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Biomechanical Recycling of Plastic Waste using *Tenebrio Molitor* Larvae

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Insect larvae eat plastics and can take part in biomechanical recycling, breaking down polymers in their intestinal tract. The objective of the research was to biodegrade waste vinyl (PVC) gloves for domestic use using *Tenebrio molitor* larvae. For this, the mass of plastic waste was recorded on days 5, 10, 15, 22 and 28 of December 2022. The larvae were measured at the beginning and at the end of the trial and its excrements too. After excrement was analyzed by FTIR. Likewise, the most dominant bacteria were isolated from the intestines of the larvae that ate the plastic waste. The results indicated a utilization rate of 12% of the PCV, a rate of specific consumption of PVC equal to 2.41 mg/larva.day. The introduction of oxygen as a consequence of oxidation and associated granmegative bacteria in the larvae's intestines were also identified in the excreta of the larvae. Se ha development the biodegrading of vinyl glove residues that can be considered a biotechnological and ecological solutions to the challenge of plastic waste.

1. Introduction

Polyvinyl chloride (PVC) is a thermoplastic polymer that is characterized by its durability and malleability and is used as a low or medium voltage material (Alsabri and Al-Ghamdi, 2020). PVC is also used in the production of highly flexible and durable vinyl gloves with applications of phthalate plasticizers (Poitou et al., 2021). The SARS-CoV-2 pandemic intensified its use in laboratories and at home, in Peru its sale is free on the streets. Unfortunately, the poor management of solid waste in the country does not favor recycling or proper disposal, ending up in street dumps (Bozek et al., 2017). Developing countries urgently require more effective management policies in the recovery and recycling of plastic (Wei et al., 2020), to prevent the loss of its economic value and generate circular economy opportunities. New research has shown that it is possible to biodegrade plastics using *Tenebrio molitor* insect larvae and recover new biopolymers by gut microbiota (Sangiorgio et al., 2021). *T. molitor* belongs to the Tenebrionidae family, of the Coleoptera order, its ability to live depends on temperature and humidity and it has become an alternative solution for the degradation of plastic waste (Wu et al., 2019; Yang et al., 2021). This process involves the depolymerization or breaking of the vinyl polymer chains, followed by the generation of oxidized intermediates and finally the mineralization to CO_2, H_2O and Cl- (Chen et al., 2022). The objective of the research was to evaluate the response of *T. molitor* larvae in the fourth stage associated with their own intestinal microbiota, during the biodegradation of vinyl glove (PVC) residues that would affect the biological processes (Peng et al., 2022).

2. Materials and methods

2.1 Breeding and adaptation and morphological development of *T. molitor* larvae

The degradation of vinyl (PVC) glove residues was treated using *T. molitor* larvae, coleopterans with the ability to chew polymers (Sanchez-Hernandez, 2021). The larvae were acquired from the company "live food

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hatchery", in a first stage and deposited in sterilized 600 ml beakers, in the Environmental Engineering Laboratory of the Cesar Vallejo University (San Juan de Lurigancho Campus) in Lima, Peru. The larvae were fed with wheat bran (Nutrimix, Peru) under controlled temperatures between 30 and 35 °C, maintained with two digital thermostats (SHT2000, China) and relative humidity between 65 and 70% (Deruytter et al., 2022). The larvae were acclimatized for 3 days, then sieved from the initial wheat bran and fasted for 24 h to empty their intestines and prepare them for the new control diet (wheat bran) and diet with plastic (gloves).

2.2 Plastic waste

Pieces of vinyl gloves were cut into 3.5 cm x 3.5 cm squares, then approximately 2 g were weighed out and added to the beakers containing 40 *T. molitor* larvae.

2.3 Experimental Procedure

The design comprised thirty experimental units arranged in 600 ml beakers, each beaker containing 40 *T. molitor* larvae (fourth instar); with an average length of 0.63 ± 0.032 cm and a biomass of 0.121 ± 0.031 g. The larvae were subjected to two diets, i) residues of vinyl gloves and ii) wheat bran (control); both under controlled conditions of temperature (30-35 °C) and relative humidity (65-70%). The average weight of each substrate was 2.0 g, with a ratio of 0.05 g/larvae. The substrates were not enriched with water or other foods or seeds, and weight loss was measured in triplicate at 0, 5, 10, 15, 22, and 28 days, by gravimetry (Sartorius analytical balance, USA). No dead larvae were recorded in the process and after 28 days the larval excrement was separated from the plastic waste; these were sieved with a 500 µm mesh (See Figure 1).

