Biodegradation of polyethylene by macro-organisms such as mealworms and greater wax moth larvae: technological application potential

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Abstract

Purpose. 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 additionally explore the technological prospects of potential future optimized biodegradation.
Methods. 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 modi, and performed gravimetric, microscopic and spectroscopic analyses. Furthermore, streamlined LCA/TEA calculations were done to evaluate the technological potential.
Results. Live larvae showed a preference for specific substrates, yet we argue by comparison to other food sources 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. Finally, there are indications that liquid alkanes are functionalized by the homogenate paste after two weeks.
Conclusions. 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 (790-1016 EUR/tonne). However, the conversion mechanism should be further elucidated for bio-functionalization of liquid alkanes as high-value application.

Keywords

Biodegradation; Larvae; Plastics; LCA; TEA

Introduction

Plastic pollution increasingly gains worldwide attention, 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 insufficiently to overcome littering. Even the biochemical degradation of polyhydroxyalkanoates (PHA), polylactic acid (PLA) or polycaprolactone (PCL) often requires specific conditions (e.g. elevated temperature), and targeted waste management schemes. Moreover, polyethylene terephthalate (PET), the most widely used polycondensate globally, is almost unsusceptible to biodegradation; only recently improved degradation was demonstrated by an engineered aromatic polyesterase [1]. 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. More general, 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 [2].

The news about accelerated biodegradation of polyethylene by larvae of the greater wax moth *Galleria mellonella*, as reported by Bombelli et al. [3], justifiably caused worldwide excitement [4]. This finding may 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. [5] pointed out in a correspondence that some of the results of Bombelli et al. [3] 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. [6] published similar work, yet with 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. They did show however the degradation of PE and PS by a shift in molecular weights determined by high-temperature gel permeation chromatography [6].

In this paper, we improved the analytical procedures of Bombelli et al. [3] by adding several blank samples to the infrared analyses, and an additional set of experiments gave more insights in the ubiquity of the observed phenomena as well as to the technological application potential. We hypothesize based on the cited literature above, that some extent of 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 to more mobile alkane moieties. In addition, we show by a streamlined LCA/TEA approach that in the foreseeable future, the potential of these findings for application as waste management option is very low. Yet, more promising would be the identification and use of microbial enzymes that may functionalize alkane moieties in general.

Materials and Methods

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. The polyethylene substrate used was commercial household low-density polyethylene (LDPE) film, marketed and sold by Carrefour supermarkets.
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

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**

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 0.1535 g, resulting and weighted after 17 h (experiment **live$_{GWM}$_1**). Similarly, a 0.2343 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 0.1887 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 2.8339, 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 (Bruker Lumos). 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. The substrates were afterwards washed with acetonitrile and dried in ambient conditions.

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 0.0648 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 197.2 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. 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 (Bruker LUMOS), 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. FTIR scans were run between 700 and 4000 cm^{-1}. For each sample the background was corrected by taking the average out of 5 or 6 measurements, depending on the sample. FTIR-microscopy 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).

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, all samples were immediately analyzed using ATR-FTIR.

Streamlined LCA/TEA

A streamlined LCA/TEA approach was followed for technological evaluation of these fundamental scientific findings, given the small number of data available and the large uncertainties accompanied with the recent findings. 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 toward mechanistic insights based on theoretical values. Because of the data availability, the discussion about 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). From Brandon et al. [6], we used the mass of individual mealworms as 75 to 85 mg (average 80 mg) per larva specimen (which closely matches the mealworms used in our experiments, which were about 90 mg per larva). Additionally, we used the full range of polyethylene consumption rates as presented by Brandon et al. [6], covering various environmental (food availability) conditions; 10 to 35 mg of PE per 100 mealworms per day. They demonstrated that the PE is converted for 49 % to CO_2, the other half ends up in fecal matter. We benchmarked the calorific intake of PE with that of mealworms bred using grains obtained from Thévenot et al. [7], to determine the theoretical maximum of plastic conversion. The amount of energy consumed to maintain a constant temperature is obtained from Oonincx and de Boer [8]. Overall, we anticipate, as shown in various literature sources, an optimal lifespan for the mealworms of approximately 60 to 70 days. By linearly interpolating the data of Zheng et al. (mealworms grow in 76 days from 1.7 mg to 176 mg), we estimate that the worms employed to degrade PE, weighing 80 mg, were grown already for 35 days [9]. From a technological and environmental point of view, it makes sense to grow the larvae on food waste. Therefore, we accounted only for the energy of maintaining a constant temperature to this process, taking a zero-burden approach for food waste, which is

