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Improving of Copper (II)-Ions Phytoextraction by Using Glycolipid Biosurfactants

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A B S T R A C T

Pollution of soil with heavy metals is a dangerous issue that endangers both the environment and human health. Phytoextraction, that implies planting contaminated area by previously selected species of plants having the potential to extract heavy metals from the soil, is common used technology. Some limitations of this technology revealed in difficulty to absorb the heavy metals, usually being in form of insoluble. For aim to increase the phytoextraction efficiency it is necessary to use agents able of solubilization of heavy metals. The aim of presented work is to test some biosurfactants for enhancing copper(II) phytoextraction by different plant species. For this purpose, the model experiments for cleaning of water and soils artificially contaminated with copper(II) ions have been carried out. The obtained results show that rhamnolipids and trehalose lipids increase the phytoextraction effectiveness significantly (2-3 times). Alfalfa with trehalose lipids are the most effective tools for cleaning soils contaminated with copper(II) ions. In this case removal of heavy metal from soil is achieved by 75%, and main part of removed copper(II) ions (approximately 60%) is translocated in upper ground parts of plant that is very important for performing phytoextraction process successfully.

Keywords: Heavy metals, Copper, Phytoremediation, Phytoextraction, Biosurfactants, Pollution

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Introduction

Heavy metals existing in environment cause the gross violation of natural equilibrium and processes of contamination of life importance ecosystems – ground and surface waters, soils, atmospheric air, vegetation (including cultivated verdure) get irreversible character, which reflects directly on the condition of health of population.

Especially dangerous and complex are the contaminations with persistent toxic substances, among which are petroleum products, heavy metals, chlorinated organic substances etc. [1]. They cause a violation of the structure, aeration, water exchange in soils, lead to changes in ecosystems and impossibility of their use in industry and agriculture.

Biological methods of restoration the environment (bioremediation, phytoremediation), that is the treatment with microorganisms and plants, are priority ones among the most promising and environmentally acceptable methods. Due to the great power of natural detoxification processes, interest in the ecological potential of microorganisms and plants has increased in the

last decades [2-6]. Microorganisms that transform organics play an important role in maintaining the ecological balance in various ecosystems and, due to their high degradation and transformation power, are successfully used for sewage and soil purification. Plants actively participate in soil and air remediation processes. Plants and microorganisms, together or individually, mainly through their powerful oxidative enzyme systems are capable to remediate environment polluted by a wide spectrum of contaminants.

Phytoremediation is a unique cleanup strategy [3-6]. The realization of phytoremediation technologies for cleaning of environment polluted with heavy metals (phytoextraction) implies planting contaminated area by previously selected species of plants having the potential to extract heavy metals from the soil. Some limitations of this technology revealed in difficulty to absorb the heavy metals, usually being in form of insoluble. For increasing efficiency of phytoextraction, it is necessary to use agents able of solubilization of heavy metals and improve their absorption by plants. Some preparations of biological origin, such as surface-active compounds bacterial origin (biosurfactants) possess metal-chelating properties that enable their application in phytoextraction of heavy metals [7].

The aim of presented work is to test some glycolipid biosurfactants for enhancing copper(II) phytoextraction by different plant species.

Materials and methods

Microbial synthesis of biosurfactants was conducted using the strains-producers of the genera *Pseudomonas, Rhodococcus and Bacillus* on optimized liquid medium with glycerol, mannitol, hexadecane and plant oil industry wastes (20 g/l) [8-11]. Monocolonial selection of the strains has been carried out for the enhancement of their activity and the improvement of the surfactant synthesis. Rhamnolipids were isolated from cultural broth via acid precipitation (10% HCl to pH 3) and following extraction of the obtained precipitate with Folch mixture (chloroform : isopropanol – 2 : 1). Trehalose lipids were isolated with the same extragent from cell mass. Polysaccharides were isolated from cultural broth via precipitation with 2 volumes of ethanol. The solvent was evaporated under vacuum. The lipids were analyzed via thin layer chromatography and HPLC. The content of rhamnolipids was determined using orcinol method, trehalose lipids – using antron method.

The isolated biosurfactants we investigated by the following parameters: surface tension of solutions, critical micelle concentration (CMC), emulsification index (E24), thin layer chromatography, UV- and IR-spectroscopy.

The following preparations of biosurfactants were prepared:

- Rhamnolipids,
- Trehalose lipids,
- Rhamnolipid biocomplex PS,
- BR1 – complex of biosurfactants produced by strain of *Rhodococcus* sp. 50,
- BP1 – complex of biosurfactants produced by strain of *Pseudomonas* sp. 6R67,
- BB1 – complex of biosurfactants produced by strain of *Bacillus* sp. 3Zu9.