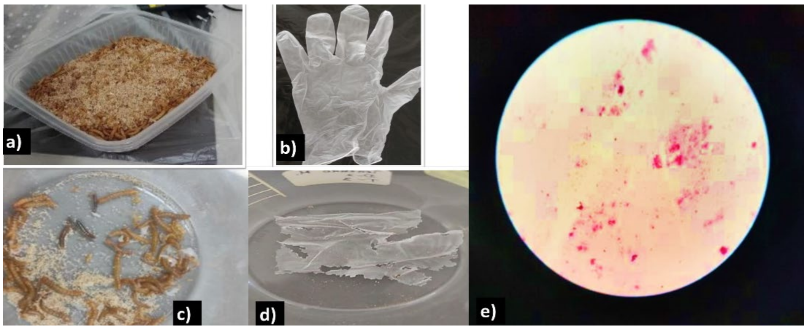

Figure 1. Evidence of: a) Adaptation of larvae in wheat bran; c) waste vinyl gloves; d) preliminary test of the biodegradation process by T. molitor larvae.; b) vinyl gloves; e) gram negative bacteria

2.4 Plastic utilization rate

The utilization rate (U) of the plastics was calculated according to Bulak et al. (2021) using the following equation:

$$U = \left(\frac{M_i - M_f}{M_i}\right) 100\ \% \qquad (1)$$

Where M_i= initial mass of plastic (g) and M_f = final mass of plastic expressed in (g).

2.5 Fourier Transform Infrared Spectroscopy (FT-IR) of Plastics

The identification of functional groups of the treated plastic glove and of the larval manure after 28 days of diets was analyzed by means of Infrared Spectroscopy (FTIR-ATR) spectra with 200 Scan in a range of 4000 to 600 nm to characterize the chemical structure in the sample (Thermo Fisher Scientific- Nicolet iS10 model, USA).

2.6 Larval intestine collection and Gram stain tests

On the 28th day, the larvae were separated from the beaker and disinfected in ethanol solution (75%) for 1 minute, then rinsed in saline solution (3 times). A scalpel was used to remove the intestine (Jiang et al., 2021) which was arranged on slides for Gram stain testing.

2.7 Data analysis method

Experimental results were analyzed using Origin-Pro 8.0 software. Statistical significance was determined using the Student's t-test and ANOVA with Tukey's post hoc test ($p < 0.05$; n = 2).

2. Results and Discussion

3.1 Rate of Utilization of PVC by the larvae of *Tenebrio molitor*

Figure 2a shows the accumulated loss of the substrate in the process, during the 28 days of the experiment. Student's t-test for this parameter demonstrated a significant difference ($p<0.05$) between the control and the vinyl plastic diet. The accumulated loss of vinyl gloves was 56.2% with respect to wheat bran. Figure 2b shows a significant consumption ($p< 0.05$) of vinyl glove residues by the larvae, the digested plastic provided a source of energy for the survival of the larvae; with a rate of use of 12%. The decreasing trend in plastic consumption after 15 days suggests a toxic effect on the larvae. Peng et al. (2023), showed that residual PVC particles generated high oxidative stress in mealworms that could cause death, because they are more difficult to purify and excrete (Jin et al., 2023). Wheat bran, on the other hand, was healthier and was reflected in a higher utilization rate (40%) (Table 1). The coincidences of loss of substrate on day 15 (0.4 ± 0.038 g for the control and 0.4 ± 0.009 g for the plastic), were due to a decompensation in the environmental conditions of the control group between 10 and 15 days.

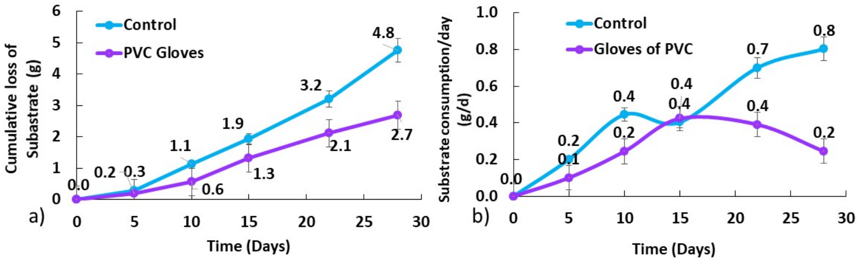

Figure 2. Feeding of T. molitor larvae: a) accumulation of substrate loss and b) Substrate consumption, period 28 days.