completely biogenic in nature. After being bred for 35 days, we assume the worms were in contact with PE for another 32 days. The functional unit of this ideal systems baseline LCA/TEA is the complete consumption of 1 tonne of PE film (without additional food) by 35-day old mealworms in an additional 32 days, resulting in 49% conversion to CO_2 and 51% excretion in fecal matter.

Results

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 0.2343 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**), 0.1887 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 2.8339 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 1). These flakes were not included in the mass balance, therefore the actual degradation rate of LDPE is certainly smaller than the aforementioned value.

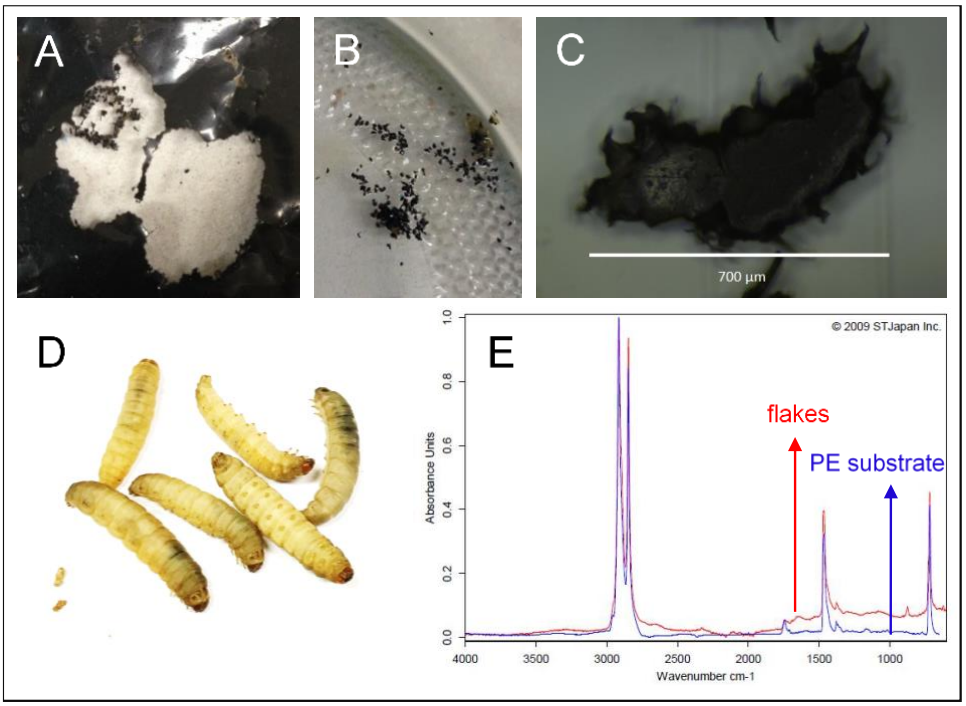

Figure 1. Results of the 9 days interaction of larvae of the greater wax moth with a piece of black low-density polyethylene from a commercial bag (experiment **live$_{GWM}$_4**). (A) one of the holes in the film made by larvae; (B) Photo of the LDPE flakes; (C) microscopic image of one LDPE flake; (D) some of the larvae specimen used; (E) FTIR spectrum 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 0.77 % 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.

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 2), 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.

