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Research paper

Engenieering Technology in Plastic Biodegradation by Large Bee Moth Larvae Depends on the Type of Polyethylene

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Abstract

The objective of this research was to evaluate the processes of biodegradation of different types of polyethylene by *G. mellonella* larvae. The determination of the types of polyethylene samples was carried out by IR Fourier spectrometry. The structure of solid household waste includes polyethylene of different types. When assessing the degree of biodegradation, it was found that holes in the polyethylene samples of different sizes formed 5.3±2.4 - 28.7±9.4 units/day and 54.8±12.6 - 1867.8±38.6 mm^2/day, and the mass of polyethylene samples, depending on the type, decreased on average by 1.5±0.5 - 51.0±6.5 µg/day. The obtained results of survival, mobility, weight gain and melanization indicate the absence of negative health effects on larvae when eating polyethylene.

Keywords: *large bee moth; Galleria mellonella; polyethylene; biodegradation; IR Fourier spectroscopy.*

1. Introduction

One of the most urgent environmental problems in the modern world is the growth of production and consumption waste: up to 98% of the materials involved in industrial production become waste. The growth of consumer goods is accompanied by an increase in the amount of household waste that has a negative impact on the environment and organisms [1, 2]. According to the UN report 2015, about two billion tons of solid domestic waste is generated on our planet annually. Polymers occupy a relatively small place in the structure of solid domestic waste, but it is plastics based on synthetic polymers that create global environmental problems caused by their stability and prolonged decomposition in the environment [3].
Polyethylene, used for the production of packages, bags, films, is a common type of solid domestic waste, presented in two forms:
- high density polyethylene, or low-pressure polyethylene (HDPE),
- low density polyethylene, or high-pressure polyethylene (LDPE).

The main ways to recycle solid household waste are to be buried in specially equipped landfills and incineration. Wastes of polymers, including packaging materials, are either buried in the ground or recycled by one of the methods: burning, pyrolysis, hydrolysis, glycolysis, methanolysis, recycling, and also by a relatively new one - biodegradation. More and more attention is given to new approaches to waste disposal using biological methods for their degradation, including the use of bacteria [4, 5]. It used to be believed that polyethylene does not lend itself to biodegradation, since it does not occur in nature. In recent years, more publications have appeared about organisms that have the ability to process, assimilate various types of polyethylene [6]. Japanese biologists have discovered a strain of bacteria capable of processing polyethylene [7]. They managed to isolate the bacterial strain *Ideonella sakaiensis* 201-F6, which hydrolyses polyethylene with the help of special enzymes. Bacteria can process a thin (0.2 mm) polyethylene film in six weeks at 30°C. Organisms not only destroy the polymer, but also use it to generate energy. Bacteria hydrolyse the polymer with enzymes - resulting in the formation of terephthalic acid and ethylene glycol, further transformations of which are well described. It was found that the mold fungus *Penicillium simplicissimum* is capable of partially utilizing polyethylene preliminarily treated with nitric acid in three months [8]. With the help of fluorescence and scanning microscopy, it was found that the growth of the bacterium *Nocardia asteroides* occurs on the polyethylene surface with its degradation for 4-7 months [9]. Inhabitants of the Indian moth's (Plodia interpunctella) intestine can decompose 100 mg of polyethylene in eight weeks [10].
The possibility of using a bee moth (*Galleria mellonella* Linnaeus, 1758) for the biodegradation of polymeric waste presents a new method in the environmental recycling of solid domestic waste. For the first time in 2017, Spanish scientists reported the ability of *G. mellonella* to process polyethylene. Researchers found that 100 larvae in 12 hours processed 92 mg of polyethylene [11]. At the same time, the features of biodegradation of different types of polyethylene by *G. mellonella* larvae have not been fully studied to date.
It is assumed that bee moth larvae were able to process polyethylene because it is similar to their usual food. Hatched larvae of *G. mellonella* feed first with honey, then beeswax and bee bread, consisting of a mixture of organic substances [12]. The composition of the wax includes the limiting hydrocarbons forming carbon bonds C-C, similar to those found in the structure of polyethylene. At the same time, it should be noted that often pathogenic microscopic fungi affecting bee hives can enter their organisms together with wax [13].
Objective of this research is to evaluate the processes of biodegradation by *G. mellonella* larvae depending on the type of polyethylene.

