Biodegradable polymers – perspectives and applications in agriculture

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Abstract The paper presents a brief overview of the results of the implementation of the project “Agropreparations of the new generation: a strategy of construction and realization”. The first part contains the analysis of the growth of the wild-type strain Cupriavidus necator B-10646 (formerly eutrophus) and the synthesis of polyhydroxyalkanoates by this strain on various substrates: glycerol, palm oil, Siberian oil seed, sunflower seed oils, and oleic acid. On refined glycerin, a highly productive process is implemented when scaling up, allowing to obtain \(128 \pm 11 \text{ g/L PHA}\). Evaluation of oils has shown that palm oil is the best carbon substrate. The second part presents the results of the development of environmentally friendly slow-release pesticide formulations. They are a degradable matrix of poly-3-hydroxybutyrate mixed with natural materials (peat, clay, wood flour), into which a pesticide (metribuzin, tribenuron-methyl, fenoxaprop-P-ethyl, azoxystrobin, epoxiconazole, and tebuconazole) has been. The developed preparations showed high activity against pathogenic fungi and weeds and had a much weaker negative effect on the soil microflora. Studies of the degradation of the developed preparations and the release of pesticides into the soil confirm their effectiveness over a long period of time, up to 90 days.

1. Introduction
Plastic pollution is a global environmental problem. Plastic is found in soils, sediments, oceans, rivers, lakes, atmosphere and animal biomass. This situation has arisen as a result of the spread of "disposable" culture, increased consumption and use of disposable packaging [1]. World plastic production has exceeded 300 million tons per year. Waste management systems do not have sufficient capacity to safely dispose or recycle plastic waste. Up to 8 million tons of plastics gets into the oceans every year. The analysis of the plastic pollution of the World Ocean showed that about 5 million tons falls on macroplastics and 1.5 million tons of microplastics [2, 3]. The threat of macroplastics has been confirmed for almost 700 marine species and more than 50 freshwater species, the ingestion of microplastics has been recorded at all trophic levels, and microplastics are increasingly found in the human nutrition system [4, 5].

One way to reduce plastic pollution is post-consumption management, which requires a significant increase in investment and custom waste management solutions [6]. Other concepts prioritize reducing the amount of plastic by replacing it with alternative products or reusing it. Recently, industrial ecology and green chemistry have become increasingly important, which open up great potential for the creation of new environmentally friendly materials, including from renewable sources [7].
Considerable attention is currently paid to polymers synthesized by living systems (biopolymers) as an alternative to synthetic plastics. A special place among biopolymers is occupied by polyhydroxyalkanoates (PHA) - degradable polyesters of monocarboxylic acids synthesized by various microorganisms. In ecosystems, PHAs are decomposed to safe products, have sufficient mechanical strength, are thermoplastic and can be processed using traditional methods [8]. PHA was discovered in 1920, but the greatest increase in interest in these biopolymers began in the 1990s, as evidenced by the surge in scientific publications that continues today. Analysis of publication activity identified about 60,000 authors with scientific interests associated with PHA [9].

The main obstacle to the wider application of PHA is the high cost of production [10]. In order to increase the economic attractiveness of these biopolymers, active research is underway aimed at: reducing the cost of carbon substrates by attracting waste from various industries, since in the cost structure of biosynthesis, the cost of a carbon substrate can reach 40-50% [11, 12]; development of more efficient fermentation strategies, optimization of biosynthesis processes [13, 14]; creation of new strains with improved characteristics such as: increased fragility of the cell wall, resistance to toxic substrates, the ability to synthesize copolymer PHA with a high inclusion of co-monomers [15, 16]; development of processes using mixed microbial cultures [17]; the search for new solvents and methods for the isolation and purification of PHA, in this case the main costs are borne by the solvents, their subsequent purification or disposal [18]; development of composite materials based on PHA, to reduce the cost of finished products [19].

One of the areas of application of PHA is the development and use in agriculture of environmentally friendly pesticides of a new generation with targeted and controlled release of active ingredients, embedded in biodegradable matrices or coated with biodegradable coatings that decompose in soil and other biological media by soil microflora to form products that are harmless to nature. The use of such formulations can reduce the amount of chemicals applied to the soil and ensure their sustained and controlled delivery during the growing season of plants, preventing the dramatic releases to the environment that occur when free pesticides are used.