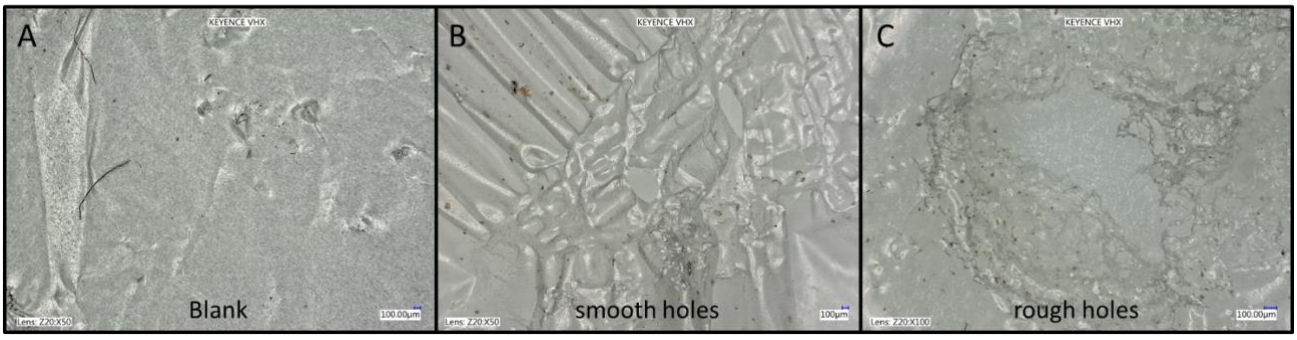

Figure 2. Microscopic imaging of LDPE cling film substrate affected zones from experiment **live$_{MW}$_1**. (A) blank LDPE cling film, (B) a zone with holes and clearly affected morphology, (C) a zone with rough holes.

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. [3] to limit any chances for mechanical tear of the LDPE substrate, as suggested by Weber et al. [5]. 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. [3], 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. [3], or the mass losses earlier detected were indeed a result of mechanical wear, instead of actual degradation, as suggested by Weber et al. [5].

Infrared spectroscopy analysis

Figure 3 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 3C). 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 3A) and the blank (Figure 3B) and removed homogenate paste (Figure 3D) 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 3 demonstrates that no significant concentration of ethylene glycol is present. More likely, the findings of Bombelli et al. [3] may indeed by caused by traces of biological material itself on the plastic films, as suggested by Weber et al. [5].

