LOW DENSITY POLYETHYLENE DEGRADATION BY SOIL MICROORGANISMS

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Introduction

In the Republic of Moldova there is an acute problem of environmental pollution by plastics, such as the low-density polyethylene (LDPE). The share of plastics in the worldwide waste volume is about 10-30%, and the rate of just polyethylene waste accumulation in the environment is 25 million tons/year [1]. From 2019 in the Republic of Moldova it has been mandatory to recycle at least 10% of plastic packaging, and from 2025 this share will increase to 20% [2]. However, there currently are no efficient technologies that could be used for the recycling/processing of low-density polyethylene (LDPE) – the basic material of the most widespread type of packaging, including single-use bags [3]. Although in the Republic of Moldova, according to the legislation [4-5], these bags are to be gradually withdrawn from circulation, nevertheless for a long time LDPE will remain among the main persistent environmental pollutants. Due to the lack of chemical and physical methods for efficient and sustainable degradation of LDPE, in the last decade attention has been directed toward development of microbiological biodegradation technologies [1, 3, 6-11], including with application of nanomaterials [12-13]. These technologies are based on the microorganisms, that can use LDPE as a source of carbon and/or energy [8, 11], and on nanomaterials that can stimulate the LDPE biodegradation. The efficiency of these technologies, among other things, depends on the identification and isolation of these microorganisms, on the elaboration of the most efficient nanomaterials, and on considering all other possible factors that can stimulate the biodegradation of LDPE. Potentially, the LDPE degrading microorganisms can be found in soils with high microbial biodiversity and/or in soils from the territories subjected to long-term pollution by plastics.

The purpose of this work was to estimate the LDPE biodegradation rates and the possibilities of their acceleration in soil from two contrasting territories from the Republic of Moldova – a virgin forest soil, and in a landfill with plastics and other contaminants.

Materials and methods

The LDPE biodegradation experiments were conducted under laboratory conditions and based on soil samples collected from two contrasting territories in the Republic of Moldova. The first soil sample was collected from a forest in the Orhei district. And the second – from a landfill near the locality of Slobozia-Dusca, the Criuleni district. Soil samples were collected in the spring of 2021 from a depth of 0-10 cm; passed through a 2 mm sieve and plant material, stones and visible organisms removed manually; adjusted to 40% water holding capacity and pre-incubated for 10 days at 25ºC in the dark, in aerated plastic bags (to prevent accumulation of CO_2) with periodic adjustment of moisture (to prevent drying). The soil organic matter content (SOM) was determined by dichromate oxidation followed by back titration of the excess dichromate [14]. The soil microbial biomass (SMB) was determined by substrate-induced respiration [15]. The basal respiration (BR) was determined by measuring CO_2 emission from soil by the Li-850 IRGA [16]. Metabolic quotient (qCO_2) was calculated as BR expressed per mg of SMB carbon.

The comparison of the properties of the selected soils indicated the presence of a relatively high ecological stress in the soil of the landfill – the microbial biomass was 27 times lower, and the metabolic coefficient (the indicator of the state of ecological stress for soil microorganisms [17]) was 99 times higher than in the forest soil (tab. 1).

Table 1. The soil parameters

N.	Territory	SOM, %	pH	W, %	SMB, μg C/g	BR, μg C-CO_2/g/h	qCO_2, C-CO_2/$mg_{biomass}$/h
1.	The forest in the Orhei district	6,71±0,02	6,9	28,1	914,2±22,4	0,39±0,03	0,42±0,03
2.	The landfill at the locality of Slobozia-Dusca	4,88±0,03	7,9	19,7	33,8±8,3	1,42±0,09	41,96±3,05

Note: SOM – the soil organic matter content, W – soil moisture content at 40% of the water holding capacity, SMB – the soil microbial biomass, BR – the basal (soil) respiration, qCO_2 – the metabolic quotient. The statistics is presented via the confidence interval at P=0,95.

The biodegradation rates were studied first within 34-36-day (34 days for the forest soil and 36 for the landfill soil) incubational experiments with introduction of LDPE, treated and untreated by different nanocomposites, into 130 g of wet soil kept in 250 mL Erlenmeyer flasks (in 3-4 replica per variant). LDPE was used in the form of two film strips cut longitudinally (190x10 mm) and transversally (210x10 mm) from a standard LDPE sheet (210x190 mm). After the end of the first incubation period soil samples were taken for measuring SMB, BR, and qCO_2. Then, the second incubation was launch by adding glucose (0.5%) into soil. This incubation lasted for 41 days. In the end of it the same microbial parameters as well as SOM and LDPE weight losses were measured. During the incubations the flasks were kept opened at the room temperature, in the dark, with periodic adjustment of moisture.

