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Technological application potential of polyethylene and polystyrene biodegradation by macro-organisms such as mealworms and wax moth larvae

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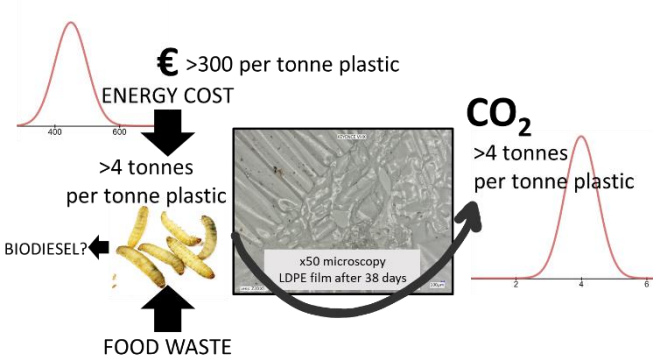
Abstract



Multiple recent reports showed accelerated biodegradation of polyethylene by employing macro-organisms such as mealworms (Tenebrio molitor) and larvae of the greater wax moth (Galleria mellonella), which seemingly chew and digest the plastic. Nevertheless, doubts regarding analytical data were published, and results are not universally transferrable. This paper aims at gaining mechanistic insights and exploring the technological prospects of potential future optimized biodegradation. We used a variety of experimental setups with both species, using both live specimens and homogenated paste, to cover a broad spectrum of potential technological setups, and performed gravimetric, microscopic and spectroscopic analyses. Live larvae showed a preference for specific substrates, yet we argue by comparison to other food sources, evidenced also by energetic uptake, that a diet of LDPE is insufficient for growth. We did not detect mass loss when homogenate paste is brought in contact with LDPE films, nor significant traces of ethylene glycol. We demonstrated that the morphology of the substrate changes after contact with live larvae, indicating some plasticizing action by an excreted liquid. This indicates a mechanism of degradation involving more than the gut microbiome alone. Using streamlined life cycle assessment and techno-economic analysis (LCA/TEA) methods, we showed that the application of these findings as either a remediation or management technology for waste plastics is highly unlikely, given the conversion to microplastics, the absence of valuable products, and the high energy cost. However, the conversion mechanism should be further elucidated for bio-functionalization of liquid alkanes as high-value application, or to mitigate plastic anomalies in composting/digesting food waste.

1. Introduction

Plastic pollution increasingly gains worldwide attention (Jambeck et al. 2015, Geyer and Jambeck 2017), and next to climate change and air quality constitutes one of the biggest environmental concerns of this time. Most plastics used are recalcitrant; they do not degrade in a reasonable time frame, or at least do not sufficiently degrade to overcome littering. Even the biochemical degradation of biobased biodegradable polymers such as polyhydroxyalkanoates (PHA) and polylactic acid (PLA), or fossil-based biodegradable polymers such as polycaprolactone (PCL), often requires specific conditions (e.g. elevated temperature), and targeted waste

management schemes. Moreover, polyethylene terephthalate (PET), the most widely used polycondensate worldwide, is almost unsusceptible to biodegradation; only recently improved degradation was demonstrated by an engineered aromatic polyesterase (Austin et al. 2018). Even more challenging is the biodegradation of carbon-carbon backbone synthetic polymers, such as saturated polyolefins (polyethylene – PE, and polypropylene, PP) and unsaturated polyolefins (polystyrene – PS, and polybutadiene – PBD), as they lack typical functional groups susceptible to (enzymatic) hydrolysis, as this is the dominant degradation mechanism. Nevertheless, slow bacterial and fungal degradation of thin PE films is achievable in landfill environments (Muhonje et al. 2018) or after treatment with mineral oxidants (Bonhomme et al. 2003). However, generally stated, targeted functionalization of alkanes is very difficult, by both classical catalytic and enzymatic pathways. To date, *Candida tropicalis* ATCC 20962 was reported to functionalize smaller, liquid alkyl chains (Craft et al. 2003).

The news about accelerated biodegradation of polyethylene by larvae of the greater wax moth *Galleria mellonella*, as reported by Bombelli et al. (2017), justifiably caused worldwide excitement (Ball 2017) and spurred further research. This finding may allegedly open up opportunities for bioremediation of littered plastic waste, or more realistically, expedite the development of biochemical recycling technologies. Inspired by this work, our laboratory initiated an effort to use the results of Bombelli et al., and design said biochemical recycling pathways, converting polyethylene into ethylene glycol, a valuable chemical. However, soon thereafter, Weber et al. (2017) pointed out in a correspondence that some of the results of Bombelli et al. (2017) were inconclusive, and raised some methodological issues: (i) the fourier-transform infrared spectroscopy (FTIR) results showing the presence of ethylene glycol may as well be due to residues of lipids and/or proteins on the polyethylene, and (ii) the mass loss may be due to mechanical action in the sample preparation. Indeed, in our view the analytical procedures may be improved, in order to demonstrate a potential enhanced degradation by larvae of the greater wax moth. More recently, Brandon et al. (2018) published similar work, yet with the claim of a full mass balance, on the degradation of PE and PS mixtures by mealworms *Tenebrio molitor*. However, we argue that the mass balances are not fully closed, as Brandon et al. did not check for residual polymeric matter in the organisms. However, they did show the degradation of PE and PS by a shift in molecular weights determined by high-temperature gel permeation chromatography (Brandon et al. 2018). Even more recent is the work of Cassone et al. (2020), who showed changes in the intestinal microbiome of larvae of the greater wax moth larvae based on their diet on polyethylene, and identified that microorganisms in the genus *Acinetobacter* likely play a role in the biodegradation. Furthermore, by using biochemical assay they showed that glycol-like moieties may be present in the treated samples. Kundungal et al. (2019) demonstrated that also larvae of the lesser waxworm (*Achroia grisella*) are able to degrade high-density polyethylene, under a range of different conditions. Nonetheless, similar to the work Brandon et al. (2018), a complete mass balance could not be established, making the interpretation of the results more complex. Polystyrene on the other hand, in addition to earlier work of Yang et al. (2015), was shown recently to also be mineralized by superworms *Zophobas atratus* at even higher rates, by the same authors (Yang et al. 2019). In that study, respiratory tests were done in order to further close the experimental mass balances.