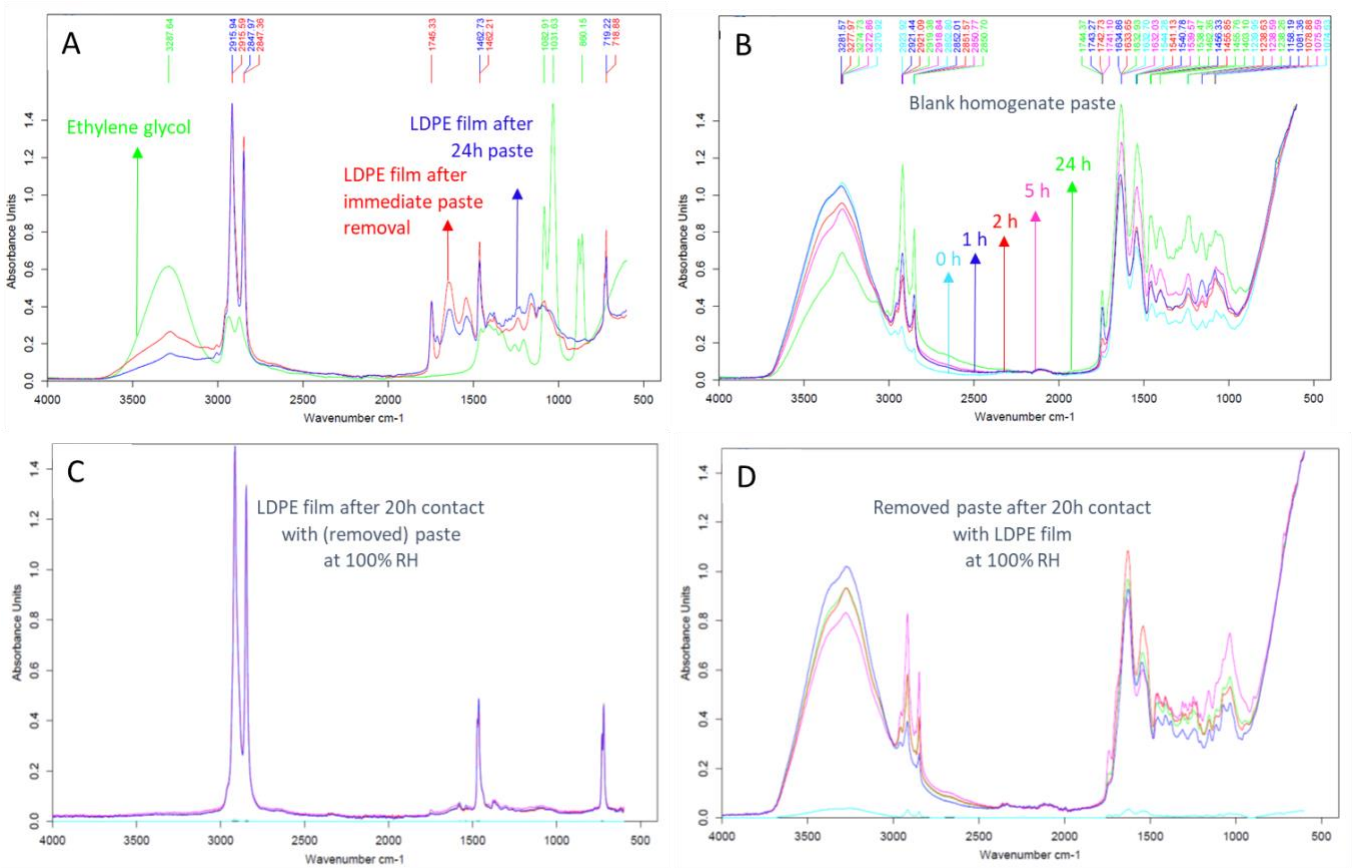

Figure 3. FTIR spectra of LDPE film after 24 h of contact with homogenate paste, with ethylene glycol shown for comparison (A), blank homogenate paste at various time intervals (B), LDPE film after 20 h of contact with homogenate in 100% RH, after washing with acetonitrile (C, experiment **paste$_{GWM}$_2**, sample analyzed times), and the homogenate paste removed from the LDPE film at the end of the 100% RH experiment (D, experiment **paste$_{GWM}$_2**, sample analyzed times). No ethylene glycol is detected in significant amounts in the samples.

Analysis of the flakes isolated from experiment **live$_{GWM}$_4** (Figure 1E), 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. [6], that LDPE is only partially digested at most.

Promising are the findings of Figure 4, giving the FTIR spectra of the four last experiments in Table 1 (**paste$_{GWM}$_paraffin**, **paste$_{GWM}$_PS**, **paste$_{MW}$_paraffin** and **paste$_{MW}$_PS**), as well as of substrate and homogenate paste blanks. In all four experiments, around 1750 cm^{-1}, distinguishable absorbance peaks appear in the treated mixture, that do not origin from the blank substrate, nor from the blank homogenate paste that underwent the same treatment. Not only does it confirms earlier findings [6,10,11], a better mechanistic understanding with identification of the responsible microorganisms and/or enzymes, may unlock the application domain of biochemical alkane functionalization.