The nanocomposites used in the study were based on iron oxide doped with cobalt or magnesium and modified by hydrophilic polymers – polyvinylpyrrolidone (PVP) or polyethylene glycol (PEG). The $CoFe_2O_4$/PEG, $MgFe_2O_4$/PEG, $CoFe_2O_4$/PVP, and $MgFe_2O_4$/PVP nanocomposites were obtained via hydrothermal synthesis from iron salts, cobalt, and magnesium at the temperature of 150° C, in water-alcohol medium, using PEG or PVP as stabilizers. The nanomaterials were in the form of a black powder. According to the confocal microscopy and SEM, the dimensions of the nanomaterials were 50-120 nm. The nanocomposites were identified by the X-ray diffraction, IR spectroscopy, atomic absorption spectroscopy, and thermogravimetric analysis.

Hydrocolloidal suspensions of the nanocomposites in the concentrations of 20 mg/L (min) and 100 mg/L (max) were obtained by treating the suspensions with ultrasound for 3 mins at 50 kHz. LDPE strips were placed into Erlenmeyer flasks with these suspensions and agitated for 1 hour at 200 rpm. Then the strips were placed on filter paper and dried for 3-5 days at the room temperature. Before introduction into soil the strips were exposed to UV radiation for 1 hour 2 times. The study included the following variants of nanocomposites: $CoFe_2O_4/PVP_{min}$, $CoFe_2O_4/PVP_{max}$, $CoFe_2O_4/PEG_{min}$, $CoFe_2O_4/PEG_{max}$, $MgFe_2O_4/PVP_{min}$, $MgFe_2O_4/PVP_{max}$, $MgFe_2O_4/PEG_{min}$, and $MgFe_2O_4/PEG_{max}$.

The statistical analysis was done with the Ms Excel 365 software.

The Results

The introduction of untreated LDPE (without the nanocomposites) into each soil did not change the soil microbial activity as compared to the corresponding controls within 34-36 incubation days (tab. 2). The treatment of LDPE with the nanocomposites resulted in several statistically significant changes (tab. 2). For example, within the variants of the forest soil there were observed 9% increases of SMB for $CoFe_2O_4/PVP_{max}$ and $CoFe_2O_4/PEG_{max}$, a 5% decrease of SMB for $MgFe_2O_4/PEG_{max}$, and 14%, 18%, and 20% decreases of qCO_2 for $MgFe_2O_4/PEG_{min}$, $MgFe_2O_4/PVP_{max}$, and $CoFe_2O_4/PEG_{max}$ respectively. All these changes were statistically significant comparing to the control variant. Within the variants of the landfill soil $CoFe_2O_4/PEG_{max}$ caused 6% and 8% decreases in SMB comparing to the untreated LDPE and control variants respectively. Also, in the case of $MgFe_2O_4/PVP_{max}$ there was a 9% increase in SMB (comparing to the LDPE variant) that coincided with 21-22% decreases in qCO_2 comparing to the control and LDPE variants respectively.

The 41-day incubation with glucose significantly decreased the SOM content in all variants (tab. 3). In the forest soil these decreases were similar in their magnitude and, consequently, did not result in significant differences between the variants including the control. The only exception was SOM in the case of $CoFe_2O_4/PVP_{max}$ that was 4% smaller than in the control. In the landfill soil the glucose introduction resulted in different SOM decreases depending on the case. The control lost 5% of the initial SOM, while

the LDPE, $CoFe_2O_4/PEG_{min}$, $CoFe_2O_4/PEG_{max}$, $MgFe_2O_4/PVP_{min}$ and $MgFe_2O_4/PEG_{min}$ variants lost even more and had 4-7% less of SOM than the control (by the end of the incubation with glucose). SOM in the rest 4 nanocomposite variants with the landfill soil was not different statistically from the control.

Table 2. The soil parameters after the incubation with LDPE, treated and untreated by nanocomposites.

