $\pm$ 0.4% of weight reduction (2500 mg PS/L in 10^8 cells/mL)	60 days

3.2. Biodegradation of PS Wastes by PS-Eating Mealworms

Yellow mealworms are the larvae of *Tenebrio molitor* Linnaeus which is a holometabolic insect, passing through the stages of egg, larva, pupa, and beetle. They are pest insects for storage and also are commercial animal feed available in pet markets and stores. Mealworms eating Styrofoam and isolation of bacteria from mealworms' gut were reported by several middle and high school students for science fairs via public media (newspapers and internet) in 2000s. However, these early studies did not get academic attention as they did not result in peer-reviewed publications or archiving of isolates.

Mealworms have chewing and eating behaviour for Styrofoam (Figures 3) and other plastic foam. The confirmation results of the biodegradation and mineralization of PS in the larval gut of the *Tenebrio molitor* Linnaeus were published by Yang et al. (2015) [39]. The mealworms were purchased from a pet market in Beijing, China. After fed with Styrofoam (5.8 g) as the sole diet for 30 days, the mealworms (500 in an incubator) consumed 31.0±1.7% of Styrofoam. A test for the determination of the survival rate (SR) over a 1 month period showed that the difference between of the SR of Styrofoam-feeding mealworms and the SR of conventional diet (bran)-feeding mealworms was not significant (average 85%). Similar results have been observed using different batch of mealworms from Harbin, China and Compton, California, the USA. The SR was approximate 81.3 $\pm$ 2.5% and 86.7 $\pm$ 3.3% for the Styrofoam-feeding mealworms from Harbin and California, respectively when Styrofoam was fed as a sole diet over one month. For one incubator containing 120 mealworms and Styrofoam was consumed by 29.9 $\pm$ 3.84% and 23.6 $\pm$ 0.1% over one month period, respectively. The consumption of Styrofoam and high SG indicated that Styrofoam-feeding could be digested and utilized as energy source to maintain their life activity.

The retention time of ingested PS or Styrofoam in mealworm gut is relatively short, estimated to be 12-15 hrs. Characterization of Styrofoam (or PS) degradation by mealworms and other insects is primarily based on the changes in chemical and physical properties of egested residues (in the frass)

from the PS-feeding insects. PS depolymerization and biodegradation were characterized by gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FTIR), solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C-CP/MS NMR) and liquid-state ^{1}H nuclear magnetic resonance (^{1}H-NMR) analysis as well as other methods [38]-[40]. GPC analysis provides information on three key indicators of depolymerization and degradation of plastic materials: the number-average molecular weight (M_n), the weight-average molecular weight (M_w), and molecular weight distribution (MWD). The M_n and M_w of the residual polymer in frass decreased significantly from those of the original Styrofoam material, indicating depolymerisation is occurring. The MWD also shifts towards to lower molecular weight [38]-[40]. FTIR, ^{1}H-NMR or ^{13}C-CP/MS NMR analysis were conducted to identify functional groups indicative of depolymerization and oxidation [38]-[40]. In addition, the release ^{13}C-labeled CO_2 from the mealworms fed with α ^{13}C-labeled and β ^{13}C-labeled PS further confirmed the biodegradation and mineralization of PS. Our recent studies indicated that supplementation of nitrogen-containing feed (e.g. bran) can enhance PS consumption and degradation by mealworms [40]. At 25 °C, the PS consumption rate can be doubled with co-feeding bran [40].
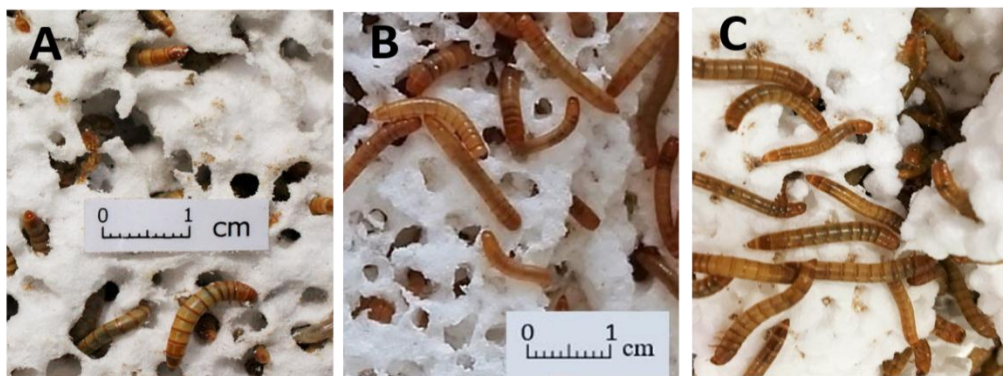

Figure 3. Styrofoam-eating mealworms from various sources (A) Beijing, China, (B) Harbin, China; and (C) Compton, California, USA.   All these mealworms are capable of PS-degradation.

To better understand the mechanism of PS-degrading by the larvae, antibiotics, such as gentamicin, were fed to mealworms to understand the activity of gut bacteria and the effect on depolymerization of the PS [38]. The mealworms fed with gentamicin for 10 days showed significantly less depolymerization and mineralization after the activities of the gut bacterial were [38], suggesting that PS degradation is likely gut-microbe-dependent. A PS-degrading bacterial strain, *Exiguobacterium* sp. strain YT2 was isolated from the guts of the mealworms tested in Beijing [38]. The single culture of strain YT2 removed PS by 7.4 ±0.4% in liquid medium containing PS pieces (2500 mg/L) during a 60 day period. GPC analysis of residual PS also confirmed PS depolymerisation by strain YT2. This has proved that PS-degrading microorganisms are present in mealworms' gut.

3.3. Future research and perspectives of microbial degradation of plastics
Mealworms degraded ingested PS in their gut up to 40-50% within the relatively short 12-15 hrs gut retention time. Further studies with antibiotics have suggested that the PS biodegradation is likely gut microbe-dependent. To date, however, no microorganisms isolated, including strain YT2 isolated from the mealworm gut, can degrade PS at the same rate as the mealworms. More research is needed to understand the mechanisms of the PS biodegradation in mealworm gut and interactions between the mealworm host and gut microbes. The mealworms' ability to biodegrade PS may expand to other plastic materials such as PE, PP, PET, and PUR and will be tested. The ubiquity of PS biodegradation by mealworms around world in relation to their gut microbiota should be investigated in order to further understand the mechanisms and select highly efficient mealworm strains and microorganisms.
Future microbiological work can focuses on isolation and characterization of more PS-degrading single cultures and define high efficiency mixed cultures (if present) from various PS-degrading mealworms.

Biodegradation of PS as well as other plastics requires breaking down or depolymerisation of plastic polymers. Further research on biochemistry can involve in the separation and identification of enzymes, especially extracellular enzymes. This could lead to production of plastic-depolymerizing enzymes for future large-scale use.

The research on PS-degradation by mealworms is just beginning to open a door for future scientific studies and development of potential biotechnologies to solve plastic pollution problem.

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