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THE WAYS TO INCREASE THE EFFICIENCY OF DEGRADATION OF PESTICIDES OF SULPHONYLUREA GROUP BY MICROORGANISMS-DESTRUCTORS

The most important component of destruction of herbicides in soil is their degradation by soil microbiota, which occurs due to the capability of microorganisms to adapt their enzymatic systems to specific substrates and to transform them. Bioaugmentation is one of the most ecological and economical methods of remediation of natural objects. However, the use of this way provides the need to receive a comprehensive information about migration, cumulation and transformations of pesticides in the environment (about dynamics) and also about factors, which increase the efficiency of focused application of bacteria-destructors (immobilization, introduction of additional substrates).

This research is focused on the study of the influence of different factors on the efficiency of degradation of pesticides of sulphonylurea group in soil. The results show that intensification of process of degradation of remaining amounts of tribenuron-methyl and metsulfuron-methyl in soil and liquid medium can be achieved by the exposure of bacteria-destructors to optimal conditions during cultivation as well as by the use of immobilized cells of microorganisms.

Key words: pesticides, immobilization, bacteria-destructors, tribenuron-methyl, metsulfuron-methyl, chromato-mass-spectrometry.

Introduction. The decisive role in growing good and stable yields belongs to herbicides (30–40% of stored crop). Average annual pesticide load on the agricultural lands of the Republic of Belarus, expressed in number of reactant of pesticides makes 0.6–1.0 kg a. s./ha [1]. Currently, for the protection of crops from weeds fourth-generation herbicides are used, the active ingredient of which is a derivative of sulfonylurea [2, 3]. The scope of application of these xenobiotics in the agricultural areas of our republic to the present time achieved e.g. on sown area of maize 80% of all herbicides used. In the agriculture of the Republic of Belarus herbicides from this group are presented 15 reactants on the basis of which 45 preparations of pesticides of sulfonylurea group (PGS) are permitted to use [4]. However, despite the low application rate (2 to 70 g/ha) appeared the data that the use of these pesticides has a negative impact on agrophytocenosis and their main components: agricultural soil, vegetation, ground and soil biota, water bodies [5]. Prolonged use of these pesticides is accompanied by adverse phenomena such as damage of sensitive crops, temporary depression of the biological activity of the soil, the emergence of resistant weed biotypes, etc. Along with pesticide residues, enough amount of persistent metabolites are found in the soil which complements the list of ecotoksikological problems associated with the use of pesticides [6]. One of the promising areas of environmental biotechnology is the introduction of active microorganisms-destructors of xenobiotics into soil contaminated by pesticides. Besides it is necessary to obtain comprehensive information on migration, cumulation and transformation of pesticides in natural environments (evolution), as well as factors increasing the efficacy of targeted use of bacteria-destructors (immobilization, making additional substrates). The result of the application of microbial products for solving the above problems is, in the first place, reducing yield losses for agricultural crops due to the phytotoxic aftereffect of herbicides residues in the crop rotations, as well as preventing the inclusion of pesticides in various migratory chains. In view of the above, the purpose of our work is the selection of the optimal culture conditions of bacteria-destructors, increasing the effectiveness of PSG degradation, as well as studying the possibility of using immobilized microorganisms for remediation of contaminated soils.

Main part. At present, a collection of soil bacteria-destructors metsulfuron-methyl (MSM) and tribenuron-methyl (TM) including 6 strains of bacteria-destructors MSM and 5 strain-destructors TM was created at the department of biotechnology and bio-ecology of the BSTU [7]. In the first stage of research were defined the optimal conditions for culturing bacteria T5, T6 and M1 which are destructors for TM and MSM respectively. The criterion for selection is the specific rate of the culture growth, using appropriate pesticides as the only carbon source. During the experiment, the following factors were varied: the temperature, degree of aeration, the concentration of the pesticide. Influence of herbicides on the growth of pure cultures of microorganisms was studied by seeding them onto dense glucose-saline medium MM9 with different concentrations of herbicides: 0.10, 0.05 and 0.01%. The results obtained after crop incubation

at 30°C for 48 h showed that the presence in a medium of herbicides at a concentration of 0.10 and 0.05% has an inhibitory effect on bacteria. In this regard, nutrient media containing 0.01% TM and MSM were used in further studies. The ratio of the microorganisms to temperature of the environment was established by culturing the bacteria-destructors in liquid medium MM9 with appropriate pesticides (0.01 vol. %). The experiments were carried out at 20, 25, 30°C. According to the results of the experiments, the curves of crop growth were built and the kinetic parameters were determined. It is established that the highest specific growth rate of cell for all tested bacteria destructors was shown at 20°C. This fact is explained by the way that these cultures are soil bacteria and have been isolated from natural sources (soil contaminated by the relevant pesticides); the annual average temperature of which is 16–18°C.