It is clear that the rapidly expanding research is only starting to understand this particular mechanism in nature, and its ubiquity towards various species and polymeric materials. In this paper, our aim is twofold; we check on the robustness and ubiquity of the claims made in global coverage of this phenomenon, and we discuss the potential for technological application based on ex-ante assessment methods. To this end, we extend the analytical procedures of Bombelli et al. (2017) by adding several blank samples to the infrared analyses, and an additional set of experiments gave more insights on the ubiquity of the observed phenomena as well as to the technological application potential. We hypothesize based on the cited literature above, that to some extent, the biodegradation of various polymer types and morphologies should be detected, but that ethylene glycol is not a detectable metabolite. Moreover, if polyolefins would be biochemically degraded, also some biochemical degradation should be observable as more mobile alkane moieties. Nonetheless, even if biodegradation would take place at acceptable rates, forming carbon dioxide as posited by Brandon et al. (2018), we hypothesize that such finding is no ground for technological waste management of polyolefin rich streams. Therefore, through combining techno-economic analysis (TEA) and life cycle assessment (LCA) methods while considering uncertain parameters, we project select environmental and economic metrics for scaling this process within a waste management system.

2. Materials and Methods

2.1 Experimental set-up

This study used three different experimental setups; (i) direct contact between live mealworm and greater wax moth larvae specimens and polyethylene films, (ii) contact between a homogenate paste of mealworms and greater wax moth larvae and polyethylene films, and (iii) mixing of homogenate paste of both species with liquid paraffin.
Larvae of the greater wax moth *Galleria mellonella* and larvae of *Tenebrio molitor* (mealworms) were used that were commercially bred on bran material. The live larvae were obtained at Amfibia hobby shop in Kontich, Belgium, and were not further conditioned. The polyethylene substrate used was commercial household low-density polyethylene (LDPE) film, as well as commercial LDPE fruit bags marketed and sold by Carrefour supermarkets. Additionally, a black commercial LDPE shopping bag was used. A wide variety of experimental conditions was chosen to screen the ubiquity of the biochemical degradation by larvae, and to potentially gain insight into the exact degradation mechanism. A summary of the various experiments and corresponding codes referred to further in the paper is presented in Table 1.

Table 1. Summary of the various experiments performed. Unless otherwise stated, all experiments were performed at room temperature (20 °C to 25 °C), ambient humidity and daily light/dark cycles, however protected from direct incident light.

Format	Species	Substrate	Time	Experiment code
Live larvae	*Galleria mellonella*	Loosely folded cling film (LDPE)	17 h	**live$_{GWM}$_1**
		Loosely folded cling film (LDPE)	89 h	**live$_{GWM}$_2**
		Folded layers cling film (LDPE)	96 h	**live$_{GWM}$_3**
		Loosely folded black bag (LDPE)	216 h	**live$_{GWM}$_4**
	Tenebrio molitor	Loosely folded cling film (LDPE)	38 days	**live$_{MW}$_1**
		Commercial fruit bag (LDPE)	38 days	**live$_{MW}$_2**
		None (blank)	38 days	**live$_{MW}$_3**
		Bran	38 days	**live$_{MW}$_4**
Homogenate	*Galleria mellonella*	Cling film (LDPE)	48 h	**paste$_{GWM}$_1**
		Cling film (LDPE) at 100 % RH	20 h	**paste$_{GWM}$_2**
		Cling film (LDPE) and blank paste	0–120 h	**paste$_{GWM}$_3**
		Liquid paraffin at 100 % RH	14 days	**paste$_{GWM}$_paraffin**
		Polystyrene (PS) powder at 100 % RH	14 days	**paste$_{GWM}$_PS**
	Tenebrio molitor	Liquid paraffin at 100 % RH	14 days	**paste$_{MW}$_paraffin**
		Polystyrene (PS) powder at 100 % RH	14 days	**paste$_{MW}$_PS**

2.2 Direct contact between live larvae specimens and polyethylene film

Multiple experiments were set up, whereby live specimens of both larvae species were brought into contact with LDPE cling film, in a 250 mL glass lab beaker closed with pierced cling film. The film used for closing the beakers was untouched, as observed visually and gravimetrically. First, 10 larvae specimens of *Galleria mellonella* were brought into contact with loosely folded cling film, weighing initially of 0.154 g, resulting and weighted after 17 h (experiment **live$_{GWM}$_1**). Similarly, a 0.234 g loosely folded film was weighted after 89 h of contact with 10 live larvae (experiment **live$_{GWM}$_2**). The effect of physical position and morphology of the film was checked by folding a 0.189 g film in four layers and laying the folded film on the bottom of the glass beaker, before contact with 10 larvae for 96 h with subsequent weighing (experiment **live$_{GWM}$_3**). Finally, to track the fate of the LDPE visually, we used a piece of a black LDPE commercial shopping back of 2.834 g, in contact with 15 live wax moth larvae, and weighed the substrate after 216 h (over 9 days) (experiment **live$_{GWM}$_4**). The substrate and isolated chewed flakes were analyzed by FTIR-ATR (Bruker

A220/D Quicksnap with diamond crystal, 4 cm^{-1} resolution, 24 scans per analysis, 24 background scans, spectral range 375..7500 cm^{-1}).
After the observation of Brandon et al. (2018), that specimens of mealworms are able to degrade polyethylene, we set up an experiment with a larger cling film sample of 2.344 g and 100 live specimens of mealworms, *Tenebrio molitor,* weighing in total 9.227 g (experiment **live$_{MW}$_1**). A second setup was made with a thin commercial fruits/vegetables grocery bag of 2.434 g with 100 mealworms weighing 9.251 g (experiment **live$_{MW}$_2**). The mealworms were left in a beaker for 38 days in a place protected from direct light contact, at room temperature. Additionally, a blank sample of only 100 mealworms (9.090 g) (experiment **live$_{MW}$_3**) and a sample of 100 mealworms (9.290 g) with 2.355 g of bran (experiment **live$_{MW}$_4**) were kept in identical conditions. The four resulting setups remained untouched for the entire 38 days, remained at laboratory room temperature (20 to 25°C) and were subject to normal day-night cycles, but protected from direct sunlight or artificial light. The film substrates were afterwards washed with acetonitrile and dried in ambient conditions.

