

Degradable Polyhydroxyalkanoates as Herbicide Carriers

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Abstract The biodegradable polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) has been used to design experimental sustained-release formulations of the herbicide Zellek Super in the form of films and microgranules. The kinetics of polymer degradation and the dynamics of herbicide release show that the rate and extent of herbicide release from the polymer matrix into the soil depends on the geometry of the carrier and the proportion of the pesticide loaded into it (polymer/pesticide mass ratio). Experiments with the creeping bentgrass (*Agrostis stolonifera* L.) show that the formulations of the herbicide Zellek Super constructed as microgranules and films can be successfully used to suppress the growth of grasses. This study is the first to demonstrate that biodegradable polyhydroxyalkanoates can be used effectively to construct environmentally friendly sustained-release PHA-herbicide systems that can be placed into the soil together with seeds.

Keywords Herbicide · Haloxyfop-P-methyl · Zellek Super · Sustained-release formulations · Polyhydroxyalkanoates · Plant growth suppression

Introduction

Rapid development of agrochemistry and the transition of agriculture to intensive technologies led to the appearance and application of a vast variety of chemicals, which can lead to their dissipation and subsequent accumulation in the biosphere [1, 2]. A new direction, aimed at reducing the risk of the uncontrolled distribution of xenobiotics in the environment, is the development of a new generation of environmentally friendly herbicide formulations with a targeted and controlled release of the active ingredient owing to the use of specific coatings and/or carriers produced from biodegradable materials. Several examples of polymeric carrier usage have been described, including ethyl cellulose [3, 4], polyurethane [5], sodium alginate [6, 7] for delivery of a number of weed and pest killing chemicals.

Polyhydroxyalkanoates (PHAs), biodegradable polyesters of microbial origin, can be potentially used for these purposes. These polymers can be processed from various phase states by a number of conventional techniques yielding products with many different geometries. PHAs are not prone to rapid chemical hydrolysis and, in turn, are degraded by microorganisms in the environment [8].

PHAs are degraded in biological media to form products innocuous to the environment: carbon dioxide and water under aerobic conditions or methane and water under anaerobic conditions. PHA biodegradation is performed by microorganisms that secrete intra- or extra-cellular PHA depolymerases, which differ in their molecular organization and substrate specificity [9]. While intra-cellular PHA depolymerases are synthesized by PHA producing bacteria and are used by them to hydrolyze their own PHA storages, extra-cellular enzymes are produced by other microorganisms to utilize PHAs usually released into the environment after death and cell lysis of PHA accumulating cells [8].

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Biodegradation of polyhydroxyalkanoates is performed by microorganisms that inhabit a specific natural environment. Among PHA degraders that have been described in the literature are representatives of various genera of bacteria [8, 10–13] and fungi [8, 11, 14–16].

Complete PHA degradation in natural or synthetic biological settings takes months, which can be very important for designing prolonged-action or time-release formulations [17–19]. In initial experiments using the pesticides HCH and lindane, we demonstrated the potential of using degradable polyhydroxyalkanoates as a matrix for embedding these compounds to enable their targeted and controlled release to the environment [20]. Recently, the ability to prepare PHA microparticles loaded with herbicide or pesticide and viable biodegradable release systems for agrochemical purposes has been demonstrated [21–23]. These previous works mainly examine the compound release kinetics in vitro. Going forward, it is important to examine the degradation and herbicide release kinetics in soil and with plants, for these formulations to be truly considered effective.

The goal of this work was to investigate PHA, specifically the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB/3HV)), as a carrier in the construction of biodegradable, environmentally friendly, sustained-release herbicide formulations that would be placed in soil together with seeds, *i.e.* in the pre-emergence phase. As mentioned previously, kinetics of biodegradation and herbicide introduction into the surrounding soil are important for the validation of the formulations produced in this work.

Experimental

Materials

The copolymer P(3HB/3HV), which, as had been previously demonstrated, can be degraded in soil more readily than the homopolymer of polyhydroxybutyrate (P3HB) [13], was used as the herbicide carrier in this work. Copolymer specimens were produced by established microbiological techniques, in accordance with the Technical Standards (TS No. 2200-001-03533441-2004, Reg. 14.12.2005 No. 068/003058) of the Pilot Production Facility at the Institute of Biophysics SB RAS, which has received the Conformance Certificate from the Russian Health Inspection (No. 24.49.05.000.M.007682.01.05 of 24.01.2005). The copolymer was synthesized by the wild-type *Ralstonia eutropha* strain B5786 [24], and PHA biosynthesis was performed as previously described [25].