This paper provides a brief overview of the results obtained by scientists from the Siberian Federal University and the Institute of Biophysics SB RAS, Krasnoyarsk, as a result of the implementation of the project "Agropreparations of the new generation: a strategy of construction and implementation".

2. PHA biosynthesis on various carbon substrates
A key element of the biotechnological process for obtaining PHA is a producer, which must be genetically pure, resistant to mechanical stress, have a high growth rate, not pathogenic, not form toxic products, and have acceptable cell wall permeability. One of the strains corresponding to these characteristics is Cupriavidus necator B-10646 (C. eutrophus B-10646). This bacterial strain showed a fairly high growth rate of 0.14 h-1 on glucose as a carbon substrate and the ability to accumulate PHA up to 80% of the cell mass [20].

Glycerin, which is formed as a waste in the production of biodiesel, has a low cost, but has insufficient purity and is a promising substrate for biotechnology. It was found that the process of PHA synthesis by the C. necator B-10646 strain on crude glycerin (80% content of the main substance) is less productive compared to the indicators on purified glycerin (99%). Comparison of the results showed the preferred use of purified glycerin, despite the costs associated with purification [21]. Optimization of the biosynthesis process for gas and nitrogen feeding under scaling conditions in a 150-liter fermenter allowed to significantly increasing productivity (Table 1).
Table 1. Productivity of the process of PHA synthesis by strain C. necator B-10646 on various substrates.

| Substrate      | Biomass, g/L  | PHA, g/L   | Process performance, PHA g/(g·h) |
|----------------|---------------|------------|----------------------------------|
| Fructose       | 130±10.0      | 107±11     | 1.8 ± 0.2                        |
| Glucose        | 150±10.0      | 124±12     | 1.9±0.2                          |
| Purified glycerol | 160±10.0    | 128±11     | 2.1±0.2                          |
| Crude glycerol | 130±10.0      | 107±11     | 1.8 ± 0.2                        |

And if previously performed experiments in a 30-liter fermenter did not show the effect of impurities in glycerin on the process of PHA biosynthesis [22]. When scaled up in a 150 liter fermenter, this effect is evident. Productivity on Crude glycerol is 13% lower than on Purified glycerol.

Vegetable oils are another inexpensive carbon source for PHA synthesis. Vegetable oils are high in carbon and are efficiently converted to PHA. The lipase activity of the wild-type C. necator B-10646 strain was studied. The absence of a delay phase during the transition to a new substrate was noted, the highest lipase activity was recorded at the beginning of cultivation, on the first day. As a result, it was found that the most balanced substrate is palm oil, the biomass yield was 6-7 g / l, the polymer content in the cell was up to 70%. The main problem for oilseed substrates is their poor solubility in aqueous media. In the same study, the effect of emulsifiers Tween 80 and Sodium cocoyl glutamate was evaluated. The addition of emulsifiers made it possible to increase the biomass yield to 8.2 g / l and the polymer content to 85%, which indicates a higher availability of the substrate for bacteria during emulsification [23].

Another interesting substrate for PHA biosynthesis is fatty acids, oleic, palmitic, stearic, etc. Many fatty acids are included in lipid fractions and can be found in fats and oils of various origins. Zhila investigated the ability of C. necator B-10646 to synthesize PHA using oleic acid as a substrate. The biomass yield was 6.5 - 7.0 g / l, the PHA content was up to 80% [24]. Physicochemical characteristics of PHA synthesized on different substrates are presented in Table 2.