2.3 Contact between homogenate paste of worms with polyethylene film

The worms of both species were homogenized using mortar and pestle, at room temperature. The resulting homogenate was then spread with a thickness of approximately 5 mm on a LDPE cling film or inert surface (microscope glass). The composite samples were kept at 30 °C at all contact times, unless stated otherwise. The homogenate was not replaced multiple times, contrary to Bombelli et al. (2017), to limit any chances for mechanical tear of the LDPE substrate, as suggested by Weber et al. (2017). However, this may limit microbial activity (drying of the homogenate), so a lower degradation might be the result. A first such experiment (experiment **paste$_{GWM}$_1**) was done using a 0.065 g LDPE cling film substrate of 10.3 cm by 10.2 cm. Homogenate paste was smeared on this substrate as described above, and left in contact for 48 h at 30 °C. Afterwards the homogenate paste, hardened at that time (the effect of drying is counteracted in the next experiment), was removed carefully and the substrate washed with water and acetone. The substrate film was weighed after drying in ambient air.
To minimize the possibility of mechanical wear of the LDPE film by multiple removals of (dried) homogenate paste (as was done in Bombelli et al. 2017), yet guarantee microbiological activity, we performed the same gravimetric experiment by keeping the homogenate paste smeared on a LDPE film of 197 mg at 100 % relative humidity for 20 h at 25 °C. The oxygen level was meanwhile kept constant to its atmospheric concentration (experiment **paste$_{GWM}$_2**). This was achieved in a solid state fermenter, which is a 150 mL jacketed vessel that has a porous solids support and allows continuous analysis (Bluesens BlueInOne Ferm) and control of O_2 and relative humidity. The substrate film was washed with acetonitrile, dried in ambient air, and subsequently weighed.
Changes in chemical composition were checked using FTIR, for samples of homogenate paste spread on different pieces of LDPE film or on an inert surface (microscopy glass) for various times (1 h, 2h, 5 h, 24 h and 120 h) at 30 °C. A blank sample of the homogenate paste, LDPE substrate and homogenate on LDPE substrate at t = 0 was isolated (experiment **paste$_{GWM}$_3**; five samples and one blank). After the aforementioned specific timespans, the homogenate was removed from the LDPE films using tweezers, without physical contact between the tweezers and the film. Afterwards, the LDPE films were analyzed by ATR-FTIR, without prior washing (to check potential formation of ethylene glycol, which would be extracted by washing). The substrate films were placed at the ATR-probe with analysis focused on the side that was in contact with homogenate. For each sample the background was corrected by taking the average out of 5 or 6 measurements, depending on the sample. FTIR-microscopy (Bruker LUMOS) was done by placing the substrate films with the contacted side upwards on the microscopy platform, indicating the analysis points randomly dispersed over the surface, and take the average spectrum out of 10 to 30 points per sample.
Also the homogenate that was removed from the substrate after 24 h was subjected to FTIR analysis, as was the blank homogenate placed on an inert surface for various timespans (1 h, 2h, 5 h, 24 h).

2.4 Mixing of homogenate paste with liquid paraffin and polystyrene powder

Approximately 25 mL of liquid paraffin was mixed in a 1:1 mass ratio with homogenate paste for both mealworms (experiment **paste$_{MW}$_paraffin**) and greater wax moth larvae (experiment **paste$_{GWM}$_paraffin**) and kept in a shaking incubator at 100% relative humidity for 14 days. The same experiment was repeated for polystyrene powder (experiment **paste$_{GWM}$_PS**) and LDPE cling film (similar to the above) (experiment **paste$_{GWM}$_LDPE**). Additionally, a blank sample of solely homogenate paste was kept in identical conditions.

At the end of the 14 days, tetrahydrofuran (THF) was added to the samples, to stop any biological activity, after which all samples were analyzed using ATR-FTIR.

2.5 Replicates

In the contact between live larvae and the LDPE films, variations between individual larvae were accounted for by using multiple specimens per test. In the greater wax moth larvae experiments, 10 to 15 specimens were used each time, as indicated in section 2.2. In the mealworm experiments, we expanded this number to 100 specimens. We acknowledge that this does not take interpopulation variations into account though; assessments of such variations can be found in recent reference works by Brandon et al. (2018) or Cassone et al. (2020). In the contact of the homogenate paste with the LDPE films, sufficient masses were selected, and FTIR measurements were done as average of 5 to 6 measurements on a single sample. In some of the samples, those resulting from experiments **paste$_{GWM_2}$**, the variation between various measurements on a single sample is shown explicitly in Figure 4C and 4D below. All measurements were done using 24 sample scans and 24 background scans.

2.6 Streamlined LCA/TEA

An ex-ante, streamlined LCA/TEA approach was followed for technological evaluation of these fundamental scientific findings, given the small data set with large uncertainties available. Very recently researchers have used LCA to study growing insects, including mealworms, as a possible supply of food and feed markets (Thévenot et al. 2018, Smetana et al. 2019). Nevertheless, approximations were made taking into account potential future optimizations. The goal of this streamlined assessment is to determine future technological prospects on the one hand, and guide mechanistic insights based on theoretical values. Because of the data available, the discussion on technological potential is restricted to mealworms (no scientific literature was found on mass and energy balances of commercial farming of larvae of the greater wax moth). We use life cycle assessment (LCA) following ISO (2006) methods to define the goal and scope of the study, construct the life cycle inventory, and evaluate select environmental life cycle impacts of treating mixed plastic waste with mealworms to digest the waste. The system boundary (Figure 1) includes the stages of mealworm growth (rearing) and plastic consumption for sustenance. The functional unit defined is 1 metric ton of treated mixed plastic waste. We estimate the life cycle impact assessment (LCIA) metrics, non-renewable energy for input of thermal and electrical energy consumed to rear and sustain the mealworms and the IPCC (Myrrhe et al., 2013) 100-year global warming potential (GWP) to calculate all cradle-to-gate processes of rearing and sustaining the mealworms via a diet of waste plastic. Data for estimating the material balance for the rearing of mealworms, such as their mass gain and energy requirements, were obtained from Zheng et al. (2013), Thévenot et al. (2018) and Ooninckx and de Boer (2012) and description of the mass balance calculations are described in Section 2 of the Appendix. However, key input data are given in Table 2. Non-renewable energy input includes the thermal and electrical energy for maintaining conditions to rear and sustain the mealworms using data from Ooninckx and de Boer (2012) and LCI data from Eco-invent v3 (Wernet et al. 2016). GWP considers the cradle-to-gate emissions related to natural gas and electricity supply and consumption in Europe. We estimate the GWP for potentially converting the lipids from the mealworms to biodiesel using insights and data from Dufour and Iribarren (2012), Mu et al. (2016), Mehta end Anand (2009), Silva et al. (2019) and Hums et al. (2018), which describe operations for fractionating and converting lipids in sewage sludge and scum from wastewater treatment facilities, and final biodiesel properties. We assume the biodiesel offsets low S diesel with GWP 93 g CO_2-eq. MJ^{-1} reported by Sorunmu et al. (2019).