To determine the optimal degree of aeration, bacteria-destructors were cultured in a liquid salt medium MM9 with a pesticide as the only carbon source under the following conditions: temperature –20°C, rocking rotational speed – 0, 50, 100 and 200 rev/min. The concentration of the pesticide in the medium was monitored by HPLC-MS method. Standard solutions of herbicides with concentration, mg/ml 0.01; 0.02; 0.05; 0.10; 0.20 were used for constructing the calibration curve. Chromatogram of the extract T5 bacteria culture fluid is shown in Fig. 1. The highest rate of cell growth of strain T5 (destructors TM) has been demonstrated at the absence of aeration when the specific rate of M1 bacteria culture (MSM destructors) was maximal at the degree of aeration 50 rev/min. Cultivation of bacteria destructors TM under optimum conditions can increase biomass growth and increases the rate of degradation of mentioned pesticide, reduces cell adaptation to xenobiotics, wherein the residual amount of the toxic substrate decreased by (14.0 ± 1.5)% compared with control rates and made 24% from initial (Fig. 2, *a*).

Concentration of MSM in culture liquid of bacteria M1, cultivated at optimal parameters, decreased not significantly and was 37% of the original (Fig. 2, *b*).

The effectiveness of pesticide degradation is greatly improved by the immobilization of cells of microorganisms-destructors, as well as adding additional co-substrates in the environment [8].

Immobilized cells have a number of advantages both to the immobilized enzymes, and to free cells, as they have higher metabolic activity, resistance to high concentrations of xenobiotics, as well as the ability to create the conditions for auto-selection of strains, exchange of genetic material [9]. One of the most commonly practiced method is to immobilize the cells by their adhesion on the support surface [10].

Sorption method of immobilization is distinguished by cheapness, versatility, lack of stress susceptibility on the cells and simplicity of implementation. The number of immobilized cells increases with the specific surface of the carrier, and because of this dispersed, fibrous, perforated materials for securing microbial cells are used.

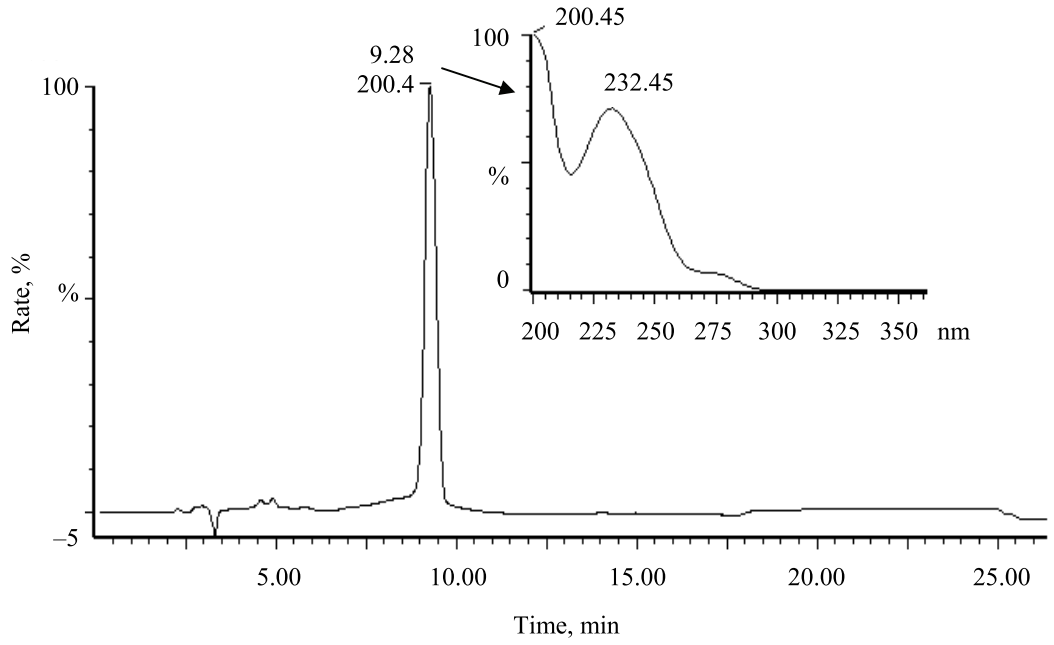

Fig. 1. Chromatogram of the extract of culture liquid T5 bacteria, cultured with TM as the only carbon source