Table 2. Properties of PHA synthesized by C. eutrophus B-10646

| Substrates      | $M_n$ kDa  | $M_w$ kDa  | D  | $C_{s}$, % | $T_g$, °C | $T_c$, °C | $T_{melt}$, °C | $T_{degr}$, °C |
|-----------------|------------|------------|----|------------|----------|----------|----------------|----------------|
| Fructose        | 220        | 750        | 3.4| 78         | -        | 88       | 175            | 293            |
| Glucose         | 365        | 920        | 2.5| 76         | -        | 92       | 178            | 295            |
| Purified Glycerol | 104         | 355        | 3.4| 50         | 2.9      | 96       | 174            | 296            |
| Refinery Glycerol | 115         | 416        | 3.6| 55         | -        | 103      | 176            | 296            |
| Crude Glycerol  | 87         | 304        | 3.4| 52         | 2.7      | 99       | 172            | 295            |
| Palm oil        | 130        | 670        | 5.2| -          | -        | -        | 171            | 268            |
| Sunflower seed oil | 190        | 780        | 4.1| -          | -        | -        | 170            | 281            |
| Siberian        | 170        | 740        | 4.4| -          | -        | -        | 170            | 271            |
The properties of PHA samples synthesized by C. necator B-10646 on three types of glycerol did not differ dramatically. The polymers, based on two types of purified glycerol, had similar values of the number average $M_n$ - 104 and 115 kDa, and the weight average molecular weight $M_w$ - 355 and 416 kDa, and polydispersity $D$ - 3.42 and 3.63, respectively. The $M_n$ and $M_w$ values for PHA obtained on crude glycerol were slightly lower, 87 and 304 kDa. These values were usually lower than those obtained earlier with fructose and glucose. Samples P (3HB) synthesized on glycerol had a reduced degree of crystallinity $C_x$ 50–55%. The ratio of the amorphous and crystalline parts became equal. The values of the melting temperature $T_m$ and degradation $T_d$ were within the previously established ranges of 172–176 and 295–296 °С, respectively. PHA obtained from oils had a reduced value of the average number weight, as a result of which, these samples are characterized by a higher polydispersity. For oleic acid, a decrease in the molecular weight of $M_n$ and $M_w$ to 94 and 381 kDa, respectively, is noted with an increase in its concentration in the medium.

The studies carried out demonstrate the potential of fat-like and oil substrates for PHA biosynthesis; fats and oils, which are waste, can become the most promising substrates. The availability of a cheap biodegradable polymer can become the basis for the design of a new generation of environmentally friendly pesticides with controlled release of active ingredients embedded in a biodegradable base or coated with biodegradable films.

### 3. PHA controlled release pesticide systems

PHA has long attracted attention as a material for designing drug delivery vehicles. Since the early 2000s, PHA has been seen as a material for the construction of controlled release systems for agricultural pesticides. The first works were aimed at studying the patterns of formation of mixtures of pesticides and PHA, presented in various forms in the form of microparticles, granules, films and tablets [25, 26]. Herbicides, metribuzin and tribenuron-methyl, were used as active substances. As a result of the work, it was found that the nature of the interaction between the active substance and the polymer matrix is of a physical nature. The study of degradation showed the dependence of the rate of degradation on the form (microparticles, films, tablets) and the content of the active substance. The most prolonged degradation of tablets was more than 60 days with a metribuzin content of 10%, the highest degradation rate was recorded for microparticles. The kinetics of the release of the active drug into water and soil was studied, which confirmed the duration of action. Studies of the effect of herbicides loaded into a polymer matrix on laboratory crops of wheat have shown greater efficiency than commercial preparations based on metribuzin and tebucanazole. So the experimental forms caused the complete death of weeds on the 30th day of the experiment. The study also notes that the use of herbicides enclosed in a polymer matrix had a positive effect on the wheat yield, which was higher compared to the positive control, where a commercial preparation was used [27, 28].

Polymeric forms in the form of films, microparticles and pellets loaded with a fungicide, tebuconazole, were investigated as fungicidal preparations. As in the case of herbicides, the interaction of the active substance with the polymer matrix occurred due to physical interaction. In studies it is reported that films degrade faster, pellets are slowest. Fungicidal activity was tested on the plant pathogen Fusarium moniliforme. The pronounced fungicidal effect was recorded for 2 weeks of the experiment and persisted for 8 weeks. The highest tebuconazole yield, up to 70%, was recorded for films and microparticles. The yield of tebuconazole from pellets was no more than 40% [29–31].

The result of the research was the development of methods and technologies for creating new formulations of agrochemicals based on a representative of degradable PHA - poly-3-hydroxybutyrate P (3HB). Methods for loading pesticides have been developed to suppress plant pathogens and destroy weeds. The influence of such factors as the geometry of the form, the concentration of pesticides, the chemical composition of the medium and the type of microbial community on the kinetics of the
release of the active substance has been established. This made it possible to start designing more complex forms containing natural fillers (birch sawdust, peat and clay).