The techno-economic analysis (TEA) estimates treatment costs and includes valorization of a possible revenue stream if converting the mealworm remains at the end of the sustenance phase to biodiesel as a value-added product from the waste treatment process. We assume the mealworms represent a low feedstock cost originating from a waste management facility and like the analysis of scum-to-biodiesel in wastewater treatment facilities by Mu et al. (2016), could be a revenue-generating activity owing to cost savings related to waste treatment. Price levels of carriers were obtained from PriceWaterhouseCoopers (2019) and the European Commission Weekly Oil Bulletin (2019).
Using data from literature, we investigate two scenarios describing the performance of the mealworms' digestion of plastic waste and estimate the thermal and electrical energy inputs to the life cycle inventory; costs for the TEA, and environmental metrics for estimating the life cycle impact assessment (Table 2). The cradle-to-gate GWP for treating the mixed plastic waste includes cradle-to-gate GHG emissions from electricity and

natural gas consumed and the CO_2 from the fugitive gas fraction of mixed plastic waste emitted by mealworms. The theoretical (T) case assumes the mealworm is able to meet its energetic need from the plastic waste and the observed (O) case uses empirical measurement from Brandon et al. (2018) of combined plastic and bran fed to the mealworms to estimate the three performance metrics. The latter work of Brandon et al. (2018) serves as a key reference, given the nearly closed mass balance. Given the fact that no proper TEA was done for biodiesel from lipids sourced from larvae, conversion costs were not taken into account. The feedstock cost is likely to be significantly lower than other biodiesel projects though, in which the share of the non-waste feedstock cost in the total cost approximates 75-90 % of the overall costs (Stacy et al. 2014).
Considering the variability among parameters that affect the three performance metrics, we use Monte Carlo simulation to estimate total energy, GWP, and cost metrics for treating the mixed plastic waste. The mass of each mealworm in the treatment process is assumed to vary according to a normal distribution (80 ± 5 μg) and putative CO_2 emissions for the mixed plastic were estimated as a range based on the digestion data from Brandon et al. (2018), see Figure A.2. A full description of the data sources and assumptions for the LCA/TEA is given in the Appendix, as are statistical data on parameters and how they were fit to probability distributions to run the Monte Carlo simulation analysis of uncertainty and the source code for calculations in MATLAB.

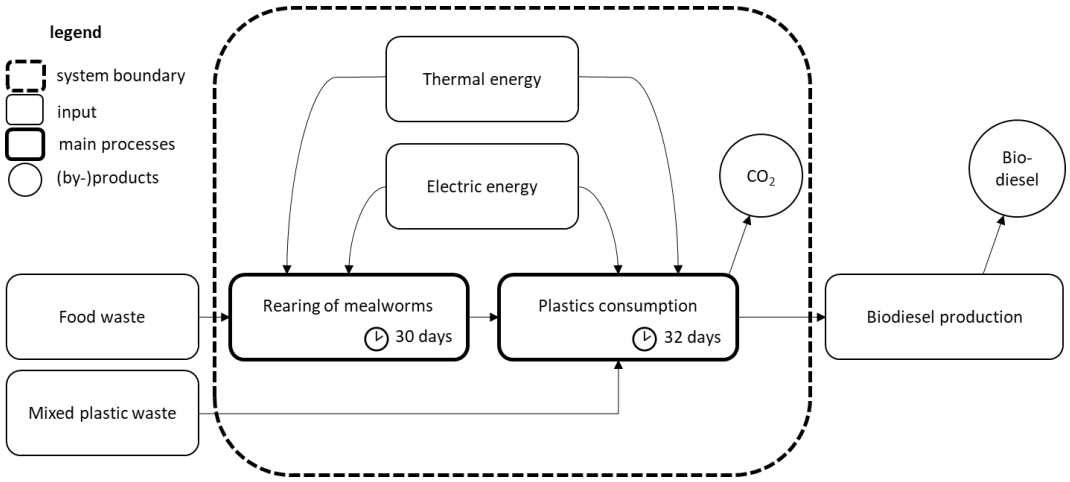


Figure 1. System boundary for cradle-to-gate environmental and economic evaluation of plastic waste treatment. The process inventories thermal and electrical input needs for three stages of treatment; a) rearing the mealworms up to 30 days with food waste; b) feeding the mealworms mixed plastic waste; and c) converting the lipid content of the mealworm to biodiesel at mealworm end-of-life.

Table 2. Scenarios investigated in the ex-ante LCA/TEA

Case	Theoretical (T)	Observed (O)
Scenario Assumptions	Mealworm is assumed to consume plastic to meet its energetic needs according to data from Thévenot et al.(2018); the digested plastic is assumed to be fully utilized.	Mealworm diet is modeled from empirical observations of plastic consumed from Brandon et al. (2018)
Energy	Electrical and thermal energy input needed to rear mealworms and treat mixed plastic waste were 55 mWh and 0.24 Wh per mealworm, respectively.	
Cost	Cost of treating plastic waste consists of natural gas (25 EUR MWh^{-1}) and electricity costs (76 EUR MWh^{-1}) and credit for potential revenue from valorization of co-produced biodiesel (0.5 to 0.7 EUR L^{-1}) from mealworm larvae remains.	
Environmental (GWP)	Estimated greenhouse gases (GHG) emitted from treatment of 1 metric ton plastic waste (kg CO_2-eq. /metric ton) included the GWP of producing and consuming natural gas (0.05594 kg CO_2-eq. MJ^{-1}), electricity (0.0665 kg CO_2-eq. MJ^{-1}), and mealworm digestion of the plastic waste (1320 ± 160 kg CO_2-eq. per metric ton mixed plastic waste). Credit was applied for offset of low S diesel (0.093 kg CO_2-eq. MJ^{-1}).	

3. Results

3.1 Gravimetric and visual observations

Two experiments (**live$_{GWM}$_1** and **live$_{GWM}$_2**) with each time 10 live larvae of the greater wax moth on a loosely folded LDPE film of 0.1535 g and 0.234 g were weighed after 17 h and 89 h, respectively, resulting in a mass loss of 4.2 % and 8.5 %. For reference, the latter mass loss corresponds to an initial rate of 0.54 mg per worm per day. In the substrate cling film about 5 to 10 holes of a few mm diameter were observed. In another experiment (**live$_{GWM}$_3**), 0.189 g of LDPE film was folded in 4 layers and spread out on the bottom of the jar. 10 live larvae were brought in contact with this film for 96 h (4 days). A mass loss of only 1.8 % was measured, likely because the larvae could not easily access the thin LDPE film. Using the fragment of a black commercial shopping bag (**live$_{GWM}$_4**), also several holes were clearly visible. Similarly to the previous experiment, the substrate film of initially 2.834 g lost only 1.8 % in mass after 9 days. The average consumption rate for these 9 days is 0.38 mg per worm per day. However, in the glass recipient black flakes of plastic smaller than 1 mm were observed (see Figure 2). These flakes were not included in the mass balance, therefore the actual degradation rate of LDPE is certainly smaller than the aforementioned value.