2. Materials and Methods

The object of the study were: various types of polyethylene, found in the structure of municipal solid waste.
The subject of the study was a bee moth (*G. mellonella*) and the ability of its larvae to biodegradation of polyethylene. Cocoons with bee moth pupae (n=200) were obtained from the experimental apiary of the Institute of Ecology and Nature Management of Kazan (Volga region) Federal University. From the pupae under the experimental conditions, adult moths and, subsequently, larvae were obtained.
The types of polyethylene samples were determined on the FT-801 IR Fourier spectrometer (Simex Analytical Equipment, Russia), the results were processed using the Ekoaudit LLC database designed for obtaining, processing and identification of IR spectra.
In the course of the study, a bioreactor was designed to produce bee moth larvae and cages for experimental studies.
Larval health indicators (mobility, melanization, survival) were assessed according to the previously described procedure [14]. The weight of larvae was measured on an analytical scale DV215CD Discovery (Ohaus, Switzerland). To visualize and take into account changes in the larvae's outer covers, a digital USB microscope DigiMicro Prof (DigiMicro, China) was used, with the MicroCapture Pro Version 2.2 software. For microscopic studies, the Biomed-3 microscope (BioMed Service, Russia), the trinocular, with the "Digital Camera for Microscope DCM310 (USB2.0)" imaging adapter and the "ScopePhoto 3.0" software were used.
The degree of biodegradation of polyethylene samples was estimated from the change in their mass (mg), as well as the number (unit) and area (mm^2) of holes formed (points of feeding) on them. Determination of the mass of polyethylene samples was carried out by weighing them on analytical scales. To determine the number and area of holes formed, the sample was scanned on a Laser Jet M1132 MFP (HPInc).
To reveal preferences of bee moth larvae in eating a particular type of polyethylene, the results were statistically processed using a single-factor analysis of variance (ANOVA). Differences between the groups were considered significant at $p<0.05$.

3. Results and Discussion

3.1 The Variety of Types of Polyethylene Samples in the Structure of Municipal Solid Waste

At the first stage of the study, 27 samples of polyethylene (Figure 1), commonly found in the structure of municipal solid waste, were selected. The samples were selected widely represented in the structure of municipal solid waste in the form of containers for various purposes. These are bags, films and other packing material, some of which are bags used in the retail chains "Bahetle", “Magnit", "Edelweis", "Aisha", "Vprok", as well as packages for food and non-food products.

Fig. 1:. Samples of polyethylene used in the study

All the samples of polyethylene were subjected to IR Fourier spectrometry (Fig. 2). The description, characteristics, dimensions and weight of the samples are presented in Table 1. Among the polyethylene samples studied, the greatest amount is represented by polyethylene for food products, 29.62% (8 of 27), 11% are samples for transportation of food products (3 of 27), 11% - an oilcloth package (3 of 27) and 7.4% (2 of 27) for storing paper towels. The remaining samples of polyethylene are presented in a single specimen and amounted to 2.7%.

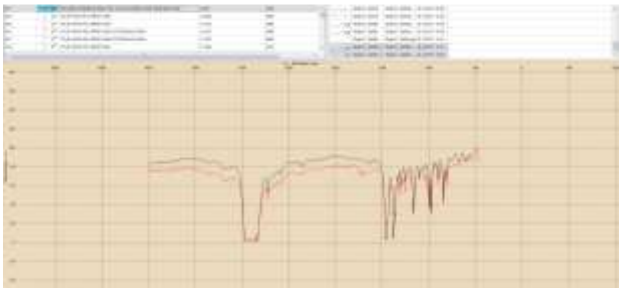

Fig. 2:. IR Fourier spectrometry of a polyethylene sample (example)