Plants. The following plant species: rape (*Brassica napus*), soybean (*Glycine max*) and alfalfa (*Medicago sativa*) were used as phytoremediators.

Model experiments. In the model experiments the soil samples contaminated artificially, as well as polluted as a result of oil pipeline accidental spills, were used.

The model experiments were carried out according to the following scheme: the suspension of microorganisms and solutions of biosurfactants was inoculated in the contaminated soil in the beginning of experiment. After different incubation times the plants were sowed in separate samples of soil. The conditions and details of each experiment are described in legends of figures.

Analysis of copper(II) ions. For analysis of Cu2+ content in plant and water samples have been determined sphectrophotometrically by measuring the absorption of the Cu(II)-dithizone (1,5-Diphenylthiocarbazone) complex at 553 nm, according to Kumar et al. [12].

Results and Discussions

For selection of biosurfactants and polysaccharides, chelating heavy metals to increase efficiency of their absorption by root

system and transportations in upper ground parts of plants, 7 preparations of biosurfactants were tested.

The following plants and biosurfactants were tested in the model experiments:

- Plants - soybean, alfalfa and rape (growing on hydroponic area);
- Copper(II) ions as model heavy metal (concentrations –100 and 200 ppm);
- Preparations of biosurfactants (concentration – 0.1 g/l):
  - o Rhamnolipids
  - o Trehalose lipids
  - o Rhamnolipid biocomplex PS
  - o BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50
  - o BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67 and
  - o BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9
  - o BB2 – complex of biosurfactants produced by strain of Baccilus spp.

For carrying out of experiments, seedlings of the plants were placed in special tubes on hydroponic media containing different concentrations of copper(II) ions and tested biosurfactants. After 7 days from the start of experiments, copper(II) contents were measured in growing medium and plant seedlings (separately in roots and upper parts). The obtained results are presented on Figures 1-4.

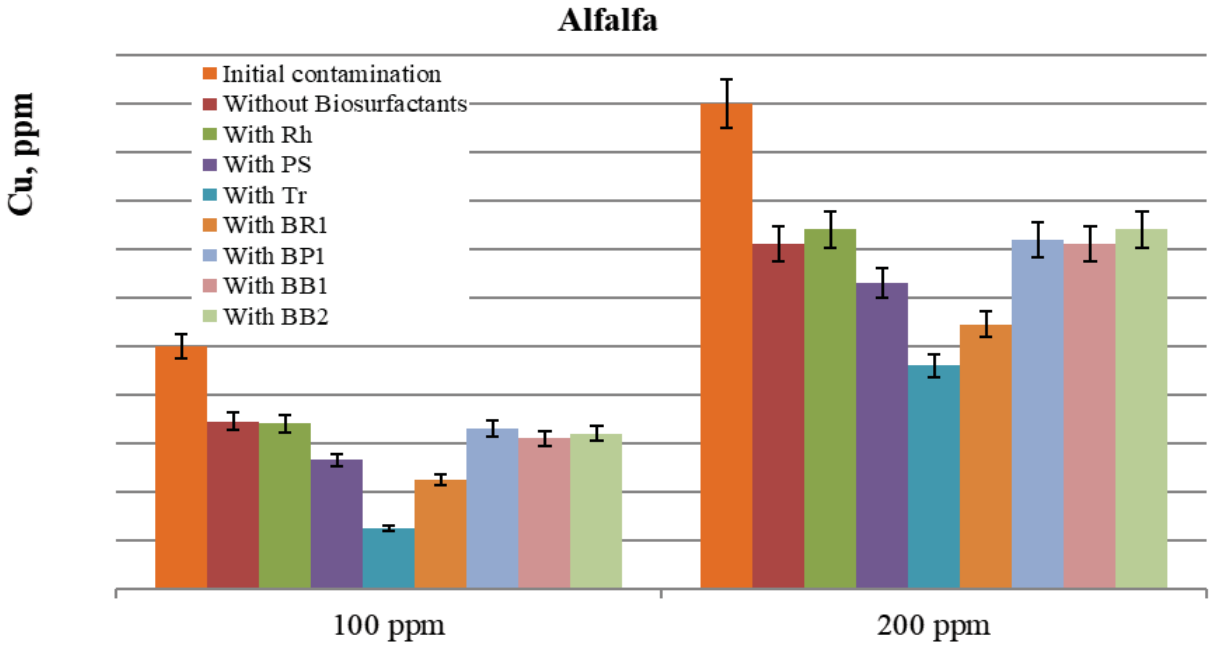

Fig. 1. The absorption of copper(II) ions from hydroponic media by alfalfa seedlings and the influence of different preparation on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