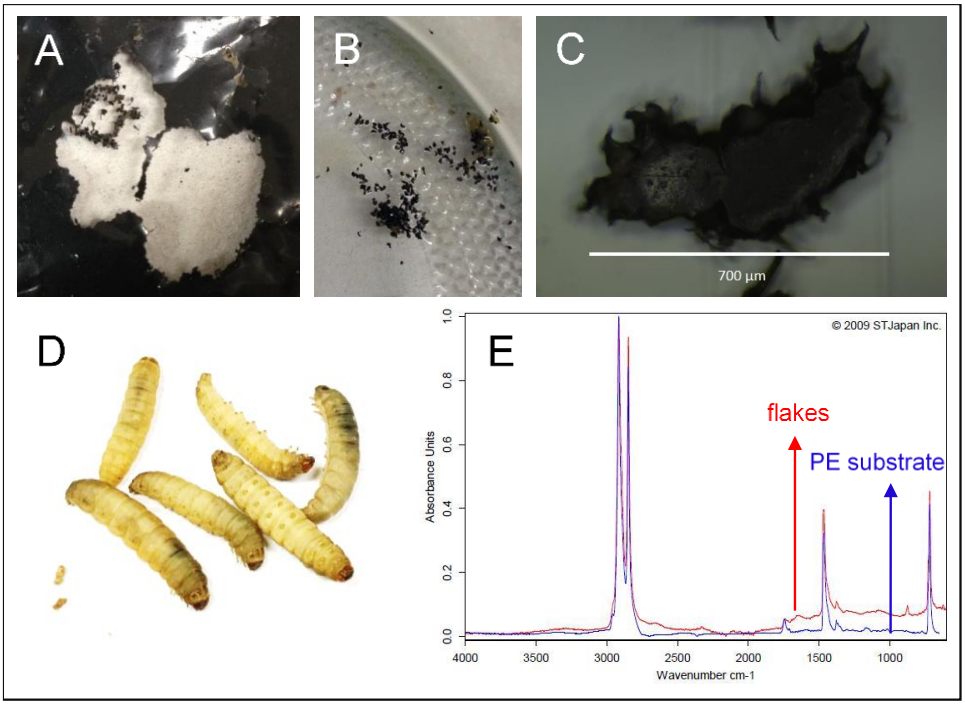


Figure 2. Results of the 9 days interaction of larvae of the greater wax moth with a piece of black low-density polyethylene from a commercial bag (experiment **live$_{GWM}$_4**). (A) one of the holes in the film made by larvae; (B) Photo of the LDPE flakes; (C) microscopic image of one LDPE flake; (D) some of the larvae specimen used; (E) FTIR spectra of virgin LDPE substrate before the interaction, and of the resulting LDPE flakes.

The LDPE substrates after contact with mealworms for 38 days (**live$_{MW}$_1** and **live$_{MW}$_2**) had a mass loss of about 0.8 % and 3.5 % respectively, corresponding to average consumption rates of 0.005 mg per worm per day for **live$_{MW}$_1** and 0.023 mg per day for **live$_{MW}$_2**. Clearly, there is a preference for specific LDPE substrates over other. These experiments are not comparable to the experiments reported by Brandon et al. (2018), since we did not remove dead specimens from the recipients, and the experiment lasted six days longer. In experiment **live$_{MW}$_1** the survival rate was only 78%, with 1 pupa and 2 adult beetles present at the end of the experiment. Additionally, it was clear that the surviving mealworms and adults cannibalized the dead specimens, as was in case in all four live mealworm experiments. The average mass of the surviving mealworms decreased from 93 mg to 75 mg (20%), excluding the pupa and beetles. In experiment **live$_{MW}$_2**, the survival rate was only 47%, with 3 pupa and 4 beetles at the end of the experiment. In this experiment, the average mass of the 40 surviving mealworms, thus excluding pupa and beetles, increased from 93 mg to 102 mg (10%). Together with the low survival rate and comparable PE mass loss, this might indicate a higher rate of cannibalism. For comparison, the mealworms without extra food source (**live$_{MW}$_3**) had a survival rate of 76% (no pupa or beetles) and decreased in mass from an average 91 mg to 79 mg (13%), the mealworms fed with bran had a survival rate of 82% (no pupa or beetles), with an average mass decreasing from 93 mg to 77 mg (17%). From these experiments, it thus seems that the mealworms that were in contact with PE substrate did not have an advantage compared to those fed with bran, or even the ones without any food source at all. It is clear though that, although experiments were not done in duplicate, conversion was less optimal than in the work of Brandon et al. (2018), therefore loose and uncontrolled replication of the technology is not evident given the effect of varying conditions.

However, the mealworms clearly made some holes in the LDPE substrate. Upon microscopic (KEYENCE VHX-6000) inspection of the affected areas of the substrates (Figure 3), edges of holes with apparent chewing patterns were visible. Yet, more interestingly, other holes had a smooth edge, and the surrounding area obtained a more heterogeneous morphology, with zones that seemed to be physically contracted, as opposed to other seemingly more flexible zones. These observations indicate that the degradation action of LDPE by mealworms may be initiated by an excreted material that affects the microstructure of the plastic substrate.

Figure 3. Microscopic imaging of LDPE cling film substrate affected zones from experiment **live$_{MW}$_1**. (A) blank LDPE cling film, (B) a zone with holes and clearly affected morphology, (C) a zone with rough holes.

Various LDPE film substrates were left in contact for different times (24 h and 48 h) at 30 °C with homogenate paste of the greater wax moth larvae. The homogenate was not replaced multiple times, contrary to Bombelli et al. (2017) to limit any chances for mechanical tear of the LDPE substrate, as suggested by Weber et al. (2017). However, this may limit microbial activity (drying of the homogenate), so a lower degradation might be the result. Nevertheless, given the results of Bombelli et al. (2017), a mass loss of approximately 2 % (1/7 of 13 %, as this resulted from 7 homogenate replacements) should be achieved at minimum. Nevertheless, after 48 h and subsequent washing/drying, the **paste$_{GWM}$_1** experiment resulted in no detectable mass loss. Similarly, experiment **paste$_{GWM}$_2**, in controlled oxygen and 100% relative humidity environment, did not result in observable mass loss after 20 h. Therefore, it appears that either the microbiome of the gut of the greater wax moth specimens was different from that of those used by Bombelli et al. (2017), or the mass losses earlier detected were indeed a result of mechanical wear, instead of actual degradation, as suggested by Weber et al. (2017).