N.	Soil	Variant	LDPE load, g/kg sol	SMB, μg C/g	BR, μg C-CO_2/g/h	qCO_2, C-$CO_2/mg_{biomass}$/h
1.	The forest soil	Control	-	352,08±6,81	0,26±0,01	0,74±0,02
2.		LDPE	1,37	360,17±18,25	0,25±0,02	0,69±0,09
3.		$CoFe_2O_4/PVP_{min}$		344.28±1.92	0.26±0. 01	0.75±0.02
4.		$CoFe_2O_4/PVP_{max}$		383.53±12.09	0.26±0.01	0.68±0.04
5.		$CoFe_2O_4/PEG_{min}$		368.83±12.11	0.24±0.01	0.66±0.04
6.		$CoFe_2O_4/PEG_{max}$		382.91±16.62	0.23±0.01	0.60±0.06
7.		$MgFe_2O_4/PVP_{min}$		339.56±10.31	0.24±0.01	0.71±0.03
8.		$MgFe_2O_4/PVP_{max}$		350.75±5.00	0.21±0.02	0.61±0.06
9.		$MgFe_2O_4/PEG_{min}$		356.03±2.90	0.23±0.01	0.64±0.01
10.		$MgFe_2O_4/PEG_{max}$		333.10±4.26	0.24±0.01	0.72±0.03
11.	The landfill soil	Control	-	35,92±1,71	0,50±0,09	7,15±0,75
12.		LDPE	1,27	35,09±0,91	0,69±0,03	7,04±0,57
13.		$CoFe_2O_4/PVP_{min}$		34.63±0.78	0.52±0.10	7.46±0.10
14.		$CoFe_2O_4/PVP_{max}$		33.95±0.82	0.63±0.18	7.71±0.37
15.		$CoFe_2O_4/PEG_{min}$		34.90±1.30	0.62±0.14	6.96±0.55
16.		$CoFe_2O_4/PEG_{max}$		33.16±0.78	0.59±0.02	6.87±0.31
17.		$MgFe_2O_4/PVP_{min}$		35.21±4.35	0.45±0.04	6.88±0.79
18.		$MgFe_2O_4/PVP_{max}$		38.36±1.88	0.57±0.06	5.56±0.73
19.		$MgFe_2O_4/PEG_{min}$		34.74±0.66	0.45±0.05	6.52±0.29
20.		$MgFe_2O_4/PEG_{max}$		33.69±2.56	0.47±0.08	7.11±0.37

Note: Control – the variant with untreated soil, LDPE – introduction into the soil of untreated low-density polyethylene, Co/MgF_2O_4 – introduction into the soil of LDPE treated by the nanocomposites stabilized with polyvinylpyrrolidone (PVP) or polyethylene glycol (PVP) and used in minimal (min) or maximal (max) concentrations. The rest of abbreviations are the same as in tab. 1. The duration of the incubation was 34 days for the forest soil and 36 for the landfill soil. The statistics are shown via the confidence interval at P=0,95.

The glucose introduction into the soil had different effects on the microbial parameters by the end of the incubation (tab. 3). In the forest soil there were observed statistically significant SMB changes in 4 cases out of 10: the LDPE and $MgFe_2O_4/PEG_{min}$ variants had a 6% increase each, comparing to the control, while the $CoFe_2O_4/PVP_{max}$ and $CoFe_2O_4/PEG_{min}$ variants had a 6% decrease each, comparing to the LDPE variant. In the landfill soil there were no statistical differences between the control and LDPE variants, while in all cases with nanocomposites SMB was always statistically higher (from +38% to +60%) than in the control, and qCO_2 was always statistically lower than both in the control (from -53% to -67%) and LDPE (from -47% to -62%) variants.

The biggest SMB was observed in the case of $MgFe_2O_4/PVP_{max}$, and the smallest qCO_2 – in $MgFe_2O_4/PVP_{min}$. The measurements of the LDPE degradation by the end of incubation with glucose revealed 0,5-2,5% decreases in LDPE mass depending on the soil and the applied nanocomposite (tab. 4).

Within the forest soil variants, the highest degradation was in the LDPE variant. The tested nanocomposites were not able to stimulate the LDPE biodegradation in this soil. In all but one nanocomposite cases the LDPE weight losses were statistically not different from the one in the variant with untreated LDPE. The loss in the $CoFe_2O_4/PVP_{min}$ variant was 46.5 times smaller. Within the landfill soil the smallest degradation rate was in the LDPE variant, and the application of nanocomposites was able to increase it statistically by 3.6-4.3 times in four variants out of 8 ($CoFe_2O_4/PVP_{min}$, $CoFe_2O_4/PEG_{min}$, $MgFe_2O_4/PEG_{min}$, and $MgFe_2O_4/PEG_{max}$).