The copolymer was recovered from bacterial biomass using dichloromethane. The recovered material was concentrated using a Rotavapor R-210 rotary evaporator (Switzerland) and precipitated in isopropyl alcohol. The polymer was dried in a laminar box at 40 °C. The 3HV monomer content in

the P(3HB/3HV) specimens used in all experiments shown here was 10 mol %. The degree of crystallinity of the copolymer was 58 %, and average molecular weight was 6.5×10^5 Da (see below).

Studies involving incorporation and subsequent time release of compounds from polymer carriers were performed with herbicide Zellek Super (active ingredient, haloxyfop-P-methyl, or (*RS*)-2-{4-[3-chloro-5-(trifluoromethyl)-2-pyridyloxy]phenoxy}propanoic acid methyl ester) (Dow AgroSciences, Austria). Zellek Super is a herbicide with systemic activity, and it is quickly absorbed by weed leaves and transported to growth points, roots, and rhizomes. The herbicide used in these studies impairs photosynthesis, causing both the aboveground plant parts and roots of the weeds to stop growing, thus leading to chlorosis. It is active towards annual and perennial grasses. In the environment, the herbicide is slowly degraded by soil microorganisms [26]; at pH levels increasing to 9, rapid chemical degradation is also observed.

Loading of herbicide

The chemicals were loaded into polymer carriers using different methods as discussed below, and experimental herbicide delivery systems were produced in the form of microgranules, and films (Fig. 1).

Polymer microgranules loaded with the herbicide Zellek Super were prepared from a solution of the herbicide and the copolymer in dichloromethane. A peristaltic pump was used to pour the solution drop by drop into the settling bath with isopropanol (precipitate), where the polymer precipitated in the form of microgranules. By varying the densities of the solutions and the needle size, we determined parameters that enabled preparation of good quality microparticles: polymer concentration in the solution = 7 % (w/v), needle size = 20G, and thickness of the precipitate layer (*h*) = 180 mm. Figure 1b shows the resulting microcapsules. Two types of microcapsules containing different proportions of herbicide were prepared. In the first batch of microcapsules, the polymer/herbicide mass ratio was 60:40 and in the second, the mass ratio was 90:10.

Polymer films loaded with the herbicide Zellek Super were prepared by polymer solution casting followed by evaporation of the solvent. A dichloromethane solution containing 2 % (w/v) of the P(3HB/3HV) copolymer was mixed with the herbicide solutions (the polymer/herbicide mass ratios in the film were 25:75 and 75:25), stirred with an overhead stirrer at a speed of 300 rpm, cast onto degreased glass and dried at room temperature for 3–4 days. Squares of 25 mm² in area (5 mm × 5 mm) were then cut from the film (Fig. 1a).

Analytical methods

Polymer content in the microgranules and films was determined by methanolysis, following a standard

