Investigation of Biodegradation of Polylactide Product Samples under Various Temperature and Soil Conditions

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Abstract. In this work, the biodegradation of polylactide films, granules and tapes at temperatures of 23 °C and 8 °C in humus and chernozem soils was investigated. It was found that under the conditions studied, polylactide products are poorly biodegradable – for 84 days of exposure in the soil, the weight loss of all products did not exceed 10%. According to the results of microbiological assessment of the microflora of soils in which destruction occurred, no destructors of this type of biodegradable polymer were identified by inoculation in nutrient media containing polymer as the only carbon source.

1 Introduction

Synthetic plastics have long conquered the world and are widely used in various industries from transparent flexible food films to durable structures and expensive medical materials. A durable and often toxic material causes environmental degradation and an increase in the greenhouse effect [1].

The introduction of modern methods of recycling plastic waste from incineration and burial to recycling is still environmentally unacceptable and requires significant labor and energy costs. Consumer concerns and industrial policies in many countries that encourage the conservation of natural resources stimulate the development, production and consumption of biodegradable polymers that can decompose into environmentally friendly components under appropriate conditions. In addition, the use of renewable carbon raw materials for the production of biopolymers has a clear connection with reducing the risk of climate change and reducing dependence on fossil resources [1].

An important feature of biodegradable polymers is a controlled or programmable biodegradation period during composting or degradation anaerobically. Using the example of polyhydroxyalkanoates (another class of biodegradable polymers), it was found that this is primarily influenced by climatic factors – temperature and humidity. A comprehensive study of the biodegradation of two types of polyhydroxyalkanoates by soil microbial communities of different composition, conducted in Siberia, showed that the decomposition of PHA is influenced by both the chemical composition of the polymer and the characteristics

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of the soil itself, the depth of the polymer in the soil, the concentration of microorganisms [2].

Polylactide is a polymer of lactic acid and its complex cyclic diesters (L, L-lactide, D, L-lactide, D,D-lactide, meso-lactide), is one of the widely used biodegradable polymers in the world [3]. Interest in polylactide has increased due to its availability and ability to break down under the influence of environmental factors [4]. It is known that the ester bonds of PLA tend to break down under the influence of temperatures, which leads to a change in the crystallinity of the polymer [5]. In this connection, the mechanical and thermal properties of polylactide products, as well as the rate of biodegradation depend on the conditions of their production [4].

However, the limited availability of accurate data on the effect of incubation temperature, soil type, and type of products from PLA on the rate of its biodegradation requires the study of these parameters to predict the rate of biodegradation of packaging products and containers from PLA at various temperatures, including in the climate of the Krasnoyarsk Territory.

The purpose of this work was to study the features of biodegradation of samples of products based on polylactide, depending on the technology of their production, soil types and temperature conditions for predicting the rate of biodegradation of packaging products and containers in various climatic conditions.

2 Materials and methods

2.1 Obtaining products from PLA

For the manufacture of polylactide products (hereinafter referred to as PLA), a granular PLA with an average molecular weight (Mw) of 119 kDa, a degree of crystallinity (Cx) of 60%, a melting point (Tmelt) of 170 °C and a D-monomer content of 7% of the NatureWorks brand (USA) was used.

Polymer polylactide films were obtained using the solvent casting method. To obtain a film with a diameter of 9.5 cm, a sample of 1.5 g of polylactide was dissolved in 40 ml of extra pure chloroform (EKROS Group of Companies, Russia) at a temperature of 50 °C. After the polymer was completely dissolved, the solution was poured onto a pre-sterilized Petri dish (d = 9.5 cm) and left in a fume hood until the solvent completely evaporated (~ 24 hours).

Polymer polylactide filament was obtained on a portable extruder (Bestfilament, Russia) at a melting temperature of 170 °C, after which it was granulated. The final granules had an average length of 7-10 mm and a thickness of 1.5-2.0 mm.

Polymer polylactide tape was also obtained using an extruder with an aluminum flat die AD 0, manufactured in the Resource Center for Collective Use "Spacecraft and Systems" of Reshetnev Siberian State University of Science and Technology with a hole width of 40 mm and a thickness of 0.1 mm. The resulting 4 cm wide tapes were cut into 1.5 cm long segments.