The main function of fillers is to reduce the cost of the resulting pesticide formulations. In addition, fillers affect the hydrophilic-hydrophobic balance (fillers: P (3HB)) and relaxation processes in the polymer matrix, which leads to a change in the kinetics of the outflow of the active substance and contributes to the degradation of forms. The physical nature of the interaction of fillers and P3HB in the mixture has been established using IR spectrometry and thermal analysis. The developed molds are pellets obtained by pressing dry powders and granules obtained by the method of wet granulation (Figure 1).

In addition to geometric dimensions, the resulting shapes differ in the packing of microparticles. In pellets, filler and P3HB particles are separated from each other, and the main strength is created by pressing force. Upon receipt of the granules, polymer deposition occurs on the filler particles, resulting in a more closed packaging. When analyzing the moisture absorption of the forms, it was found that granules are more resistant to water than pellets. Pellets with clay and peat as fillers disintegrated 3 and 5 hours after the start of the test. Pellets with sawdust retained their shape, but moisture absorption was about 80%. The maximum moisture absorption of granules is also fixed for a mixture with sawdust, no more than 35%.

Further, the degradation of the obtained forms in the soil was investigated in laboratory conditions (Figure 2).
Figure 2 shows that Granules degrade faster than Pellets. The residual mass of Granules on day 35 was 45–60%, and Pellets 58–80% of the original. Samples P (3HB) / wood flour and P (3HB) / peat with 50% filler showed the highest weight loss, but these samples are characterized by low strength. Thus, using various methods of making molds and varying the amount of filler, it is possible to control the rate of degradation of the carriers. The molds developed in this study can be used to make degradable packaging, greenhouse accessories and fertilizer and pesticide delivery systems. [19].

On the basis of the developed forms, preparations with slow release of fungicides (azoxystrobin, epoxiconazole and tebuconazole) and herbicides (metribuzin, tribenuron-methyl, and fenoxaprop-P-ethyl) have been created. Infrared spectroscopy showed no formation of chemical bonds between the components in the experimental formulations. The presence of fungicide and herbicides in pellets and granules had a significant impact on degradation. The degradation time in all variants increased to 83 days. The residual weight of pellets with herbicides on the 83rd day was 45–75%, with fungicides 65–80% of the original. The residual mass of granules with herbicides on the 83rd day was 60–80%, with fungicides 55–70% of the original. As with no pesticide loading forms, granules degraded slightly faster than pellets. Investigation of the release of pesticides from granules and pellets into the soil under laboratory conditions allowed confirming the prolonged action of the developed forms. For granules, the maximum concentration of the pesticide in the soil is reached on day 20 and is maintained until the end of the experiment. For pellets, the maximum concentration is reached at 48 days. For forms with fungicides, the highest yield was recorded for granules and pellets containing tebuconazole, 40 -50%. For forms with herbicides, the highest yield was recorded for granules and pellets containing metribuzin, 70 -80%. Of great importance for the yield of pesticides is their solubility in water. For example: the rapidly dissolving metribuzin and tribenuron-methyl were released faster than the less readily soluble fenoxaprop -P-ethyl. No reliable influence of the type of filler on the outflow of drugs was recorded. The class of pesticides and the technology of making molds have the greatest influence. The antifungal activity of fungicides deposited in granules and pellets has been investigated in vitro. All formulations have been found to have a significant inhibitory effect on Fusarium verticillioides; the colony size of the fungus was smaller in the presence of the developed Granules and Pellets than in the control group without fungicide, but comparable to the size of the colonies in the control group containing pure fungicide [32, 33].

The herbicidal activity of preparations with prolonged action of herbicides metribuzin and tribenuron-methyl was studied on laboratory crops of Triticum aestivum wheat and Hordeum vulgare barley with weeds of various species, as well as on weed thickets. The formulations created effectively suppressed all studied weed species. The biological efficiency of herbicidal preparations against intact plants in thickets of wheat and barley infected with weeds was close to 100%, which significantly exceeded the effect of their free forms. More effective weed control using embedded herbicides was favorable for the growth of crops, the aboveground biomass of which was 15% higher than that of crops treated with free herbicides [34]. Thus, all formulations are able to function for a long time in the soil, providing a gradual and sustainable delivery of pesticides.