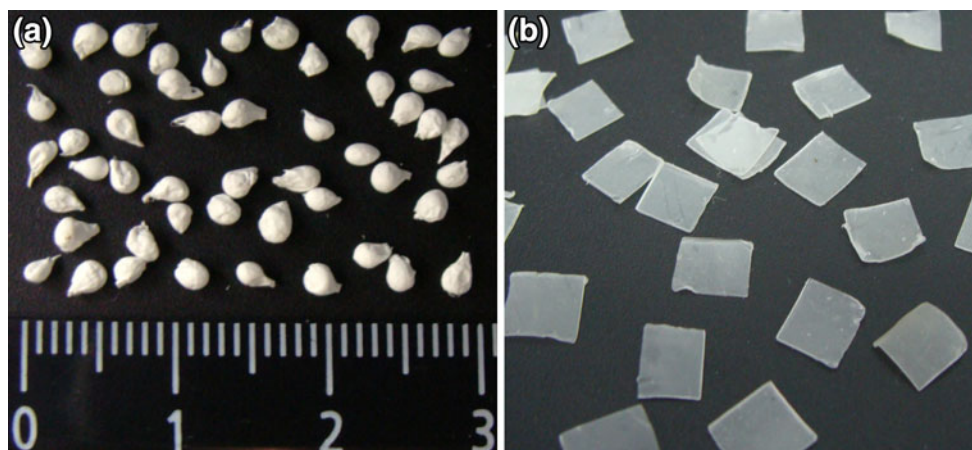
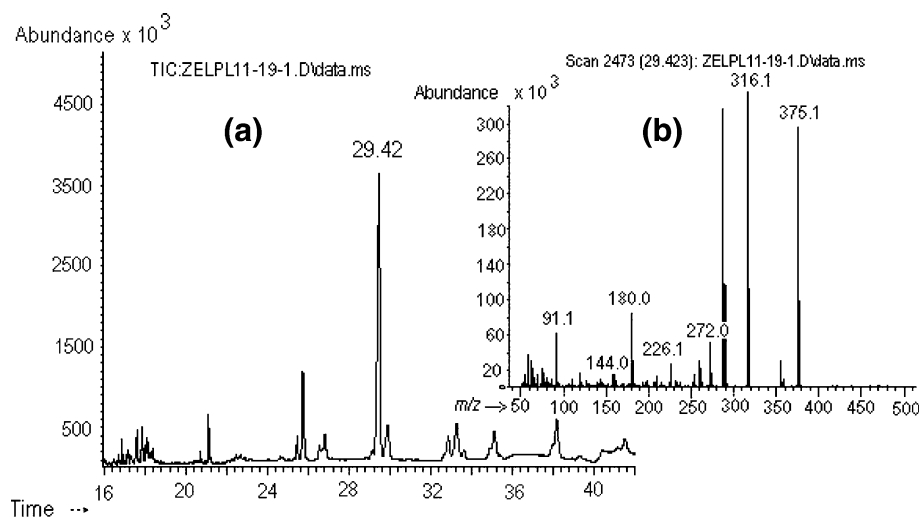


Fig. 1 Photographs of the designed PHA/pesticide formulations: microgranules (a) or films (b). Formulations were fabricated according to Materials and Methods

Fig. 2 The chromatogram (a) and mass spectrum (b) (inset) of Zellek-super detected following extraction from the soil



procedure [27]. Analysis of 3-hydroxyalkanoate methyl esters was performed on a gas chromatograph–mass spectrometer model 6890/5975C (Agilent Technologies, USA) on a HP-FFAP capillary column (30 m length, 0.25 mm internal diameter), with benzoic acid as the internal standard. Percentages of the monomers in PHA were quantified based on mass spectra, and verified by taking $^1\text{H-NMR}$ spectra of copolymer solutions in CDCl_3 (Advance III 600 NMR spectrometer, Bruker Corporation, Bremen, Germany).

Physicochemical properties of PHAs were examined using X-ray structure analysis, and high-performance liquid chromatography as discussed below

X-ray structure analysis and determination of crystallinity of PHA samples were performed using an X-ray spectrometer (D8 Advance, Bruker Corporation, Bremen, Germany) (graphite monochromator on a reflected beam)

in a scan-step mode, with a $0.04\text{ }^\circ\text{C}$ step and exposure time 2 s, to measure intensity at point. The instrument was operating at $40\text{ kV} \times 40\text{ }\mu\text{A}$.

Molecular weight (M_w) and polydispersity (PD) were determined by gel permeation chromatography at a temperature of $40\text{ }^\circ\text{C}$, using a Waters chromatographic system (Waters 1515 isocratic pump, a Reodyne 7725i injector, and a Waters 2414 refractometric detector and Waters Styragel HR4E and HR5 columns USA). Chloroform was used as the eluent, with a flow rate of 0.8 mL/min . The system was calibrated using low polydispersity polystyrene standards supplied by Fluka (Switzerland).

All data presented in this work were analyzed statistically by conventional methods, using the standard software package of Microsoft Excel.

The herbicide Zellek Super was extracted with ethanol from the total mass of the soil, after the specimens were removed, and purified prior to measuring its concentration in soil. Then, after a series of standard procedures

(concentration, washing, pH stabilization, and dehydration) [28], the herbicide concentration was determined using gas chromatograph–mass spectrometer model 6890/5975C (Agilent Technologies, USA) with a HP5SM capillary column (30 m length, 0.25 mm internal diameter). Analytical standard haloxyfop-methyl (Fluka/Riedel, Germany) was used to plot the calibration curve. The chromatogram and mass spectrum of Zellek Super extracted from a soil sample are shown in Fig. 1.