Table 1. Mass of the substrates and morphological development of the larvae at the beginning and end of the tests and the mass of plastics remaining in frass of the T. molitor larvae (mean ± SD; n = 2).

Parameter\Substrate	Control	Vinyl gloves
Initial mass of substrate (g)	2.007 ± 0.0019	2.0247 ± 0.0286
Final Mass of substrate (g)	1.204 ± 0.012	1.7806 ± 0.058
Utilization (w/w %)	40 ± 0.649	12.06 ± 1.609
Plastic remnants in frass (w/w %)	No measure	22.44 ± 3.18
Initial mass of 1 larvae (g)	0.121 ± 0.031	0.12 ± 0.033
Initial Length of 1 larvae (cm)	0.63 ± 0.032	0.66 ± 0.035
Final mass of 1 larvae (g)		0.0288 ± 0.0094
Final length of 1 larvae (cm)		1.337 ± 0.4324

Table 2. Comparison of results with recent research on biodegradation of PVC plastics

PVC product	Description	Reference
PVC Gloves residue	Usage rate: 12.06%; 28-day survival: 99%; cumulative waste loss from PVC gloves: 2.7 g	Present research
Rigid PVC microplastic (MP) powders (70–150 μm)	Survival at 5 days: 80% and 36.62 mg MP/100 larvae.day. Droppings contain 34.6% residual PVC and chlorinated organic carbons.	Peng, et al. (2020)
PVC	Larval mortality rate (7.90 ± 1.10%)	Jin et al. (2022)
PVC -MP	Consumption of 16.99 -28.49 mg/100 larvae.day and 63.13 - 91.2% survival in 28 days	Wu et al., (2019)
PVC plastic pipe pieces (10 mm)	Consumption: 13.57 mg/100 larvae.day. The larvae lost 19% of mass during 21 days.	Bozek et al. (2017)
PVC -MP	PVC weight loss: 10%. Reduction of the mass of larvae in 25 days: 30%	Espinoza et al. (2022)

On the morphological development of the larvae of *T. molitor*, Table 1 shows the weights and lengths of the larvae at the beginning and at the end of the treatment. The larvae that fed on the vinyl glove residues decreased by 24% of the initial weight and increased their length to twice the initial one. Regarding the survival of the larvae, all were maintained until the end of the test and exceeded the ranges of other studies (Peng et al., 2022; Wu et al. 2019) above 60% (Table 2). Likewise, utilization rates were similar to those reported by Bozek et al. (2017) and Espinoza et al. (2022) between 10 - 13.57%, but lower than those reported by Peng et al. (2022) and Wu et al. (2019) with rates between 16.99 and 36.62%, for the biodegradation of PVC microplastics.

The great flexibility and elasticity of PVC gloves make it difficult to bite and crush these materials (Peng et al., 2022). The intestinal microbiota reported in the experiment was made up of gram-negative bacteria of the genus coccus (Figure 1 e) that would significantly favor the initial depolymerization of PVC and its biodegradation (Peng et al., 2022) since a part of the ingested plastic was converted into CO_2 and another part was assimilated by the cells, reflected in the growth of the larvae until they reached lengths of 1.37 cm but with a decrease in their body mass (0.0288 g); evidenced in previous studies (Bulak et al., 2021; Peng et al., 2022; Bozek et al., 2017; Jin et al., 2022; Espinosa et al., 2022). Yang et al. (2015) and Bulak et al. (2021) have suggested that larvae subjected solely to plastic without access to water suffer from dehydration and significant loss of fatty tissue. The reduction in the weight of the larvae would be related to the presence of toxic C-Cl groups (Wu et al., 2019; Bozek et al., 2017), typical of Polyvinyl Chloride or phthalates (Poitou et al., 2021), used as plasticizers. Part of the waste was expelled in the feces, due to the formation of microplastics and less toxic derivative compounds. The specific consumption rate calculated in this research was 2.41 mg of plastic/day•larvae (Figure 2 a), showing a high performance that can be improved with the addition of natural nutrients.