3.2 Infrared spectroscopy analysis

Figure 4 gives an overview of various FTIR spectra taken from experiments **paste$_{GWM}$_1**, **paste$_{GWM}$_2** and **paste$_{GWM}$_3**. When subjecting an LDPE cling film to ATR-FTIR, only very small differences with the spectrum of virgin LDPE film can be observed, which are situated mainly at wavenumbers ranging 1550 to 1700 cm^{-1} (Figure 4C). However, given the gravimetric results above, large variations were not expected. Nonetheless, this area is of interest given the potential contributions of carbonyl and carboxylic groups to the absorbance, indicating a potential biochemical oxidation of polyethylene, albeit very slightly. Yet, we cannot exclude ingression/diffusion of homogenate paste biomolecules (e.g. lipids or free fatty acids) into the polymer, giving rise to the respective absorbance. It can be seen from both the unwashed film after contact with paste (Figure 4A) and the blank (Figure 4B) and removed homogenate paste (Figure 4D) that the biomass itself indeed has a strong absorbance at 1550 to 1700 cm^{-1}. Moreover, with the use of proper blanks and standards, Figure 4 demonstrates that no detectable concentration of ethylene glycol is present. This is contrast to the findings of Cassone et al. (2020), who found glycol-like moieties in excreta, but it may suggest that macro-organisms are required for such conversion. More likely, the findings of Bombelli et al. (2017) may indeed be caused by traces of biological material itself on the plastic films, as suggested by Weber et al. (2017).

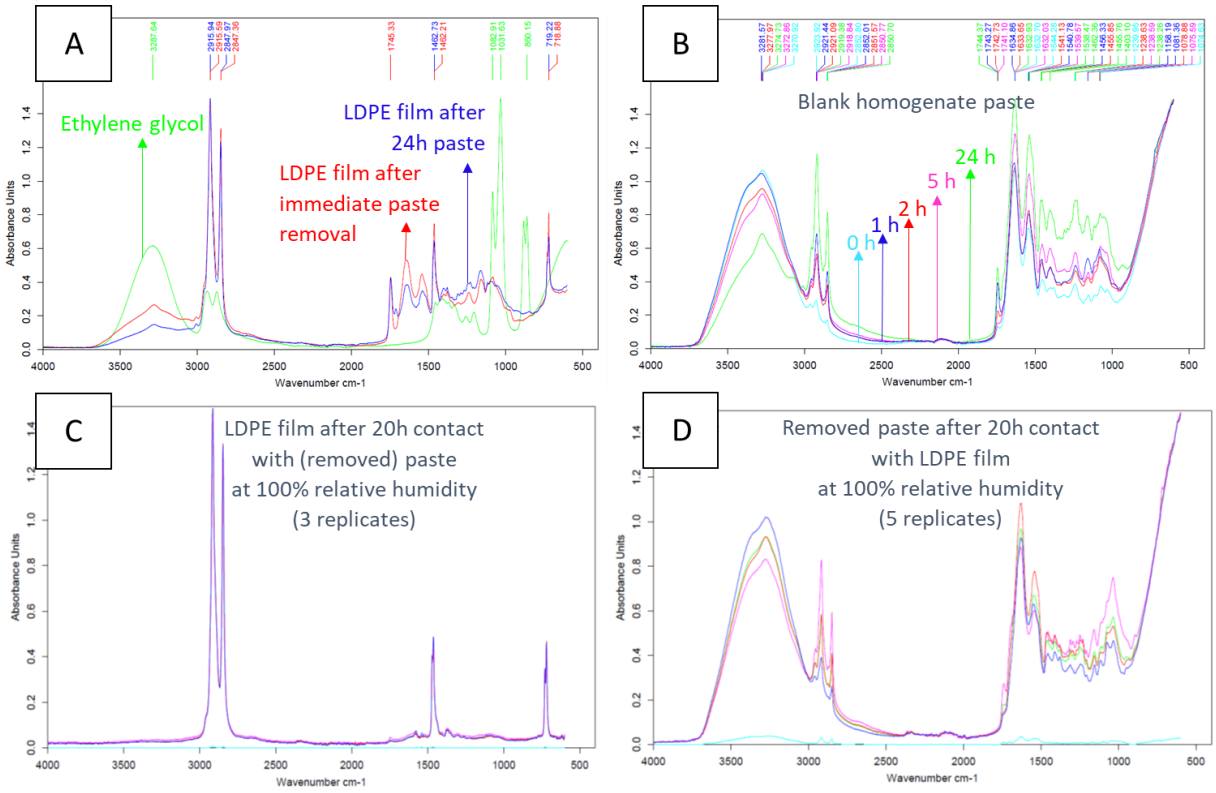


Figure 4. FTIR spectra of LDPE film after 24 h of contact with homogenate paste, with ethylene glycol shown for comparison (A), blank homogenate paste at various time intervals (B), LDPE film after 20 h of contact with homogenate in 100% relative humidity, after washing with acetonitrile (C, experiment **paste$_{GWM_2}$**, sample analyzed 3 times), and the homogenate paste removed from the LDPE film at the end of the 100% relative humidity experiment (D, experiment **paste$_{GWM_2}$**, sample analyzed 5 times). No ethylene glycol is detected in the samples.

Analysis of the flakes isolated from experiment **live$_{GWM}$_4** (Figure 2E), using a black shopping bag fragment as substrate, showed only very little indication of potential biochemical degradation, around wavenumber 1650 cm^{-1}. However, here also the observed absorbance can be due to traces of biological material on the LDPE flakes. Analysis of fecal matter (not shown) has shown clearly the presence of LDPE therein as well, supporting one of the observations of Brandon et al. (2018), that LDPE is only partially digested at most. We did not perform high-temperature gel permeation chromatography to check potential changes in molecular mass distributions though.

The direct contact between homogenate pastes and paraffin (**paste$_{MW}$_paraffin** and **paste$_{GWM}$_paraffin**) as well as polystyrene (**paste$_{MW}$_PS** and **paste$_{GWM}$_PS**) did not yield conclusive results. Any peaks present in the infrared spectra (Figure A1 in Appendix) in the region of 1750-1600 cm^{-1}, which could potentially be due to carbonyls resulting from biodegradation, might be attributed to changes in the blank homogenate paste (likely degradation of the biomass itself) or to residual traces of THF instead.