2.2 Experiment on the aging of products made of PLA in model soil ecosystems

For an experiment on modeling degradation in different types of soil at different temperatures in vitro, two types of soil were taken – humus and chernozem. Humus soil was purchased in a retail store in Krasnoyarsk and had an initial humidity of 88.0%. This type of soil was characterized by high absorbency and visually low bulk density due to the large amount of organic residues in the composition. The chernozem soil was purchased from Sibpromservice-K LLC and had an initial humidity of 14.5%. This type of soil had a low
absorbency and high bulk density compared to humus soil. The indicators of ash content, humus, potassium content, phosphorus and nitrogen for the types of soils used are presented in Table 1.

**Table 1.** Chemical properties of the used types of soil.

<table>
<thead>
<tr>
<th></th>
<th>Ash content, %</th>
<th>Humus, %</th>
<th>Total potassium, %</th>
<th>Total phosphorus, %</th>
<th>Nitrate nitrogen, ppm</th>
<th>Ammonium nitrogen, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humus</td>
<td>17.73</td>
<td>33.483</td>
<td>0.59</td>
<td>0.121</td>
<td>3.830</td>
<td>5.776</td>
</tr>
<tr>
<td>Chernozem</td>
<td>82.97</td>
<td>13.883</td>
<td>1.02</td>
<td>0.132</td>
<td>1.009</td>
<td>3.108</td>
</tr>
</tbody>
</table>

100 mg of the corresponding product in three repetitions for each type of soil and temperature was placed in plastic containers pre-filled with 45 g of experimental soil, and then filled with an additional 45 g of soil on top, which totaled 90 g of soil in each container.

Some of the containers were incubated at a temperature of 23 (± 3) °C (hereinafter referred to as temperature 1) in a dry ventilated room. The other part of the containers was incubated at a temperature of 8 (± 3) °C in a Biryusa 118 household refrigerator (Krasnoyarsk Refrigerator Plant Biryusa JSC, Russia). Soil moisture in containers was maintained by regular watering (at least twice a week) with tap water at the rate of 25-65 ml per container (depending on temperature) and loosening. The experiment was carried out for 3 months with 6 experimental points every two weeks. The relative residual mass of PLA products at each point n for each repetition was determined by the formula (1):

\[ \Delta m_n\% = \frac{m_n - m_0}{m_0} \cdot 100 \% \]  

where \( m_n \) is the mass of the sample after exposure in the soil, \( m_0 \) is the initial mass of the sample before laying in the soil.

For the obtained values, the average of several repetitions for each point was found.

### 2.3 Determination of the microbiological composition of soil microflora

Determination of the microbiological composition of the soil microflora of the soil before exposure was carried out by plating dilutions of soil suspension on solid elective media. From one repetition of one experimental group, 1 g of soil was removed sterically (over an alcohol lamp) and placed in 100 ml of sterile water. The flask was incubated for 1 hour at a temperature of 32 °C and 150 rpm in an ES-20 incubator shaker (Biosan, Russia). After incubation, successive dilutions up to 10-7 were made from the flask with suspension. Next, cultures were made for bacteria from dilutions 10-5, 10-6 and 10-7, for fungi from dilutions 10-2, 10-3 and 10-4. The cultures were plated on agarized elective media of meat-peptone agar (MPA), Sabouraud, starch-ammonia agar (SAA) and Ashby's agar, intended for the isolation of ammonifiers, fungi (micromycetes), actinomycetes and nitrogen fixers, respectively. Ready-made media of MPA and Sabouraud (Russia) were used. Starch-ammonia agar and Ashby's agar were prepared independently according to preparation instructions. Starch-ammonia agar had the following composition, g: starch – 10, (NH₄)₂SO₄ 4 – 2, K₂HPO₄ 4 – 1, MgSO₄ 4 – 1, CaCO₃ 3 – 3, agar-agar – 20, distilled water – 1000 ml. Ashby's agar had the following composition, g: sucrose – 20.0; K₂HPO₄ 4 – 0.2; MgSO₄ x 7H₂O – 0.2; NaCl – 0.2; FeSO₄ 4 – 0.1; CaCO₃ 5.0; distilled water – 1000 ml.