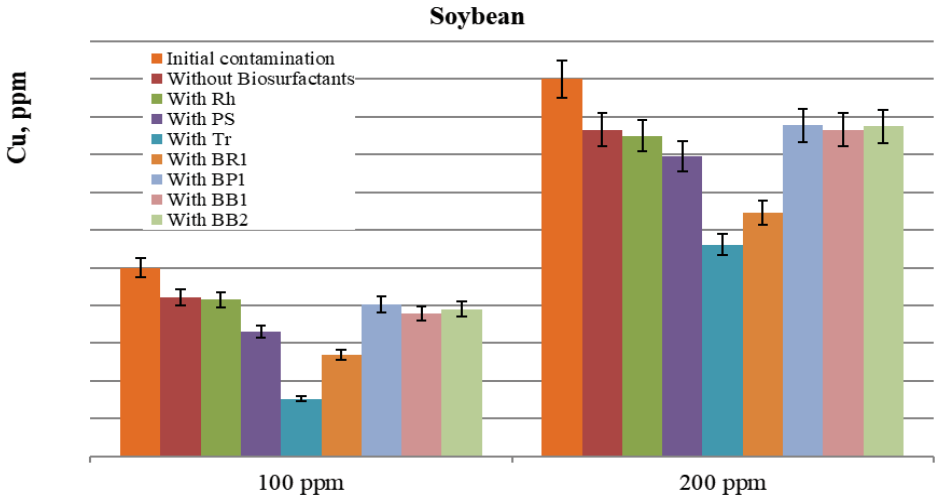

Fig. 2. The absorption of copper(II) ions from hydroponic media by soybean seedlings and the influence of different preparation on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

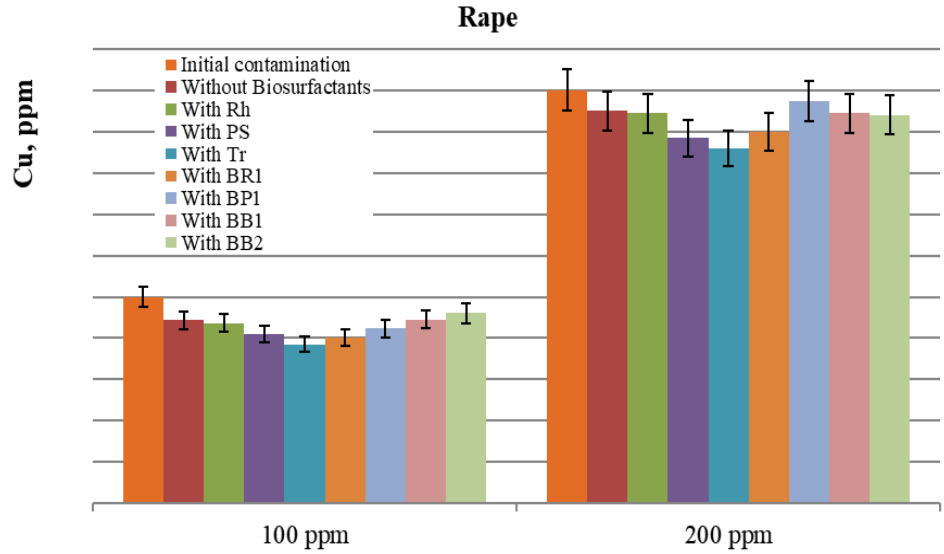

Fig. 3. The absorption of copper(II) ions from hydroponic media by rape seedlings and the influence of different preparation on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants p roduced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

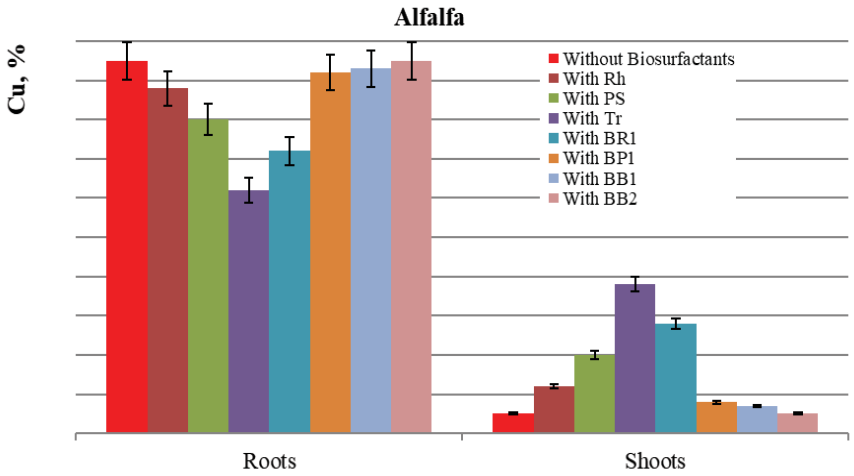

Fig. 4. The distribution of absorbed from hydroponic media copper(II) ions between roots and shoots of alfalfa seedlings and the influence of different preparations on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