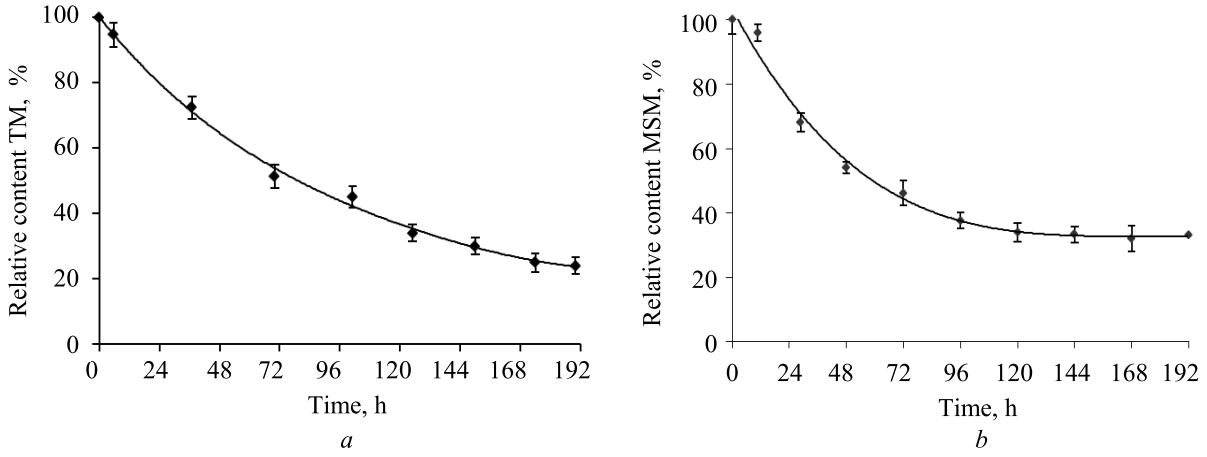

Fig. 2. Kinetic curves of pesticide degradation of sulfonylurea group by bacteria-destructors: *a* – TM; *b* – MSM

When used microbiological degradation in the processes of cleaning soils from PGSM, most commonly used natural carriers are peat, charcoal, etc. This approach eliminates the secondary contamination of the soil, besides peat initiates the development of both native microorganisms-destructors and introduced active cultures. In general, carriers may have both positive and negative effects on the physiology of microorganisms.

In this connection, the next step was microbial degradation investigation of TM and evaluation of the efficiency of the given process performed by bacteria destructors T5 in model soil system by both immobilized cells and free ones. The peat (deposit "Turshovka" selection time 03/09/2014, the degree of decomposition 20%, Ø 0,5–1,0 mm) was selected as the carrier. Laboratory test of method for bioremediation of soil contaminated with TM under model conditions, was carried out in glass Petri dishes. Cultures of microorganisms-destructors immobilized on peat, were introduced into soil contaminated with TM in an amount of 0.01% according to the following scheme:

– soil + peat + culture;
– soil + peat + pesticide;
– soil + culture + pesticide;
– soil + peat + culture + pesticide.

Contaminated soil was composted for 28 days at constant temperature corresponding to optimal for a given culture (20°C) and humidity 60% (of the total moisture capacity). Soil samples for microbiological and physico-chemical studies were selected on the day of the experiment and during the experiment in 1, 7, 14, 21 and 28 days. During the experiment, the content of the herbicide in the soil, as well as the number of bacteria-destructors were controlled. Chloroform was used for extraction xenobiotic from the soil. Determination of the concentration of herbicides in soil samples were determined by HPLC-MS method. The total number of microorganisms in the course of the experiment was determined by Koch plate method. The experimental results are presented in Fig. 3.