Next, 0.1 ml of the appropriate dilution was introduced into a Petri dish with a frozen sterile agarized medium, and then thoroughly rubbed with a pre-calcined / sterile spatula. The cultures were incubated in a thermostat for 3-7 days at a temperature of 32/25 °C. After incubation, the dishes were viewed, colonies were counted, their description was made, the total microbial count (TMC) was calculated, and, if possible, the species of the grown
bacteria was determined by matrix-activated laser desorption/ionization with time-of-flight separation (MALDI-TOF) using MALDI-TOF MS Microflex (Bruker Daltonics, Germany). Bacterial species were identified using the automated MALDI TOF MS system with Bruker Daltonics flex Control 2.4 software (Build 38). The identification of bacterial species was based on the analysis of the mass spectra of protein molecules characteristic of this type of microorganisms.

2.4 Allocation of PLA destructors

Isolation of PLA destructors was carried out by plating dilutions of swabs from the surfaces of PLA products incubated in a soil medium onto solid elective media. To obtain swabs, the corresponding products from the PLA after removal from the soil were placed in 100 ml of sterile distilled water and incubated in a shaker incubator for 1 hour at a temperature of 32 °C and 150 rpm. Further, dilutions up to $10^{-6}$ were made from the obtained suspensions. The cultures were made from dilutions $10^{-4}$ and $10^{-6}$ for bacteria and from dilutions $10^{-2}$ and $10^{-3}$ for fungi into two types of agarized media containing PLA as a carbon source. The media were prepared independently according to preparation instructions (Table 2). The culture was taken similarly to clause 1.4. The cultures were incubated for 14 days at a temperature of 32 °C and 25 °C for bacteria and micromycetes, respectively, periodically scanning the dishes to control excessive growth and the appearance of "clean zones" characteristic of media containing polymers as the only carbon source.

Table 2. Composition of mineral media for isolation of fungal and bacterial destructors of PLA.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity, g (per 1000 ml of DW)</th>
<th>Component</th>
<th>Quantity, g (per 1000 ml of DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$</td>
<td>4.4908</td>
<td>NaNO$_3$</td>
<td>2.0</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>4.6846</td>
<td>K$_2$HPO$_4$</td>
<td>1.0</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>1.0</td>
<td>MgSO$_4$×7H$_2$O</td>
<td>0.5</td>
</tr>
<tr>
<td>MgSO$_4$×7H$_2$O</td>
<td>0.5</td>
<td>KCl</td>
<td>0.5</td>
</tr>
<tr>
<td>Iron Citrate</td>
<td>0.05</td>
<td>CaCO$_3$</td>
<td>3.0</td>
</tr>
<tr>
<td>CaCl$_2$×2H$_2$O</td>
<td>0.005</td>
<td>Fine PLA powder</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.05</td>
<td>Agar-agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine PLA powder</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar-agar</td>
<td>15.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After incubation, the isolated colonies were counted and the titer of microorganisms (CFU/(g · ml)) was calculated according to the formula:

$$CFU = \frac{(a \cdot b)}{(c \cdot d)}$$

where $a$ is the average number of colonies on a Petri dish, $b$ is the dilution used for culture, $c$ is the volume of suspension used (ml), $d$ is the mass of the product (g).

The results obtained for various products and various conditions were compared, the type of grown bacteria was determined using the automated MALDI TOF MS system with Bruker Daltonics flex Control 2.4 software (Build 38). The identification of bacterial species was based on the analysis of the mass spectra of protein molecules characteristic of this type of microorganisms.
3 Findings

3.1 Determination of the microbiological composition of soil microflora

To assess the soil biodegradation of samples of products from PLA under model conditions, a study of the microbiological composition of two types of soils (humus and chernozem) was carried out. The results of the culture tests are shown in Figs. 1 and 2.

![Fig.1. Photos of Petri dishes with various media for determining the number of microorganisms of various ecological and trophic groups in humus before laying products from PLA: 1 - MPA, 2 – SAA, 3 – Ashby's agar, 4 – Sabouraud agar.](image1)

![Fig.2. Photos of Petri dishes with various media for determining the number of microorganisms of various ecological and trophic groups in chernozem before laying products from PLA: 1 - MPA, 2 – SAA, 3 – Ashby's agar (no growth), 4 – Sabouraud agar.](image2)
The total number of microorganisms of the studied ecological and trophic groups (Fig.3) was higher for humus soil and amounted to $3.8 \cdot 10^8$, for chernozem $1.4 \cdot 10^7$ CFU/g. The differences in the number of microorganisms are mainly due to the higher content of ammonifiers and actinomycetes in humus (a difference of two orders of magnitude in both cases) and the absence of nitrogen fixers in chernozem.

Fig.3. The number of microorganisms of various ecological and trophic groups in soils of two types before laying products from PLA for the study of their soil biodegradation under model conditions.