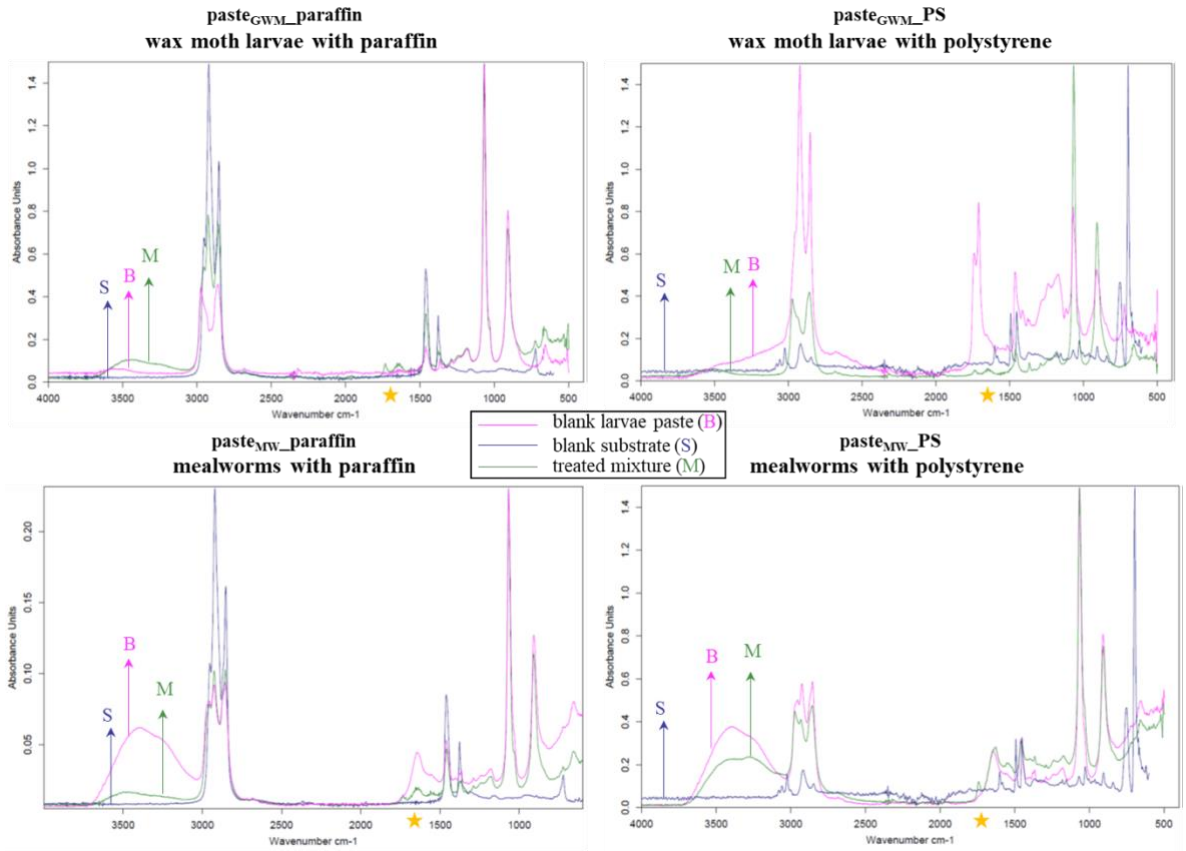

Figure 4. FTIR spectra of homogenate paste of greater wax moth (top) and mealworm (bottom) homogenate paste mixed with paraffin (left) and polystyrene (right), and blank homogenate paste after two weeks at 100% RH, together with spectra of blank paraffin and polystyrene substrate.

Streamlined LCA/TEA: ideal systems baseline

The energetic uptake through grain based nutrition for mealworms reared for proteins, is about 2529 kcal (10 581 kJ) per kg of worms over a period of 11 to 13 weeks [7]. Averaged (12 weeks) this means about 126 kJ per kg of worms per day, or approximately 9.5 J to 11 J per worm per day, assuming an average mass of the worms of 75 mg to 85 mg at the start of plastics consumption after 32 days [6]. From that moment onwards, we assume that the mealworms do not gain mass as a result of plastics consumption, in line with own findings and those of Brandon et al. [6]. In case the worms are fed with food waste on top, no additional environmental or economic impact is accounted for. The consumption of plastics (PE) ranges between 5 and 35 mg per 100 worms per day (equaling a potential energetic uptake between 215 J and 1505 J per 100 worms, or between 2 J and 15 J per worm) [6]. It was shown that of the consumed PE, 50% ends up in the fecal matter [6], therefore limiting the actual energy uptake to a theoretical 1 J to 7.5 J per worm per day. This value is still a theoretical maximum, as the mass balance of Brandon et al. [6] was not completely closed by measurements; CO_2 emissions were deduced by calculations, and the biological material (the bodies of the worms at the end of the experiment) were not analyzed for traces of plastic fragments or converted moieties. This low energetic value, together with the strong retardation of growth (the mealworms did not gain significant body mass, if any, during the experiments), demonstrate that the nutritional value of PE is insufficient for mealworms. However, assuming an optimal growth rate when fed with grains, we can assume the energetic uptake from Thévenot et al. [7] as a theoretical maximum, which leads to a maximum of 9.4 J, and hence 0.22 mg PE per worm per day. Since Brandon et al. [6] have estimated PE digestion at 49 % by mass, their observed upper limit of PE consumption, about 0.35 mg per worm per day, is only slightly lower than the theoretical maximum which would be 0.45 mg per worm per day assuming 49% digestion. This may support the observations of Brandon et al. [6] regarding the digestion of PE.