Experimental designs

Degradation of the polymer matrix and time course of action of the constructed xenobiotic-polymer systems were studied in laboratory scale experiments. The microgranules or films loaded with the herbicide were placed in fine mesh synthetic fabric (mill gauze) covers and inserted into the 250-cm³ containers, each of them containing 100 g of wet environmental garden soil. The containers were incubated in a constant-temperature cabinet at 25 °C at constant soil moisture (50 %). All values presented were calculated for dry soil. A total amount of 79 ± 5 µg or 159 ± 4 µg of polymer granules containing 32 ± 2 µg or 15 ± 1 µg (total amounts) Zellek Super, respectively, were added per g dry soil. For PHA films, 82 ± 21 µg polymer containing 18 ± 2 µg Zellek Super (total amounts) were added per g dry soil. Throughout the course of the experiment, the covers with polymer/herbicide samples ($n = 3$ of each type) were removed from the containers at intermittent time points; the samples were removed, mechanically cleaned to remove residual soil, washed with distilled water, and dried at 40 °C for 24 h. Biodegradation of the polymer matrix was evaluated based on the residual polymer content, molecular weight (M_w and M_n), and the degree of crystallinity (C_x). The samples were weighed using a Mettler class 4 precision balance (Mettler-Toledo, USA). The experiments with microgranules and films loaded with Zellek Super were carried out for a duration of 42 days. All samples were set up in triplicate.

Microbiological study

Soil microbial communities were examined using conventional methods of soil microbiology [29]. The total bacteria counts were determined on fish peptone agar. Microscopic fungi were isolated on wort agar. Soil suspension (10^4 – 10^7 dilutions) was used for inoculations, which were performed in triplicate. Inoculated plates were maintained for 3–7 days at a temperature of 30 °C prior to counting bacteria and for 7–10 days at 25 °C prior to counting micromycetes.

Effectiveness of the sustained-release Zellek Super-polymer system

The effectiveness of the herbicide loaded in microgranules and films was evaluated using a model plant (weed), creeping bentgrass (*Agrostis stolonifera* L.), which is an annual grass. *A. stolonifera* L. seeds were planted in garden soil (100 g) placed in 500 cm³ containers (30 g of seeds per 1 m²). The specimens prepared and described above were placed into the treatment containers. The positive control plants were sprayed with an aqueous solution of the herbicide at day 19 of the experiment (in the beginning of the tillering phase): 1 ml of the herbicide per 0.1 m². Plants grown in the soil without herbicide application served as the negative control. The experiment was conducted in the laboratory, under natural photoperiod, at room temperature. Soil moisture content was maintained at 50 %.

Plant productivity was estimated as a biomass increase. Vegetative parts of plant specimens were cut off at 10, 20 and 30 days after starting the experiment, dried at 105 °C, and dry biomass weight was measured.