3.2 Experiment on the aging of products made of PLA in model soil ecosystems

Figure 4-6 shows the appearance of the studied products made of PLA after exposure in the soil for 70 days. All products, regardless of the incubation temperature and the type of soil used for exposure, after three months of exposure did not show visible changes in appearance and structure characteristic of other biodegradable polymers during this exposure period [6]. The expected changes in the structure, cracks, fragmentation were not detected, from which it can be concluded that during this period the degradation processes were just beginning.

Fig.4. Appearance of PLA films before and after exposure in different types of soil at different temperatures: 1 – humus, 23 °C; 2 – humus, 8 °C; 3 – chernozem, 23 °C; 4 – chernozem, 8 °C
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Fig. 4. Appearance of PLA films before and after exposure in different types of soil at different temperatures: 1 – humus, 23 °C; 2 – humus, 8 °C; 3 – chernozem, 23 °C; 4 – chernozem, 8 °C

Fig. 5. Appearance of PLA granules before and after exposure in different types of soil at different temperatures: 1 – humus, 23 °C; 2 – humus, 8 °C; 3 – chernozem, 23 °C; 4 – chernozem, 8 °C

Fig. 6. Appearance of PLA tapes before and after exposure in different types of soil at different temperatures: 1 – humus, 23 °C; 2 – humus, 8 °C; 3 – chernozem, 23 °C; 4 – chernozem, 8 °C

This conclusion was confirmed by the results of determining the relative residual mass of the products (Fig. 7-9) after exposure in the soil. Figure 7 shows the decrease in the mass of PLA films after exposure in different types of soil at different temperatures. It can be seen that in conditions of humus soil in both temperature regimes, as well as in conditions of chernozem at a moderate temperature, the mass of products for 84 days of exposure, the mass of films of PLA decreased by no more than 10%.
Figure 7 shows the decrease in the mass of PLA films after exposure in different types of soil at different temperatures. After 84 days of exposure in the soil, a significant decrease in mass (by 16\%) was noted for granules aged in chernozem soil conditions at moderate temperature. In other conditions, no significant changes in mass loss were observed.

Figure 8 shows the decrease in the mass of PLA granules after exposure in different types of soil at different temperatures. During 70 days of exposure in different types of soil, the loss of mass practically did not occur. Both in the case of tapes and in the case of other products, it can be concluded that, in general, the studied products from PLA slowly degrade over short periods of time in these types of soil and at these temperatures. It is probably necessary to increase the exposure time or use soils with a more diverse microbiological composition.
3.3 Allocation of PLA destructors

Figure 10 shows the results of microbiological cultures for the determination of bacterial destructors of PLA 14 days after exposure in different types of soil at different temperatures. It can be seen that a large number of microorganisms was characteristic of the products, after exposure in humus soil at 23 °C and 8 °C. The largest number of bacteria was typical for samples of PLA granules (1.5 \( \cdot \) 10^8 CFU/g), then for PLA films (5.5 \( \cdot \) 10^7 CFU/g). In chernozem at 23 °C, the number of bacteria for tapes and films did not exceed 2.5 \( \cdot \) 10^7 CFU/g. In the case of chernozem conditions and a temperature of 8 °C, there was no growth. From this it can be concluded that the lowered exposure temperature negatively affects the soil microflora, the type of soil also determines microbial activity during exposure. However, it is worth noting that among the grown colonies there were no colonies with clear zones formed as a result of the degradation of PLA present in the medium. This suggests that there are no PLA destructors among the grown colonies or the destruction processes are still poorly expressed.

Figure 11 shows the results of microbiological cultures for the determination of fungal destructors of PLA 14 days after exposure in different types of soil at different temperatures. In contrast to the cultures for the determination of bacterial destructors, in this case, the growth of microorganisms was observed for all experimental groups. In particular, under
humus conditions at 23 °C, the largest number of micromycetes was observed in PLA granules – up to $8.35 \times 10^5$ CFU/g. At the same time, the corresponding values for films and tapes were at the level of $7.1 \times 10^5$ and $5.4 \times 10^5$ CFU/g, respectively. In humus at 8 °C, the number of microorganisms for all products decreased slightly, but in general remained in the same order. It is also worth noting that for the conditions of chernozem at 8 °C, comparable indicators of the number of micromycetes were characteristic for all products. In chernozem at 23 °C, as well as in humus at the same temperature, the largest number was shown for PLA granules ($9.5 \times 10^5$ CFU/g). The numbers of micromycetes for the remaining groups of products remained in the same order.