3.2 Functional groups in vinyl glove residues and excreta from *T. molitor* larvae

Figure 3, show infrared spectra in vinyl gloves and *T. molitor* larvae excreta.

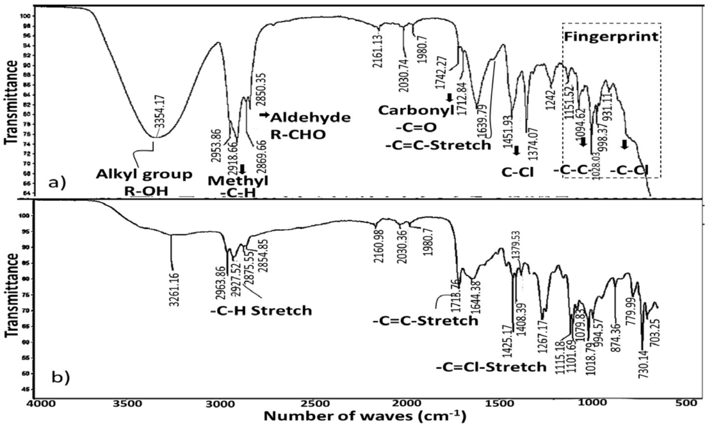

Figure 3. Infrared spectra of: a) vinyl gloves and b) T. molitor larvae excreta

The infrared spectrum (FTIR) of the vinyl gloves and of the excreta expelled from the *T. molitor* larvae showed modifications in the chemical structure and the incorporation of oxygen in the biodegradation process of PVC (Figure 3a and 3b). Regarding vinyl gloves, three characteristic peaks were observed at 690 cm^{-1} (stretch –C–Cl), 1094.62 cm^{-1} (stretch –C–C–) and 2963 cm^{-1} (stretch –C– H). The spectra of the droppings were compared with those reported by Wu et al. (2019). Peaks of new functional groups assigned to the stretching vibrations of CH (CH_2) were formed at 2927.52 cm^{-1}; variable angle vibrations of the O-H plane and C=O stretching at 1644.38 cm^{-1}. A peak assigned to the CH_2 cutting vibration is also observed at 1408.39 cm^{-1}; and C-O stretching vibration peaks at 1079.83 cm^{-1}; variable angle vibration of CH_2 at 1018.79 cm^{-1}, in addition there was oxygen incorporation evidenced by R-OH stretching (peak: 3261.16 cm^{-1}) and C-O stretching between 1000 and 1200 cm^{-1} (Brandom et al., 2018). Peng et al. (2022) considered that the appearance of carbonyl, hydroxyl and carboxylic acid groups constitute a fundamental preliminary stage in a process of oxidation and biodegradation of plastics, while the C-Cl peaks with less intensity in the droppings show a partial mineralization of PVC (Wu et al., 2019).

3.3 Applicability of *T. molitor* larvae to other types of plastic waste

The authors believe that this method of biodegradation of synthetic PVC gloves by *T. molitor* is applicable to other plastic wastes. polyethylene terephthalate (PET); polystyrene (PS); high density polyethylene (HDPE); low density polyethylene (LDPE); polypropylene (PP); they are formed by carbon atoms, long chains and others with the presence of benzene rings that include heteroatoms in the main chain (Mohanan et al., 2020). The difference in densities characterizes its recalcitrant nature and according to Mohanan et al. (2020) follow the following order: PVC(1.31-1.45) ≥ PET(1.35) > PS(1.03-1.09) > HDPE(0.94-0.97) > LDPE(0.94-0.93) > PP(0.90-0.91). Jin et al. (2022) demonstrated that these larvae, in addition to degrading PVC, also depolymerized and biodegraded PE and PS; this is because the intestinal microbial flora of the larvae is adaptive and they biodegrade chemically different plastics (Brandon et al., 2018). Bulak et al. (2021) confirmed a degradation order for PS <PU< < PE, while Bożek et al. (2017) pointed out that PVC is always more complicated to depolymerize and degrade in a similar way to PS. These results established a theoretical-practical guide for future applications and research on the biodegradation of other plastic waste by *T. molitor* larvae.