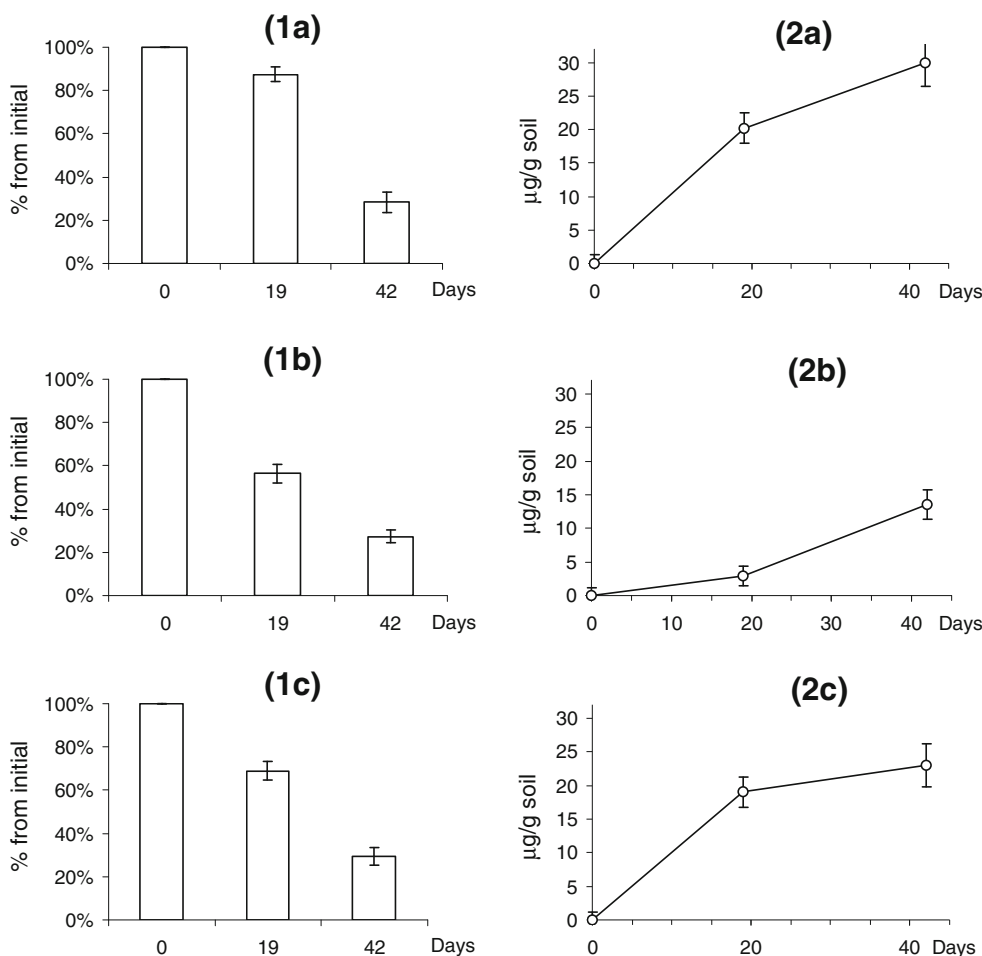
Results

Dynamics of biodegradation of P(3HB/3HV) microgranules and films and release of the herbicide Zellek Super to the soil

PHA microgranules containing the herbicide Zellek Super were placed into the soil for examination of polymer degradation and concomitant herbicide release. Upon degradation of the samples in soil, the weight loss for the microgranules varied depending upon the proportion of the herbicide in the granules (Fig. 3). At day 19 of the experiment, the residual mass of the copolymer in microgranules with a polymer to herbicide ratio of 60:40 was 87 % (Fig. 3, 1a), while in the granules with a polymer to herbicide ratio 90:10, a lower residual mass was observed, amounting to 56 % (Fig. 3, 1b). At day 42 of the experiment, however, the residual masses of the granules were almost equal to each other, amounting to 28 % of their initial masses. The mean specific rate of polymer matrix degradation over the 42 day experiment was about 0.03 mg polymer/day.

As the polymer matrix was degraded, the molecular weights of the specimens decreased (Table 1). At the same time, the polydispersities of the specimens increased, suggesting an increase in the number of fragments with different degrees of polymerization. These observations suggested the necessity for further examination of polymer properties during the course of degradation. We subjected the specimens to X-ray structure analysis to observe

Fig. 3 Degradation dynamics of the P(3HB/3HV) matrix (films and microgranules) (1) and the release profile of the herbicide Zellek Super (2): (a) microgranules with the polymer to herbicide ratio 60 : 40; (b) microgranules with the polymer to herbicide ratio 90:10; (c) polymer films with the polymer to herbicide ratio of 75:25



whether the degradation of the polymer matrix changed the proportions of the ordered and disordered phases, *i.e.* the degree of crystallinity, of the polymer. There are published data suggesting that depolymerizing enzymes preferentially attack amorphous regions of polymers rather than their ordered phases [8, 30, 31]. Assuming these observations are accurate, as the polymer is degraded and its amorphous phase is more rapidly disintegrated, the polymer that remains must exhibit an increase in its degree of crystallinity. Analysis of X-ray spectra of the PHA specimens that had been buried in the soil exhibited an increase in their degree of crystallinity, suggesting preferential disintegration of amorphous phases of the copolymer (Table 1).

The greater the proportion of the herbicide in the granules, the higher its release rate into the soil (Fig. 3). The concentration of the herbicide in soil, measured at the conclusion of the experiment, from microgranules that contained the greater proportion of Zellek Super (40 mass %) was twice as high as that of soil containing microgranules that initially contained 10 mass % of Zellek Super, amounting to 30 µg/g dry soil compared to 15 µg/g dry soil (compare Fig. 3a, b). The mean release rate of the

herbicide from the granules with the copolymer to herbicide ratio 60:40 was 0.7 µg/day/g soil.