Table 3. The soil parameters after the 41-day incubation with glucose.

N.	Soil	Variant	SOM, %	SMB, µg C/g	BR, µg C-CO_2/g/h	qCO_2, C-CO_2/$mg_{biomass}$/h
1.	The forest soil	Control	6,21±0,13	709,56±16,59	0,99±0,14	1,40±0,21
2.		LDPE	6,18±0,10	752,57±9,73	1,02±0,05	1,26±0,18
3.		$CoFe_2O_4$/PVP_{min}	5.98±0.18	743.52±78.17	0.75±0.15	1.02±0.31
4.		$CoFe_2O_4$/PVP_{max}	5.95±0.12	705.21±12.67	0.89±0.03	1.27±0.04
5.		$CoFe_2O_4$/PEG_{min}	6.05±0.12	704.69±21.48	0.92±0.17	1.31±0.28
6.		$CoFe_2O_4$/PEG_{max}	6.05±0.12	738.99±35.29	0.82±0.08	1.11±0.15
7.		$MgFe_2O_4$/PVP_{min}	5.98±0.14	716.88±49.81	0.82±0.15	1.14±0.20
8.		$MgFe_2O_4$/PVP_{max}	6.09±0.07	724.32±20.72	0.98±0.05	1.35±0.07
9.		$MgFe_2O_4$/PEG_{min}	6.05±0.12	751.55±12.50	0.76±0.13	1.01±0.18
10.		$MgFe_2O_4$/PEG_{max}	6.40±0.14	751.33±37.09	0.84±0.35	1.11±0.45
11.	The landfill soil	Control	4,62±0,05	459,41±40,65	2,39±0,17	5,26±0,93
12.		LDPE	4,36±0,05	523,97±90,51	2,41±0,68	4,66±1,72
13.		$CoFe_2O_4$/PVP_{min}	4.53±0.03	652.94±120.76	1.24±0.65	1.84±0.62
14.		$CoFe_2O_4$/PVP_{max}	4.79±0.24	690.92±119.89	1.55±0.21	2.26±0.28
15.		$CoFe_2O_4$/PEG_{min}	4.28±0.07	635.40±85.09	1.57±0.11	2.49±0.34
16.		$CoFe_2O_4$/PEG_{max}	4.31±0.12	665.40±90.08	1.40±0.11	2.13±0.31
17.		$MgFe_2O_4$/PVP_{min}	4.28±0.09	723.64±79.70	1.29±0.36	1.76±0.33
18.		$MgFe_2O_4$/PVP_{max}	4.47±0.27	733.02±91.70	1.33±0.47	1.85±0.77
19.		$MgFe_2O_4$/PEG_{min}	4.43±0.09	725.19±65.22	1.37±0.39	1.90±0.57
20.		$MgFe_2O_4$/PEG_{max}	4.53±0.09	714.70±41.07	1.36±0.22	1.90±0.30

Note: See the notes for tab. 1-2.

Table 4. The LDPE degradation

Nr.	Soil	Variant	Applied LDPE weight, g	LDPE weight loss, mg	LDPE degradation, %
1.	The forest soil	LDPE	0,134±0,005	3,100±1,978	2,299±1,408
2.		$CoFe_2O_4$/PVP_{min}	0.125±0.003	0.067±0.131	0.05±0.11
3.		$CoFe_2O_4$/PVP_{max}	0.129±0.006	3.283±3.163	2.54±2.40
4.		$CoFe_2O_4$/PEG_{min}	0.133±0.002	2.800±3.941	2.11±2.99
5.		$CoFe_2O_4$/PEG_{max}	0.127±0.009	1.433±1.641	1.08±1.23
6.		$MgFe_2O_4$/PVP_{min}	0.127±0.006	1.167±2.287	0.88±1.72
7.		$MgFe_2O_4$/PVP_{max}	0.123±0.005	0.500±0.837	0.39±0.66
8.		$MgFe_2O_4$/PEG_{min}	0.125±0.001	1.300±0.765	1.03±0.60
9.		$MgFe_2O_4$/PEG_{max}	0.127±0.002	3.067±1.139	2.40±0.86
10.	The landfill soil	LDPE	0,136±0,004	0,800±0,564	0,582±0,392
11.		$CoFe_2O_4$/PVP_{min}	0.130±0.005	3.050±0.889	2.34±0.66
12.		$CoFe_2O_4$/PVP_{max}	0.129±0.006	1.917±1.443	1.46±1.05
13.		$CoFe_2O_4$/PEG_{min}	0.136±0.006	3.350±0.493	2.46±0.26
14.		$CoFe_2O_4$/PEG_{max}	0.131±0.005	2.133±2.417	1.61±1.79
15.		$MgFe_2O_4$/PVP_{min}	0.130±0.002	1.733±1.077	1.33±0.82
16.		$MgFe_2O_4$/PVP_{max}	0.135±0.005	1.950±1.318	1.43±0.92
17.		$MgFe_2O_4$/PEG_{min}	0.133±0.007	3.350±1.225	2.50±0.77
18.		$MgFe_2O_4$/PEG_{max}	0.135±0.005	2.833±1.454	2.09±1.07