3.4 Waste Management Strategies for PVC Gloves

Table 3 shows some plastic waste management strategies that include vinyl gloves. Pyrolysis as part of chemical recycling (Wang et al. 2022) is suitable for the production of alternative fuels (Aragaw, Mekonnen, 2021; Kumar et al., 2023), as long as the technology includes an emission control system (Jang et al., 2006). Despite the segregation of waste (Benson et al. 2021), the application of the 5Rs (Kumar et al., 2023) also contributes to a circular economy. The application of these residues as aggregates for construction has already been reported (Albiajawi et al., 2021). However, the search for alternative methods of treatment with an ecological approach is a challenge in future research. The application of *T. molitor* larvae and other insects requires exploring the chemical processes that involve the biodegradation of plastic waste, intermediate products, the response of the type of larva and its microbial dynamics, among other aspects.

Table 3. Waste Management Strategies for PVC Gloves

Alternative	Description	Reference
Pyrolysis PP and PVC, can be easily transformed into fuel energy through pyrolysis.	Endothermic peaks of 431 ℃ (PVC) and 175 ℃ (PP) generate a rate of 75% liquid fuel, char (10%) and non-condensable gases.	Aragaw, Mekonnen (2021)
Pyrolysis Alternative fuel production	Produces: 70–80% plastic pyrolysis oil, 10–15% biochar and gaseous fuel.	Kumar, et al. (2023)
Chemical upcycling	Recycling of polymer waste through cascade hydropyrolysis-hydrogenation generates alternative fuels with improved properties	Aragaw, Mekonnen, (2021); Wang et al. (2022)
Waste Segregation	Waste Segregation: Thermal with Autoclaving, sterilization and Final Disposal; Irradiative (Microwave); Chemical Disinfection; Biological (use of Enzymes)	Benson et al. (2021)
Incineration	Incineration requires control of toxic emissions into the air (dioxins and furans). It is urgent before minimizing and recycling waste.	Jang et al. (2006)
Application of the 5Rs	5R (refuse, reduce, re-use, re-adapt and recycle)	Kumar, et al. (2023)
Substitution in concrete mixes	Ground latex gloves, used to replace coarse aggregates in concrete mixes, significantly improve the water absorption of concrete samples.	ALbiajawi et al. (2021)

4. Conclusions

This research demonstrated that *T. molitor* larvae can depolymerize and biodegrade vinyl glove residue as the sole food source for 28 days with full survival. The biodegradation was evidenced by the formation of the functional groups R-OH, O–C and C=O identified in the excreta of the larvae, likewise a partial mineralization of the C-Cl group typical of PVC was produced. This process has been supported by the predominance of intestinal gram-negative bacteria that synergize with *T. molitor*.

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References

ALbiajawi M.I., Alkasawneh R.W., Mostafa S.A., Johari I., Embong R., Muthusamy K., 2021, Performance of sustainable concrete containing recycled latex gloves and silicone catheter under elevated temperature, Journal of King Saud University-Engineering Sciences.

Alsabri A., Al-Ghamdi S.G., 2020, Carbon footprint and embodied energy of PVC, PE, and PP piping: Perspective on environmental performance, Energy Reports, 6, 364-370.

Aragaw T.A., Mekonnen B.A., 2021, Current plastics pollution threats due to COVID-19 and its possible mitigation techniques: a waste-to-energy conversion via Pyrolysis, Environmental Systems Research, 10, 1-11.

Benson N.U., Bassey D.E., Palanisami T., 2021, COVID pollution: impact of COVID-19 pandemic on global plastic waste footprint, Heliyon, 7(2).

Bożek M., Hanus-Lorenz B., Rybak J., 2017, The studies on waste biodegradation by Tenebrio molitor, In E3S web of conferences (Vol. 17, p. 00011), EDP Sciences,

Brandon A.M., Gao S.H., Tian R., Ning D., Yang S.S., Zhou J., Wu W.M., Criddle C.S., 2018, Biodegradation of polyethylene and plastic mixtures in mealworms (Larvae of Tenebrio molitor) and effects on the gut microbiome, Environ. Sci. Technol., 52, 6526–6533.

Bulak P., Proc K., Pytlak A., Puszka A., Gawdzik B., Bieganowski A., 2021, Biodegradation of different types of plastics by Tenebrio molitor insect. Polymers, 13(20), 3508.

Chen J., Wu J., Sherrell P.C., Chen J., Wang H., Zhang W.X., Yang J., 2022, How to Build a Microplastics-Free Environment: Strategies for Microplastics Degradation and Plastics Recycling, Advanced Science, 9(6), 2103764.