Degradation of polymer films with the polymer/herbicide ratio 75:25 occurred at a higher rate than that of the granules (Fig. 3, 1c). At day 19 of the experiment, the residual mass of the copolymer in these films was 69 %. At the end of the experiment, the residual mass of the polymer was about 30 % of its initial mass, and the specific biodegradation rate was 0.08 mg/day, which was more than twice as high as that of the microgranules. The herbicide release rate was comparable in microgranules. For the first

Table 1 Comparative characterization of P(3HB/3HV) (10 mol. % 3HV) specimens before and after soil exposure

Examples	Parameter		
	M _w (kDa)	Polydispersity, M _n	Crystallinity, C _x (%)
Microgranules (Initial)	650 ± 85	2.01 ± 0.11	58
Microgranules (19 day)	470 ± 35	2.56 ± 0.35	68
Films (Initial)	650 ± 85	2.01 ± 0.11	58
Films (19 day)	380 ± 45	2.76 ± 0.42	69

19 days, 18 $\mu\text{g/g}$ soil of the herbicide, or 90 % of the amount loaded into the films, was released (Figs. 3, 2c).

Given these observations, our results demonstrate that the P(3HB/3HV) carriers of the herbicide Zellek Super, as designed in this study, enable a sustained release of the herbicide into soil, with carrier geometry being the major factor for the rate of release.

Effectiveness of the sustained-release Zellek Super-polymer system

To the best of our knowledge, no group has examined effectiveness of weed killing using the PHA/herbicide matrices. Thus, we examined the timed release of Zellek Super, from microgranules and films, on the model plant (weed), the creeping bentgrass *Agrostis stolonifera* L. The form of the herbicide carrier significantly affected the response of *Agrostis stolonifera* L. (Figs. 4 and 5). However, regardless of whether the herbicide Zellek Super was loaded in microgranules or films prepared from P(3HB/3HV), the herbicide loaded into biodegradable polymer carriers was more effective than the herbicide when traditionally applied as a spray to plants during the tillering phase (the positive control; Fig. 4, number 2). Moreover, the effect of the herbicide applied as a spray had not been observed until day 19 after the treatment (Fig. 5).

In the experiment with using polymer microgranules (Fig. 4, number 3, 4), we observed a few weed shoots at day 10 (about 15–20 % of those in the positive control). At later observation times, as the polymer matrix was gradually degraded and the herbicide was released to the soil and delivered to the plant roots, the number of the shoots decreased: they wilted and became dry. Spraying of the plants with standard doses of Zellek Super was not as effective (Fig. 4, number 2), as the herbicide was applied to the plants during the tillering phase, and the plants stopped growing 3–5 days after spraying, i.e. 23–25 days after the weed was planted. The sprayed plants were measured to be 1.5–2 cm in height.

As can be seen in Fig. 4, application of the herbicide loaded into polymer films produced even more effective results than those obtained in the experiment with herbicide-loaded microgranules. No weed growth was observed in the experiment with the herbicide-loaded films applied to the soil (Fig. 4, number 5). In this experiment, plant growth was demonstrated to be completely suppressed.

Changes in the abundance of microorganisms in treatments with different herbicide formulations

In order to be able to compare the numbers of rhizosphere bacteria in the control and treatment soils, we examined the soil before planting the seeds.