In her work, Prudnikova investigates the effect of herbicides (metribuzin, tribenuron-methyl, fenoxaprop-P-ethyl) and fungicides (tebuconazole, epoxiconazole, azoxystrobin) applied to the soil as free pesticides or in the form of sustained-release pellets. Pesticides loaded into pellets had a much weaker negative impact on soil microflora, including functional groups of microorganisms and primary P (3HB) destructors. In some cases, there is a stimulating effect, in contrast to free pesticides. The study showed that experimental formulations of pesticides with slow release retain the potential for degradation of microflora, which favorably affects the removal of xenobiotics from the biosphere. The microbial taxonomic composition of pellets soil samples is more diverse than soils treated with free pesticides. Compared with untreated control soil, the use of Pellets with introduced pesticides resulted in an increase in the proportion of Bacillus, Pseudomonas and representatives of the type Actinobacteria. Pseudarthrobacter, Rhodococcus, Stenotrophomonas and Variovorax were also found in experimental soil samples, which are classified as destructors of complex organic compounds and
polymers. Pellets loaded with fungicides had a similar effect on pathogenic fungi as free fungicides. Fusarium numbers were declining; plant pathogens of the genera Alternaria, Pythium and Verticillium were not detected. Pellets loaded with herbicides had no effect on soil micromycete diversity compared to negative control soil samples. Almost all pesticides embedded in the matrix stimulated the growth and development of copiotrophic bacteria. The probable reason for this stimulation was the presence of an additional substrate for microorganisms capable of hydrolyzing P (3HB) and its degradation products, components of wood flour, peat, and humic acids. The use of clay as a filler did not affect the number of microorganisms in the soil [35].

The efficacy of Granules (P3HB / wood flour / metribuzin) and Granules (P3HB / wood flour / tribenuron-methyl) has been tested in weed-infested field crops. Micro-field experiments were carried out on the meadowchernozem thick heavy clay-loam soil at the field laboratory of Krasnoyarsk State Agrarian University located at the city of Krasnoyarsk during the growing season of 2019. The results of the presented studies in the field confirm the previously obtained laboratory data. In the field, granules with embedded metribuzin and tribenuron-methyl confirmed prolonged herbicidal activity. Metribuzin in test soil samples was fixed throughout the experiment from May to August. Its concentration increased to 30 mg / m2 in July and decreased to 15 mg / m2 by August. The concentration of tribenuron-methyl from May to July was kept at the level of 0.15–0.20 mg / m2, and in August traces of tribenuron-methyl were found in experimental soil samples. In control soil samples where free pesticides were used, the concentration of metribuzin decreases from 30 mg / m2 to 10 mg / m2 by July, and in August metribuzin is found in trace amounts. Tribenuron-methyl is less stable than metribuzin. In control soil samples, it was recorded only at the initial stage, in May. The researchers emphasize that the positive effect from the use of granules was not recorded at the initial stage of the experiment in June, but already in July the number of weeds decreased to the level of positive control, in which the free pesticide was applied. And by August, in crops using prolonged forms of pesticides, the number of weeds becomes significantly lower than in control crops [36].

4. Conclusion
An important scientific task is to create a fundamental basis for the construction and use of new generation agrochemicals in agriculture in order to reduce the risk of uncontrolled spread and accumulation of chemical products of the technosphere in the biosphere. In particular, PHAs can act as carriers or delivery vehicles for fertilizers or pesticides. However, the use of biodegradable polymers in agriculture is constrained by their high cost. One way to reduce the cost of PHA is to use inexpensive carbon substrates. Promising substrates are oils and fatty acids, which are formed as waste in some technological processes and provide a high PHA yield per unit of carbon substrate consumed, up to 70%. The availability of inexpensive PHA allowed us to start developing methods and technologies for creating new formulations of agrochemicals based on degradable PHA. The positive assessment of the effectiveness of the use of sustained-release preparations was confirmed in laboratory conditions: in soil ecosystems with known properties, containing higher plants infected with plant pathogens and weeds, it allowed to proceed to field trials

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