Deruytter D., Coudron C.L., Claeys J., 2022, The Effects of Density on the Growth and Temperature Production of Tenebrio molitor Larvae, Sustainability, 14 (10), 6234.

Espinoza P.J., Ordonez G.J., Castaneda-Olivera C.A., Benites A.E.G., 2022, Environmental Biotechnology: Biodegradation of Microplastics with Larvae of Tenebrio Molitor and Galleria Mellonella, Chemical Engineering Transactions, 93, 187-192.

Jang Y.C., Lee C., Yoon O.S., Kim H., 2006, Medical waste management in Korea, Journal of environmental management, 80(2), 107-115.

Jiang S., Su T., Zhao J., Wang Z., 2021, Biodegradation of polystyrene by Tenebrio molitor, Galleria mellonella, and Zophobas atratus larvae and comparison of their degradation effects, Polymers, 13(20), 3539.

Jin L., Feng P., Cheng Z., Wang D., 2023, Effect of biodegrading polyethylene, polystyrene, and polyvinyl chloride on the growth and development of yellow mealworm (Tenebrio molitor) larvae, Environmental Science and Pollution Research, 30(13), 37118-37126.

Kumar A., Pali H. S., Kumar M., 2023, A comprehensive review on the production of alternative fuel through medical plastic waste, Sustainable Energy Technologies and Assessments, 55, 102924.

Mohanan N., Montazer Z., Sharma P.K., Levin D.B., 2020, Microbial and enzymatic degradation of synthetic plastics, Frontiers in Microbiology, 11, 580709.

Peng B.Y., Sun Y., Wu Z., Chen J., Shen Z., Zhou X., Wei-Min Wu, Zhang Y., 2022, Biodegradation of polystyrene and low-density polyethylene by Zophobas atratus larvae: Fragmentation into microplastics, gut microbiota shift, and microbial functional enzymes, Journal of Cleaner Production, 367, 132987.

Peng B.Y., Sun Y., Zhang X., Sun J., Xu Y., Xiao S., ... & Zhang, Y. (2023). Unveiling the residual plastics and produced toxicity during biodegradation of polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) microplastics by mealworms (Larvae of Tenebrio molitor). Journal of Hazardous Materials, 452, 131326.

Poitou K., Rogez-Florent T., Lecoeur M., Danel C., Regnault R., Vérité P., Monteil C., Foulon C., 2021, Analysis of Phthalates and Alternative Plasticizers in Gloves by Gas Chromatography–Mass Spectrometry and Liquid Chromatography–UV Detection: A Comparative Study, Toxics, 9(9), 200.

Sanchez-Hernandez, J.C., 2021, A toxicological perspective of plastic biodegradation by insect larvae, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 248, 109117.

Sangiorgio P., Verardi A., Dimatteo S., Spagnoletta A., Moliterni S., Errico S., 2021, Tenebrio molitor in the circular economy: a novel approach for plastic valorisation and PHA biological recovery, Environmental Science and Pollution Research, 28(38), 52689-52701.

Wang J., Jiang J., Meng X., Lam S.S., Ragauskas A.J., Wang Y., 2022, Catalytic cascade upcycling single-use natural rubber glove wastes into fuels via a two-stage pressurized fixed-bed reactor, Fuel Processing Technology, 238, 107490.

Wei R., Tiso T., Bertling J., O'Connor K., Blank L. M., Bornscheuer U.T., 2020, Possibilities and limitations of biotechnological plastic degradation and recycling, Nature Catalysis, 3(11), 867-871.

Wu Q., Tao H., Wong M.H., 2019, Feeding and metabolism effects of three common microplastics on Tenebrio molitor L., Environ Geochem Health, 41:17–26.

Yang Y., Yang J., Wu W.M., Zhao J., Song Y., Gao L., Yang R., Jiang L., 2015, Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Chemical and physical characterization and isotopic tests, Environ. Sci. Technol., 49, 12080–12086.

Yang L., Gao J., Liu Y., Zhuang G., Peng X., Wu W.M., Zhuang X., 2021, Biodegradation of expanded polystyrene and lowdensity polyethylene foams in larvae of Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae): Broad versus limited extent depolymerization and microbe-dependence versus independence, Chemosphere, 262, 127818.