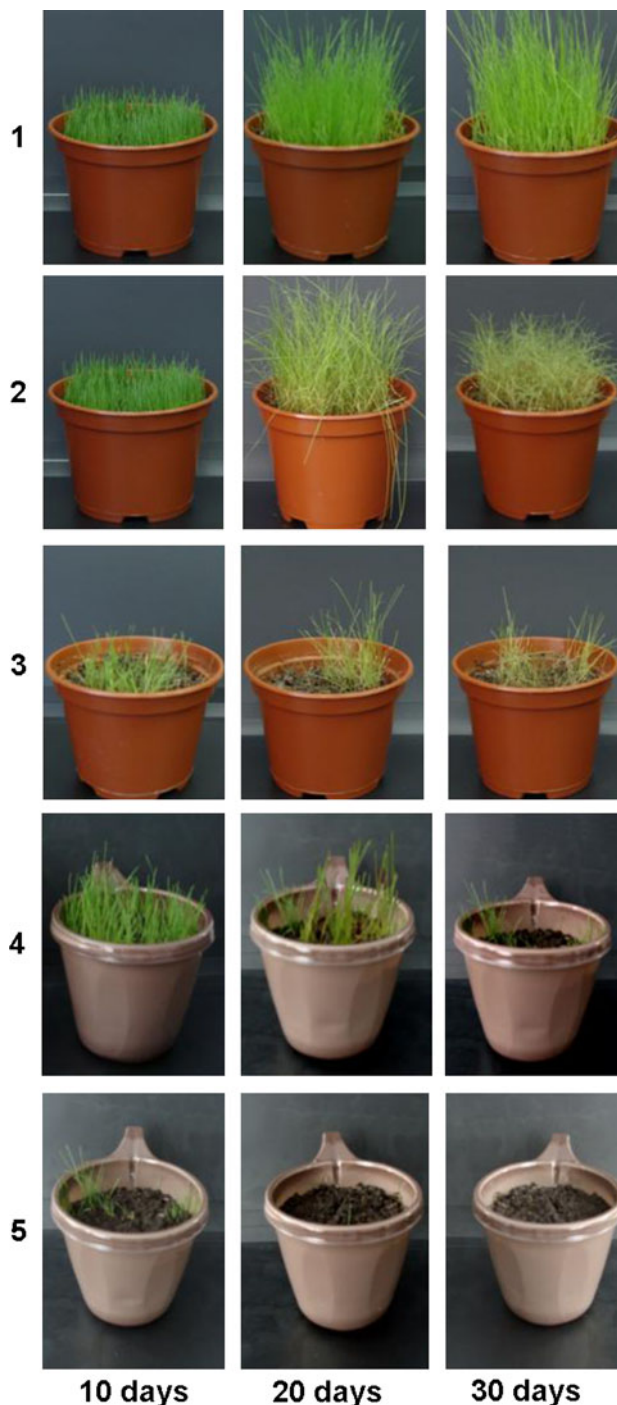


Fig. 4 Suppression of the growth of the plant *Agrostis stolonifera* L. by the sustained-release formulation of the herbicide Zellek-super loaded into the P(3HB/3HV) matrix shaped as microgranules with the polymer to herbicide ratio 90:10 (3) and 60:40 (4) or films with the polymer to herbicide ratio 25:75 (5). Negative (intact) control, untreated plants (1). Positive control, plants sprayed with a Zellek Super herbicide solution (2)

Analysis of soil microflora at the time of planting showed that the total number of heterotrophic bacteria was $(17 \pm 2.7) \times 10^7$ CFU in 1 g of air dry soil (Table 2). By

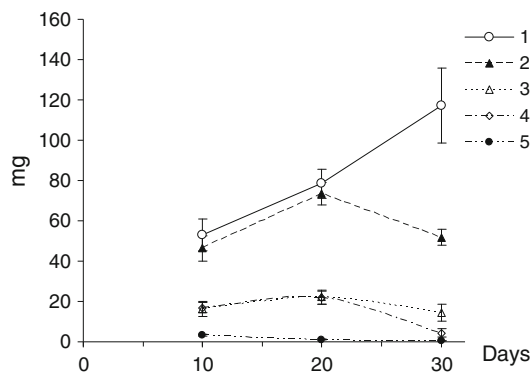


Fig. 5 Growth of the plant *Agrostis stolonifera* L. after addition of the PHA incorporated herbicide Zellek Super, mg of dry vegetative part: 1—negative control (untreated plants); 2—positive control (plants sprayed with a herbicide solution); 3—microgranules, the polymer to herbicide ratio 90:10; 4—microgranules, the polymer to herbicide ratio 60:40; 5—films, the polymer to herbicide ratio 25:75

Table 2 Total number of microorganisms in experimental samples

No.	Sample	Total number, CFU in 1 g of soil	Percentage relative to control, %
Bacteria (fish peptone agar)			
1	Initial soil	$(17 \pm 2.7) \times 10^7$	11
2	Control	$(158 \pm 8.3) \times 10^7$	100
3	Zellek Super, P(3HB) films	$(50 \pm 1.4) \times 10^7$	32
4	Zellek Super, P(3HB) granules	$(153 \pm 2.6) \times 10^7$	97
5	Zellek Super, spraying	$(0.56 \pm 1.6) \times 10^7$	0.4
Micromycetes (wort agar)			
6	Initial soil	$(18 \pm 2.8) \times 10^3$	69
7	Control	$(26 \pm 3.3) \times 10^3$	100
8	Zellek Super, P(3HB) films	$(43 \pm 4.4) \times 10^3$	165
9	Zellek Super, P(3HB) granules	$(28 \pm 3.5) \times 10^3$	108
10	Zellek Super, spraying	$(10.6 \pm 2.2) \times 10^3$	41

the end of the experiment, the number of bacteria in the control had increased almost tenfold, amounting to $(158 \pm 8.3) \times 10^7$ CFU in 1 g of soil. In the sprayed samples, the number of bacteria was 280 times lower than that in the control soil: $(0.56 \pm 1.6) \times 10^7$ CFU in 1 g of air dry soil. The number of bacteria in the samples with the herbicide loaded in polymer granules was similar to the number of bacteria in the control sample, taking into account experimental error.