![Fig. 11. Titer of fungi 14 days after exposure in different types of soil at different temperatures: 1 – humus, 23 °C; 2 – humus, 8 °C; 3 – chernozem, 23 °C; 4 – chernozem, 8 °C.]

In the case of cultures on fungal destructors, as well as for cultures on bacterial destructors, no clean zones were found on the surface of the agarized medium. This means that there are no PLA destructors among the micromycetes.

According to the data on the determination of the generic/species belonging of microorganisms isolated during inoculation for the determination of PLA destructors by the MALDI-TOF method (not presented), among the isolated microorganisms were the genera *Pseudomonas*, *Pichia*, *Lactobacillus*, *Penicillium*, *Acinetobacter*, *Bacillus*, *Staphylococcus*, *Streptomyces*, *Arthrobacter* and *Acidovorax*. Specific species included *Acinetobacter calcoaceticus*, *Pseudomonas brassicacearum/kilonensis*, *Acinetobacter iwoffii*, *Bacillus pumilus*, *Streptomyces griseus* and *Acidovorax delafieldii*.

### 5 Discussion

When considering degradation processes, it is necessary to take into account that polymers are destroyed either throughout the entire volume of the material (volumetric erosion), or only at the boundary of the material with the environment (surface erosion) [7]. The visible reduction of polymer products in size during degradation occurs as a result of surface erosion; with volumetric erosion, the samples retain their shape, but become more porous throughout the volume until they finally collapse mechanically [8]. Which type of degradation prevails depends on the relative rate of water diffusion through the material compared to the rate of bond hydrolysis. The main factors influencing these rates are the hydrophobicity of the polymer, roughness, crystallinity and molecular weight. For example, PLGA undergoes volumetric erosion, which means that the diffusion of water through the entire volume of the sample is faster compared to the rate of hydrolysis of ester bonds [9]. P(CPP-SA), on the other hand, undergoes surface erosion, which means that the hydrolysis of anhydride groups...
at the polymer-water interface occurs faster than the diffusion of water molecules through the material [10].

According to literature data, microbes that can decompose PLA belong mainly to actinomycetes, a family of filamentous bacteria that produce antibiotics, usually found in soil. Most Actinobacteria that decompose PLA belong to Pseudonocardiaceae, and other families include Micromonosporaceae, Streptomycetaceae, Streptosporangiaceae, Thermomonosporaceae, etc. and Bordetella petrii [11]. In addition, there have been reports of degradation of PLA oligomers (molecular weight up to 1000 Da) of Fusarium moniliforme and Penicillium roqueforti and degradation of PLA Amycolatopsis sp [12,13,14].

As for the fungal degradation of PLA, the process occurs at the ends of the PLA chain. Microorganisms consume the monomer at the end of the chain, which leads to polymer depolymerization [8]. Among the fungi most often mentioned as capable of assimilating DL-lactic acid (the product of the destruction of PLA) are the species Fusarium monilforme, Penicillium roqueforti, Tritirachium album, Aspergillus fumigatus and Thermomyces lanuginosa [15].

The above-mentioned species were not found in the microflora of the used soil types. On the one hand, this may indicate their absence in the soil, since these species are rarely found in the soil microflora (with the exception of the genera Penicillium and Fusarium). On the other hand, it is possible that within the framework of this experiment it was not possible to isolate such destructors due to the fact that the nutrient medium used was not suitable for their isolation. In this case, it is likely that more specific elective environments may be required that would take into account the metabolic needs of the intended destructors.

6 Conclusion

When studying the biodegradation of the obtained products from PLA in humus and chernozem soil at moderate and low temperatures, it was found that they are poorly biodegradable under these conditions – the weight loss for 84 days did not exceed 10%. In the study of the microbiological composition of the soils in which the exposure took place, no specific PLA destructors were naturally identified. In general, the rate of biological decomposition of polylactide and composites based on it should be evaluated for a sufficiently long time (at least a year), since the PLA destructors in the soil, as a rule, are few and insufficiently active. A high rate of destruction can be stimulated by maintaining constantly high humidity and optimal (at least 20 °C) soil temperature, as well as additional application of mineral fertilizers. To accelerate the destruction, additional introduction of natural strains-destructors of this group of polymers is possible.

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References