Study TM degradation (introduced number of 1 mg/g of dry soil) in model experiments with sterile soil showed that the concentration of TM for 28 days virtually unchanged, indicating that no chemical interaction of the compound with the soil components. TM degradation in soil containing immobilized introduced bacteria-destructors cells is already noticeable on the 7th day. TM is decomposed in the soil fast enough and after 14 days its residual amount was 15%, and in a month it was present in trace amounts (0.3%).

In the process of biodegradation TM in soil it was detected the presence of intermediate such as saccharin and 2-hydroxy-4-methyl-6-dimethylamino-1,3,5-triazin but they were subjected to further transformation rather fast.

The activity of the bacteria-destructors introduced into soil without prior immobilization, was much lower. Adaptation period of the bacteria was about 14 days, the decomposition of TM went quite slowly and in a month it made 39% of the original when the further decomposition of xenobiotic didn't occur.

In addition to the analysis of changes in the TM concentration in soil samples the development of introduced culture was examined as well. The number of CFU per 1 g of dry soil at the beginning of the experiment with immobilized cells was 6.1×107 cells/g. An analysis of the introduced crop growth showed that after the first seven days of exposure the number of CFU decreased by far, and then monotonically increased and was maximal on the 14th day of the experiment. Then there was a decrease of this indicator, due to the depletion of the main feed medium.

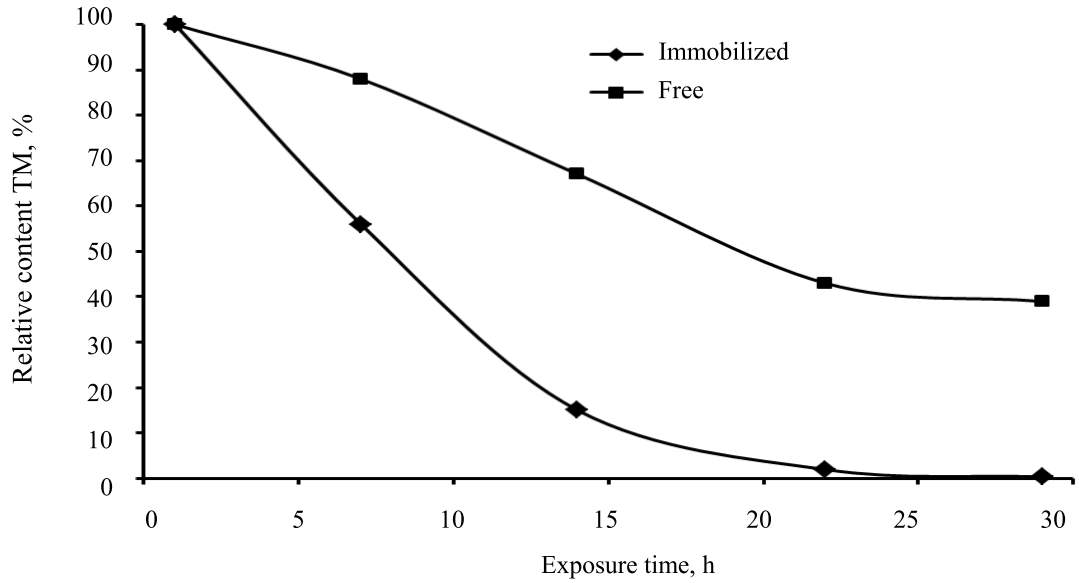

Fig. 3. Degradation dynamics TM by free and immobilized bacteria-destructors T5 in model-contaminated soil

Comparative analysis of the number of cells in model soil systems with TM and in the model soil system without TM showed that the maximum bacterial concentration of the destructor strain in the soil with TM is above the maximum concentration of mentioned microorganisms in the soil in the absence of herbicide.

This indicates that TM doesn't not have an inhibitory effect on the growth of bacteria-destructors cells, and vice versa, it is a growth substrate.

Conclusion. Thus, it was found that immobilized on peat the cells of strain T5 are able to carry out full degradation of TM in model-contaminated soil with high efficiency, which makes it possible to use these bacteria in soil bioremediation technologies polluted by this herbicide. The paper also experimentally determined the optimal conditions for culturing of bacteria destructors TM and MSM (the following parameters are defined: the degree of aeration, temperature, the initial concentration of the herbicide, the composition of the culture medium).

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