Changes in the number of micromycetes were similar to those in the number of bacteria. Analysis of the initial soil showed that micromycetes totaled 18 ± 2.8 thousand CFU in 1 g of air dry soil. By the end of the experiment, the number of micromycetes in the control soil had increased by 1.4 times, reaching 26 ± 3.3 thousand CFU in 1 g of air

dry soil. Spraying with the herbicide inhibited the development of rhizosphere micromycetes: their number decreased by 2.5 times, to 10.6 ± 2.2 thousand CFU in 1 g of air dry soil. Similarly to the number of bacteria, the number of micromycetes was not significantly affected by the addition of the herbicide loaded into polymer granules.

Discussion

The material used to prepare carriers for herbicide and pesticide formulations must have such properties as biodegradability, environmental safety, long-term (for weeks and months) retention in nature, controlled degradation to nontoxic products, processability by available techniques, and compatibility with the substances loaded into it. Above all, decreased complexity of formulation would allow for a less expensive product, making environmentally friendly pesticide application systems more readily available.

This study was the first to investigate PHAs, linear thermoplastic and bio-based polymers, as carriers (matrices) for herbicides, and their application in the soil. As these polymers are not prone to chemical hydrolysis in most liquid media and are degraded as a result of true biological degradation, systems based on them can function in the soil for extended time. The microbial component of soil is an active agent in the processes of hydrolysis and degradation of organic substances. Many soil microorganisms can degrade PHAs and utilize them as growth substrates [8]. Degradation rate of the PHA matrix can be altered by varying the shape of the carrier and the proportion of the substances loaded into it, thus controlling the rate of substance release and delivery. PHAs have been scarcely used thus far to construct environmentally friendly formulations of pesticides. Analysis of the available literature revealed that research on this concept is in its infancy. In previous studies, P(3HB/3HV) microspheres were prepared as a delivery system for the herbicides atrazine [21] and ametryne [22]. The effect of the herbicides on the morphology and size of the microspheres was characterized and the herbicide release profiles in aqueous medium were examined. The genotoxicity of atrazine-loaded P(3HB/3HV) microspheres was decreased in relation to atrazine alone [21]. Microspheres of pure polymers of PHB and poly(ϵ -caprolactone) loaded with malathion pesticide were fabricated and examined in another work [23]. The effect of the pesticide on the morphology and particle size of the biodegradable microspheres was examined. The test for controlled release in an aqueous medium shows that release of malathion was improved depending on the poly(ϵ -caprolactone) content. These results demonstrate viable biodegradable herbicide and pesticide release systems, using PHA for agrochemical purposes.

Previously, we demonstrated potentialities of the copolymer P(3HB/3HV) as a carrier for two different pesticides (HCH and lindane) [20]. Experimental sustained-release formulations of HCH and lindane were prepared by cold pressing, but the specimens loaded with Zellek Super in this work were prepared by the solvent evaporation (films) and micro-dropping (granules) techniques. The investigation of polymer degradation kinetics and herbicide release dynamics showed that the release of herbicides from the polymer matrices into the soil depends on the shape of the carrier and the proportion of the herbicide loaded into it. Release of the substance occurs slowly, as the polymer is degraded by soil microflora. Both for microgranules and films the degrees of crystallinity increased with duration of soil exposure, suggesting similar preferential disintegration of the amorphous phases of the polymer.

The rate of herbicide release into the soil can be regulated by varying the polymer/herbicide ratio. Experiments with the creeping bentgrass (*Agrostis stolonifera* L.) demonstrated that the formulations of the herbicide Zellek Super constructed as either microgranules or films can be successfully used to suppress the growth of grasses.

The increase in the number of soil bacteria during the course of plant growth should be associated with root exudates released by plants and serving as nutrients for bacteria. Spraying with the herbicide solution exerted the most significant inhibitory effect on the rhizosphere bacteria. The herbicide loaded into polymer granules had the least effect on the microorganisms of soil samples. A similar dependence was observed for the numbers of micromycetes.

Thus, encapsulation of the herbicide Zellek Super in the polymer matrix reduced its inhibitory effect on soil microorganisms while retaining its effectiveness against the test plant.

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