3.3 Streamlined LCA/TEA: ideal systems baseline

We provide a first outlook of the technology potential of the new findings by using LCA/TEA. The scope and system boundary (Figure 1) are related to the rearing of larvae on food waste for their first 30 days, after which they consume plastics – PE or PS or combinations thereof – for an additional 32 days. All of this time, a habitable environment should be created for the larvae, leading to a consumption of energy in the form of electricity as well as natural gas, as shown by the work of Oonincx and de Boer (2012). As shown in Table 2, we consider both an "observed" scenario (O), using data on plastics consumption from Brandon et al. (2018), as well as a "theoretical" scenario (T), considering a normal energetic nutritional intake translated to a

maximum amount of plastics digested. In the observed scenarios of Brandon et al. (2018), we can see an average plastics consumption of about 25 mg per 100 mealworms per day. Taking into account the calorific value of PE of 44.60 MJ/kg (Walters et al. 2000), this corresponds to an energy uptake of approximately 11 J per mealworm per day. On the other hand, Thévenot et al. (2018) reported an energy intake of 2529 kcal per kg of mealworms over a period of 11 to 13 weeks, when reared on a diet of wheat bran, cereal flours, meals and sugar beet pulp. Taking into account the average mass of mealworms of 80 mg each (Brandon et al. 2018), the energy uptake also equals approximately 10 J per mealworm per day. Therefore, theoretically, mealworms would only require 0.22 mg of PE each day to fulfil their nutritional energy demand. Yet, it was reported (Brandon et al. 2018) that the mealworms do not grow on a diet of plastics. In contrast, they tend to lose weight, as was shown in our own experiments as well (see section 3.1). Considering the indirect mass balance of Brandon et al. (2018), it appears that on average only 41 % of the plastics seems to be digested, which is clearly in line with the energetic uptake as explained here.

The results of the economic analysis, shown in Figure 5A, are further disaggregated into the rearing and plastics digestion phase (i.e. treatment), the valorization of larvae biomass into biodiesel and the final, net resulting cost of the overall treatment (which is a sum of the treatment and valorization). Keeping in mind that the overall aim and thus functional unit is the treatment of one metric ton of PE, and only an economic benefit is attributed to biodiesel, without considering the cost of this valorization. Nevertheless, the results clearly show that even the observed plastics degradation rates are too little to overcome the high operational cost of energy. The total costs of this energy would well exceed 1000 EUR per tonne of PE. Even a revenue from biodiesel, not considering the costs of extraction and chemical conversion, are insufficient to obtain a viable technological route for plastic waste management. In the very best case we could imagine (theoretical net cost and impact), whereby larvae were bred on a zero-burden food waste, consuming what we consider the theoretical maximum amount of plastic, with valorization of the biomass as biodiesel (without considering costs of conversion), and without labor, logistics or capital costs, the net treatment cost would still very likely exceed 300 EUR per tonne, which already is much higher than the gate fee for Waste-to-Energy plants. Moreover, collected plastic waste fractions could also be processed to refuse derived fuels, having an even higher market value.

The carbon footprint of the waste treatment process is likewise very high (Figure 5B), between 4 and 8 tonnes of CO_2-eq. per tonne of plastic treated, for the theoretical and observed scenario, respectively, with an interquartile range of about 1 tonne. This is not surprising, as the only offset CO_2 emissions are from the limited amount of biodiesel that may be produced. The dominant share of the impact comes from energy sources, as shown in the adjusted Figure A.3 in the Appendix. In essence, if larvae of any kind would be able to grow themselves by the energetic uptake from plastics, which is demonstrated by Brandon et al. (2018) not to be evident, still this would be a very circumstantial way of energy recovery, rather than taking more direct routes.

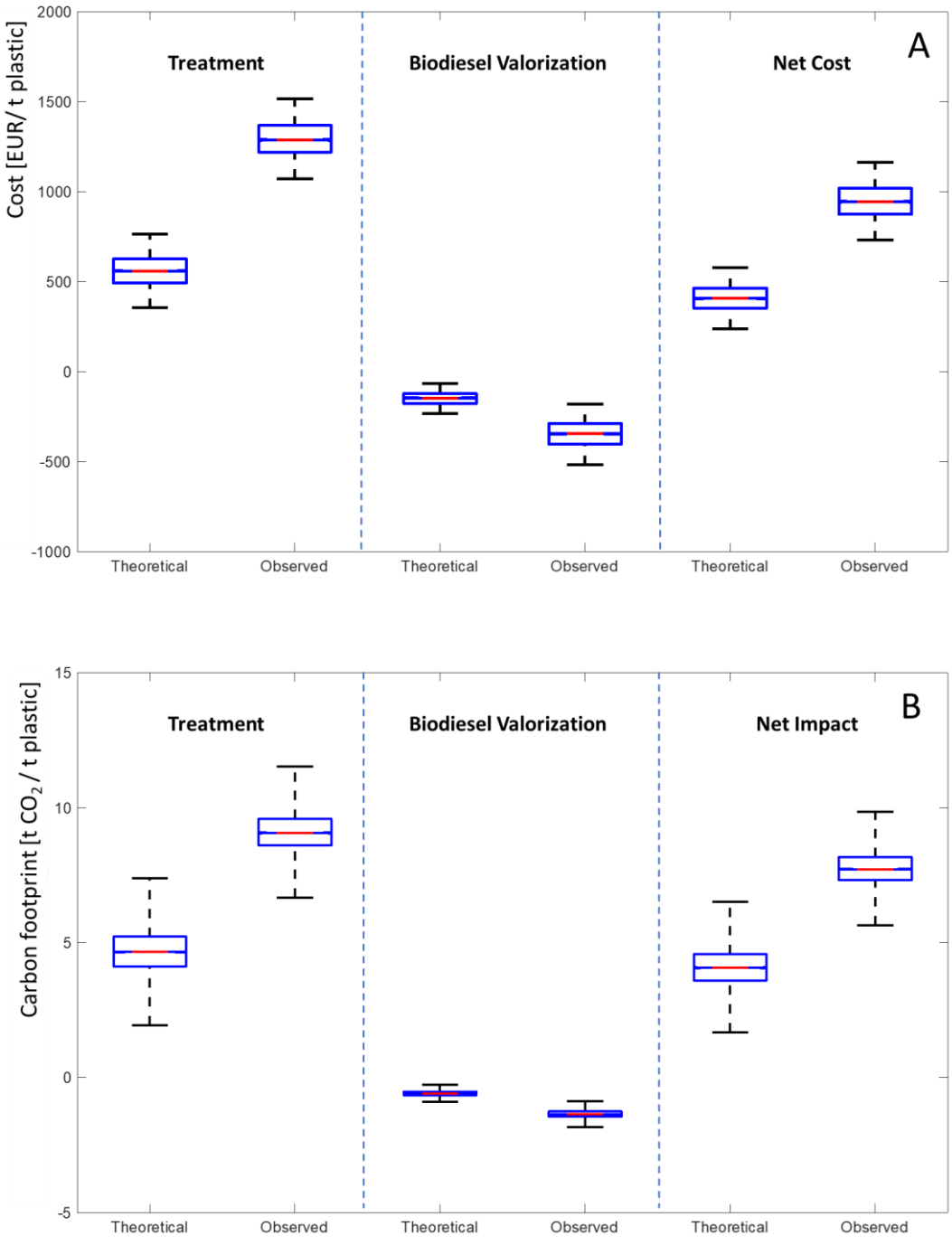


Figure 5. Treatment cost (A) and carbon footprint (B) of the treatment of one tonne of PE waste by mealworms, disaggregated into treatment costs or impact, valorization revenues or credits and the net results (which the sum of treatment and valorization), both for a theoretical and observed scenario. The boxplots represent the medians, lower and upper quartiles, and minima and maxima.