Table 1:. Characteristics of the types of polyethylene samples used in the study

sample No. (n – quantity, pc.)	Type of polyethylene by IR spectrometric analysis and the percentage of coincidence	Purpose of use	Total area, cm^2	Total weight, mg
1 (n=1)	64,5% cta 87 fo470 polypropylene	Food storage	1100	5280
2 (n=1)	51,5% cta 87 co475 polypropylene ground cover fill	Food storage	360	1737
3 (n=1)	60% amaco/olefin (amaco of yarn) yel 22.5u polypropylene nbs-ho 256	Food storage	896	4652
4 (n=1)	81,1% phillips/olefin (marvess) tow 18.4u polypropylene 3den nbs-f0249	Paper towel storage	800	3000
5 (n=1)	65,6% parvan 4550, f.n. 3519	Paper towel package	1466	8352
6 (n=1)	48% calcium carbonate, 85% concentrate carbonic acid, calcium salt	Food transportation	3500	6655
7 (n=1)	62,3% unbranched polyethylene, selfrein forced lupolen 6021 d	Transportation of clothes from a dry cleaner	5684	11436
8 (n=1)	62,3% astro turf / polypropylene green / std 416	Shoes transportation	2666	1700
9 (n=1)	68% escorene ld-105	Food transportation	2886	5250
10 (n=1)	41,4% sealite 43, f.n. 3679	Food transportation	3744	16600
11 (n=1)	55,6% baylon	Transportation of various items	2320	17960
12 (n=1)	60,2% petrothene na 143	Household chemicals transportation	3900	9340
13 (n=3)	62,7% cta 87 co473 polypropylene primary carpet backing	Cigarette wrapping film	1619	4280
14 (n=4)	77,9% petrothene lr 734	Food storage	9048	5640
15 (n=1)	62% armodur transpatent	MSW storage and transportation	4602	2310
16 (n=2)	74,9% cta 87 eo 398 olefin (polypropylene)	File for stationery	2852	2790
17 (n=1)	69,7% unbranched polyethylene, selfrein forced lupolen 6021 d	Package of various items	2028	3420

18 (n=5)	71% linear polyethylene hostalen gf 4750	AIRplus air package	2280	5050
19 (n=1)	68,7% vybar 260	Oilcloth package	1872	7790
20 (n=2)	74,7% petrothene lr 734	Food storage	3588	2440
21 (n=1)	64,9% escorene ld 151 cf 1	Oilcloth package	1800	6910
22 (n=5)	74,7% cta 87 ao 461 polyethylene laminated to craft paper	ZIP LOCK package	990	2600
23 (n=1)	77,3% plastic bag	Oilcloth package	1960	11560
24 (n=1)	82,9% cta 87 eo 398 olefin (polypropylene)	Packaging bag	1260	3100
25 (n=1)	73,8% poly(ethylene)	Food storage	768	440
26 (n=1)	73,2% a-c 6	Food storage	672	470
27 (n=6)	54,2% npe 953	Food storage	29760	68100
Total			9.443 m^2	218862

Based on bivariate analysis, multiple pregnancy, menopause, smoking, hyperglycemia, hypertension and hyperlipidemia were more prevalent among cases (P<0.001 for all). However, the results suggested no significant difference in taking OCPs between cases and controls (table 1
The largest number of polyethylene samples also had the largest area and weight. The area of food package polyethylene was 4.6 m^2, and weight 27,459 mg. The area of polyethylene waste for food transportation was 1.013 m^2 and had a mass of 21,850 mg, the area of the oilcloth package was 0.563 m^2 and the weigh – 26,260 mg. It is interesting that some samples of polyethylene with its share of not more than 3% of the total number of packages had a large area and weight. For example, polyethylene for transportation of various items had an area of 0.568 m^2 and weighted 17,960 mg.

3.2 Cultivation of *G. mellonella*

Three bioreactors were designed and installed to grow and maintain a moth culture. The bioreactor is a plastic cube with a lid, with dimensions: height - 31 cm, width - 23 cm, length - 28 cm, volume: 19,964 cm^3 (0.02 m^3). To provide air circulate holes of 10 x 5 cm covered with a metal net with the help of Scotch tape (the mesh cells did not exceed 0.2x0.2 cm in size) were cut out on the lid and on one wall.
In each bioreactor, the feed mixture was added in a proportion of 1:3:3 - bee bread: beeswax: slumgum and 100 cocoons with pupae of *G. mellonella* (Figure 3). Within 52 days, the entire cycle of development of the wax moth passed through the bioreactors. It has been established that *G. mellonella* is successfully cultivated under artificial conditions of the bioreactor in low light, at a temperature of 25 - 30°C and in humidity of 67-80%.