Note: See the notes for tab. 1-2.

Discussion

The absence of statistically significant changes in the microbial parameters after the initial soil incubation with the introduced untreated LDPE showed that the microorganisms of the two tested soils could hardly use LDPE as a source of carbon and/or energy. This agrees well with the general knowledge that polyethylene is extremely recalcitrant to degradation by microorganisms, and that under usual soil conditions it takes years for the biodegradation to come close to 0.5% [18]. The negative implications for the environment in this case are obvious. However, the obtained results also demonstrated that soil amendments with glucose and LDPE treatment by iron oxide-based nanocomposites may considerably accelerate LDPE biodegradation.

Glucose is a source of easily degradable organic carbon that, being introduced into soil, can stimulate the mineralization of the recalcitrant forms of carbon within SOM [19]. This phenomenon is known among the so-called soil priming effects and, in our case, its presence was manifested in the statistically significant SOM decreases in absolutely all variants on day 41 after the glucose introduction. Although not usually used for stimulating LDPE biodegradation, priming effects were suggested as a strategy for degradation of soil organic pollutants [20]. According to our results, the glucose induced priming effects are the best explanation for the increased LDPE weight losses in at least some variants. For example, within the forest soil the second-best degradation rate was observed in the LDPE variant, which also had the highest SMB. The statistically significant 6% increase of SMB in this variant comparing to the control could not result from the initial ability of the soil microorganisms to actively degrade LDPE since, as it was already mentioned, the introduction of LDPE did not cause any SMB stimulation before the incubation with glucose. The fact that by the end of that incubation the SOM content was practically identical to the control one implied that this increase could not result from higher rates of SOM degradation too. Thus, there is good evidence that the glucose induced priming effects stood behind both observed phenomena – the increased microbial biomass and the high LDPE degradation rate. To the best of our knowledge this is one of the first demonstrations of the possibility of stimulating LDPE biodegradation in soil via glucose amendments.

Iron oxide nanoparticles and nanocomposites (as the ones used in this study) are known for their ability to stimulate biodegradation of LDPE. They, among other things, may contribute to capturing O_2 in the LDPE hydrocarbon chain and through that stimulate its further bioactive hydrolysis [12]. The obtained results showed that the tested nanocomposites could indeed substantially increase the LDPE degradation rate, but their influence depended on the soil and on whether the soil was amended with glucose or not. The nanocomposites were ineffective in the forest soil conditions, and in one case they even significantly inhibited the LDPE biodegradation. In the landfill soil variants, judging by the absence of any positive impact on SMB comparing to the control, they were quite inefficient too. But the things changed radically after the landfill soil was amended with glucose. All the nanocomposite variants had significantly better microbial parameters comparing to the control, and in 4 out of 8 cases the LDPE degradation rate was up to 4.3 times higher than in the variant with the untreated LDPE. The facts that by the end of the incubation with glucose the biodegradation was much smaller in the variant with the untreated LDPE, and that the microbial biomass of the latter was not statistically different from the control, showed that neither the glucose amendments nor nanocomposites could efficiently stimulate the LDPE degradation by themselves, and that their efficiency became visible only when they were used together. Again, to the best of our knowledge, this is the first-time demonstration that glucose amendments can stimulate the ability of iron oxide-based nanocomposites to stimulate the LDPE biodegradation in soil.

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