4. Discussion

In this work, we have shown that the degradation of polyethylene, in the sense of chewing and ingesting or creation of holes, by both living specimens of the greater wax moth larvae (*Galleria mellonella*) and of mealworms (*Tenebrio molitor*) takes place at somewhat similar rates than the one reported by Brandon et al. (2018). However, a large variation is reported, and certainly not all of the chewed LDPE is digested. Low density polyethylene was found both in excreted matter, and in separate, rather clean flakes. Our comparison to a theoretical energy uptake of mealworms shows additional insights to the mass balance; This might be attributed to prior treatment of the used specimens with antibiotics, affecting the behavior as shown by Yang et al. (2019), by a different microbiome of the organisms, or by slightly different experimental conditions. Nonetheless, our results question the ubiquity of observations in earlier published work, as suggested recently by Cassone et al. (2020). In contrast with the findings of Bombelli et al. (2017), the degradation of the LDPE films by a homogenate paste only was observed to be negligible in short time frames. Supporting the suggestions of Weber et al. (2017), we argue that earlier rapid degradation rates may be due to mechanical wear, and we have not identified significant traces of ethylene glycol in either the homogenate paste, the surface of the treated film or the excrements, when compared to proper blank samples. Yang et al. (2014) discovered that such degradation is also achieved by bacteria isolated from the gut of waxworms, yet the degradation rates are much lower than those reported by Bombelli et al. (2017).

The highest rate of reduction of the mass of LDPE film, in terms of chewed mass (holes), was observed for wax moth larvae - 0.54 mg per worm per day - and was calculated based on the initial 89 h only. This value is higher than the upper value reported by Brandon et al. (2018) for mealworms. The fact that the values provided by Brandon et al. (2018) are consistent with the theoretical maximum consumption may provide additional mechanistic insight, and does not seem coincidental. The small flakes of LDPE identified are from an environmental point of view very important; they impede the downstream beneficial use of the residues in case of a waste treatment based on digestion by worms, and additionally constitute a risk of spreading microplastics if these findings would be employed as a remediation strategy.

It appears that the consumption of PE serves for life subsistence, rather than for growth, at best. Therefore, mealworms or wax moth larvae have to be reared first, and can consecutively be employed for PE degradation. Even if the theoretically maximum complete degradation rate of approximately 0.22 mg of PE per worm per day would be reached, maintaining the living conditions for the 4 (best case) to over 10 tonnes of worms required for treatment of 1 tonne of PE would require excessive amounts of energy; therefore *such waste management system does not seem to be techno-economically feasible* nor will it in the near future. Integration with the treatment of food waste is complicated by the excretion of non-digested microplastics, therefore entering the food or feed chain should be restricted. Moreover, it is doubtful whether at any point in the future legislators will allow insect species grown on waste to enter the food chain, given the associated risks for public health. The options for the residues of such treatment are thus limited; likely they are to be incinerated with energy recovery. Even in the case of biodiesel production from the larvae, where we for the sake of simplicity omitted the impact of conversion operations, environmental or economic benefits should not be attributed to the plastics conversion, as larvae on a diet of plastics only do not gain mass. In that respect, the yield of lipids, as a precursor for biodiesel, is not caused by the plastics, but rather to the other parts of the diet of the larvae. The extraction or upgrading of lipids from larvae to building blocks other than biodiesel is unreported thus far, but may be explored in the future.

From a technological point of view, far more interesting is unravelling the degradation mechanisms that take place, from which knowledge can be transferred to other waste management technologies and beyond. For instance, composting/digestion installations could be inoculated with microbial cultures potentially capable of degrading plastics, to mitigate plastics anomalies present in e.g. food waste. Additionally, if microbiological (or even enzymatic) activity only would do the degradation, also targeted functionalization of alkane moieties should be feasible, allowing to obtain high-value molecules. The question remains though, whether the functionalization could be done by the microbiological processes only at adequate rates, in absence of live macro-organisms, as this is undemonstrated. However, we have shown evidence of excreted material that alters the morphology of LDPE substrate. An hypothesis is that such excreted material diffuses into the structure and acts as a plasticizer, facilitating subsequent biochemical degradation. This could mean that the degrading action of the micro-organisms in the gut of the larvae is insufficient to achieve the reported conversion rates. This

might also explain why we, nor other reports thus far, were able to reproduce the degradation experiments with using homogenate paste only.

5. Conclusions

Experimentally, we have on the one hand confirmed some of the findings of earlier work, that both larvae of the greater wax moth (*Galleria mellonella*) and mealworms (larvae of *Tenebrio molitor*) chew on polyethylene films and make holes by doing so. Additionally, indications were found that in the immediate surroundings of the holes in the polyethylene films, the morphology of the plastic seems to have changed. Further study is needed, but if confirmed, the mechanism of relatively rapid biodegradation would involve more than the gut microbiome alone. We could not detect any noteworthy changes of the polyethylene film substrates by either gravimetry or infrared spectroscopy after contact with the homogenate biomass paste alone, which might indicate that either the specimens were different to those in earlier work, or in turn confirm that living macro-organisms are needed for the degradation. Nevertheless, also in the experiments with live larvae, the survival rate is low, and the average mass of the specimens decreases, hence these species do not seem capable of growing on a diet of plastics. The incomplete degradation of the plastics was demonstrated by residual small flakes in the recipients – raising concern on microplastics generation – and by the calculation the energetic intake compared to that of a regular diet.

From a techno-economic point of view, the observation that larvae chew, ingest and potentially biodegrade plastics has little value related to the development of dedicated plastic waste management strategies. Raising the larvae is too slow and too costly, especially with regard to the little potential value that is created. Predominantly the larvae, requiring energy for habitable conditions, convert plastics into CO_2, with no added value. If conversion to glycol-like moieties would be further corroborated though, the results of our study would change. Alternatively, deploying these species as an environmental remediation strategy is unlikely, as other feed sources are preferred. Additionally, the larvae could convert macroplastics to microplastics in the environment, which is problematic and studies to date do not exclude the possibility of this unintended consequence.
Interesting though is the scientific unraveling of the exact degradation mechanisms, so that they could potentially be used in the development in new, green methods for the targeted functionalization of alkane moieties, or for advanced mitigation of plastics present in food waste during composting or digesting. This would require though that the fast degradation as reported can be attribution to a single micro-organism or enzyme, or a stable microbiological culture. However, it would be rather problematic for technological prospects of this kind if the involvement of macro-organisms such as these larvae is required for the observed high rates of biodegradation.

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