Assuming a fair digestion of PE by the mealworms, the highest consumption rate (0.35 mg per worm per day) reported by Brandon et al. [6], or the aforementioned postulated theoretical maximum of 0.45 mg per worm per day, the digestion over a 32-day timespan is still only 14 % to 18 % of the mealworms' body mass. Therefore, to treat 1 tonne of PE waste, between 5.5 and 7.1 tonnes of reared mealworms are required.
Oonincx and de Boer [8] estimated the energy consumption of rearing mealworms for protein production, predominantly to yield a climate controlled, ventilated station in the Netherlands. Converted, they estimated a consumption of 55 mWh of grid electricity per worm, 0.24 Wh of natural gas per worm and 0.22 L of water per worm. This means that for 1 tonne

of PE, between 3.8 MWh and 4.9 MWh of electricity are required, and 17 MWh to 21 MWh of natural gas. Given the current price of electricity for large consumers of about 76 EUR/MWh, treating 1 tonne of PE waste, for which 5.5 to 7.1 tonnes of mealworms are required for a 67-day period (35 days rearing for subsequently 32 days of PE digestion), the cost of electricity alone for this treatment would be 290 EUR to 370 EUR, which is much higher than most other waste treatment technologies. Additionally, assuming a natural gas price for non-household consumers of 30 EUR/MWh, the cost of the treatment increases with another 500 EUR to 642 EUR. Combined, the cost of the energy alone for the proposed treatment of PE by mealworms is between 790 EUR and 1016 EUR per tonne. The cost of land, capital goods, labor and various upstream processes (e.g. waste sorting) are not included.

As products of the treatment, about 1.54 tonnes of emitted CO_2 (49 % of PE is converted [6]) are emitted and 0.51 tonnes of PE remains in the fecal matter. It appears based on the available data ([6], this paper) that PE is consumed for maintenance, not actually for growth, meaning that no carbon is stored in biomass. However, the mealworms themselves can be valorized as biodiesel [9]. It was reported that 34.2 g of biodiesel can be produced from 701 g of fresh mealworms of 176 mg individual mass [9]. Therefore, the 5.5 to 7.1 tonnes of mealworms used for treating 1 tonne of PE could yield 0.27 to 0.35 tonnes of biodiesel, although the lipid content in mealworms fed with PE is expected to be lower. It is clear that the production price of diesel, 0.5 to 0.7 EUR/L, yielding 162 to 294 EUR revenue from biodiesel per tonne of PE treated, does not suffice to overcome the high energy cost.

From an environmental perspective, it is very unclear, and at this point rather unlikely, that PE is effectively converted into biomass, and more specifically lipids, that may be converted to biodiesel. It seems thus far that if PE is consumed, the energetic intake is barely sufficient to sustain the organism. Therefore, the products from such treatment that can be directly attributed to PE are effectively limited to CO_2, and excreted plastics. Therefore, no environmental benefit can be identified when benchmarked against other degradation phenomena, or against incineration.

Discussion and conclusions

In this work, we have shown that the degradation of polyethylene 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. LDPE was found both in excreted matter, and in separate, rather clean flakes. The highest rate of reduction of the mass of LDPE film 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. [6] for mealworms. The fact that the values provided by Brandon et al. [6] 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.

In contrast with the findings of Bombelli et al. [3], 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. [5], 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.

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 degradation rate of approximately 0.45 mg of PE per worm per day would be reached, maintaining the living conditions for the 5.5 to 7.1 tonnes of worms required for 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. The options for the residues of such treatment are thus limited; likely they are to be incinerated with energy recovery.

From a technological point of view, far more interesting is unraveling the degradation mechanisms that take place, from which knowledge can be transferred to domains other than managing plastic waste. On the one hand, we have shown in this paper indications 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. On the other hand, we have shown indications of biochemical oxidation upon prolonged contact of homogenate paste (in other words, the worms' microbiome) with both liquid paraffin and polystyrene. Not only does this confirm that biodegradation takes place to at least some extent, more importantly it indicates that a functionalization of alkane moieties takes place. If the exact oxidation mechanism by either enzymes or microorganisms or a combination thereof is elucidated, high value applications may be developed.

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