Fig. 3:. Bioreactor with fodder and pupae of *G. mellonella*

3.3 Polyethylene Biodegradation Experiment

One of the above bioreactors was filled with all the polyethylene samples listed in Table 1. The experimental conditions were the same as for the cultivation of a large bee moth. The experiment lasted for 90 days, during which biodegradation of various types of polyethylene was observed. To estimate the extent of biodegradation, the mass of polyethylene samples, the number and the area of the formed holes were measured in the course of the experiment.
The development cycle of *G. mellonella* in the experimental bioreactor continued, similar to that in the bioreactor free of polyethylene. Despite the sufficient amount of food in the bioreactor, large bee moth's larvae also ate polyethylene of all the types presented (Figure 4).

Fig. 4:. Samples of polyethylene with formed holes - the result of a process of biodegradation by *G. mellonella* larvae

When assessing the degree of biodegradation, it was found that holes in the polyethylene samples of different sizes formed 5.3±2.4 - 28.7±9.4 units/day and 54.8±12.6 - 1867.8±38.6 mm2/day, and the mass of polyethylene samples, depending on the type, decreased on average by 1.5±0.5 - 51.0±6.5 µg/day. It should be noted that polyethylene in the body of bee moth larvae decomposes to ethylene glycol [11], which confirms the biodegradation process.

3.4 Assessment of Health Indicators of Larvae and Imagos of *G. Mellonella*

The experiment revealed that 84.8±8.3% of the larvae in the bioreactor with polyethylene remained alive, which does not differ significantly from the indicator in the bioreactor without polyethylene - 85.8±7.9% (p>0.05). Consequently, eating polyethylene does not affect the survival rate of larvae. The average mass of live larvae in the pre-pupal stage was noted at the level of 182.4±26.3 mg and 188.7±28.2 mg, respectively (p>0.05). It follows that polyethylene does not affect the growth rate of larvae. Most individuals from both bioreactors were active without stimulation, single individuals showed activity after stimulation. The processes of melanization of the outer cover of *G. mellonella* larvae were not detected (Fig. 5a). However, larvae with the processes of orange pigmentation (Figure 5b), which persisted in adult individuals were found in the bioreactor with polyethylene (Fig. 5c).

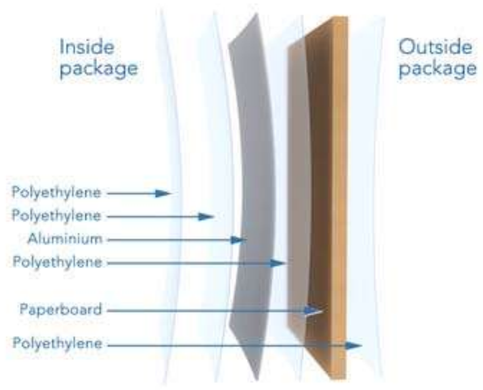

Fig. 5: *G. mellonella* after eating orange polyethylene: larvae without pigmentation (a), larvae (b) and imago (c) with pigmentation

Thus, the conducted experiments indicate that there is no toxic effect of polyethylene on the investigated health indicators of *G. mellonella* larvae. The noted processes of orange pigmentation are most likely due to eating polyethylene with such a dye.

4. Conclusion

Among the polyethylene samples studied in the structure of municipal solid waste, the largest number is represented by polyethylene for food storage (29.6%), for transportation of food products (11.0%), and oilcloth package (11.0%). When assessing the degree of biodegradation, it was found that holes in the polyethylene samples of different sizes formed 5.3±2.4 - 28.7±9.4 units/day and 54.8±12.6 - 1867.8±38.6 mm2/day, and the mass of polyethylene samples, depending on the type, decreased on average by 1.5±0.5 - 51.0±6.5 μg/day. Polyethylene in the body of bee moth larvae decomposes to ethylene glycol, which confirms the biodegradation process. The obtained results of survival, mobility, weight gain and melanization indicate the absence of negative health effects of larvae from eating polyethylene. However, there were noted processes of orange pigmentation, which are most likely due to eating polyethylene with such a dye.

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