The obtained results show that after penetration in plants, copper(II) ions are located mainly in the roots of the plant. Among the tested plants, alfalfa revealed the highest ability to uptake copper(II) ions (coefficient of bioaccumulation equals 78 mg per kg of dry biomass). After alfalfa, came soybean (64 mg/kg) and rape (37 mg/kg).

Trehalose lipids sharply increase the process of translocation of copper(II) ions in the upper parts of plants, but in case of high concentrations of copper (100 and 200 ppm), so much quantity of heavy metal penetrates into plants that it becomes lethal to them. In case of using rhamnolipid biocomplex PS similar results are observed only at 200 ppm concentration of copper(II) ions. In case of biopreparation BP1 the increasing of uptake of copper(II) ions and intensification of their translocation in upper parts of plants is observed. Other 4 preparations do not affect absorption of copper(II) ions by plants and their translocation.

The optimal concentration of tested biosurfactants equals 0.1 g/l.

The model remediation experiments in laboratory and greenhouse conditions for cleaning of soils artificially contaminated with heavy metals (copper(II) ions) have been carried out.

The model experiments were carried out according to the following scheme:

1. The soil samples were dried, sieved and artificially contaminated with different concentrations of heavy metals (50 and 200 ppm according to content of metal ions in dried mass of soil). Heavy metals were added on soil in the form of copper(II) acetate water solution.
2. The plants (soybean and alfalfa) were sowed in separate samples of soil.
3. For intensification of copper phytoextraction, after 3 weeks from the beginning of experiments, the following preparations were added to soil samples (concentration 0.1 g/l) as metal chelating agents:
   a. Rhamnolipid biocomplex PS
   b. Trehalose lipids
   c. Biopreparation BP1 (complex of biosurfactants produced by strain of Pseudomonas sp. 6R67)
   d. EDTA (for comparison of biopreparations effects with chemical chelating agent)

The model experiments were carried out during 30 days at greenhouse conditions (temperature 22-27°C). On 10th, 20th and 30th days of experiment the content of copper in soil samples was measured. After finishing the experiments, copper contents were measured also in plant seedlings (separately in roots and upper parts) (Table).

Table. *Concentration of copper(II) ions in soil during remediatin experiments by using alfalfa and different chelating agents. Initial concentration of copper(II) ions was equaled 50 ppm*

Chelating agent	Concentration of copper(II) ions in soil, ppm				Concentration of copper(II) ions in dried plant organs, ppm	
	Initial	After 10 days	After 20 days	After 30 days	In roots	In shoots
Control[1]	50	44	35	27	21	n.d.[2]
PS[3]		37	30	24	18	7
Tr[4]		18	15	12	11	16
BP1[5]		42	37	32	12	5
EDTA[6]		6	3	1	5	42

[1] – without chelating agents

[2] – n.d. – not detected

[3] – PS – Rhamnolipid biocomplex PS

[4] – Tr – Trehalose lipids

[5] – BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67

[6] – EDTA – Ethylenediaminetetraacetic acid

As it seen from obtained results, using plants as phytoextractors without chelating agents has less effectiveness in case of contamination of soil with copper(II). Chelating agents increase the effectiveness of cleaning the soil from heavy metals significantly (2-3 times). The effectiveness of tested preparations for their application for enhancing of copper phytoextraction increases in the following order:

Biopreparation BP1 < Rhamnolipid biocomplex PS < Trehalose lipids < EDTA.

It is worth noting that tested biopreparations are much less effective than chemical chelating agent EDTA.

Alfalfa with biopreparation of trehalose lipids are the most effective tools for cleaning soils contaminated with copper(II) ions. In this case 75% removing of heavy metal from soil is achieved, and main part of removed copper(II) ions (approximately 60%) is translocated in upper ground parts of alfalfa.

Conclusion

The obtained results allow drawing following conclusions:

- Among the tested plants, alfalfa revealed the highest ability to uptake copper(II) ions (coefficient of bioaccumulation equals 78 mg per kg of dry biomass).
- Rhamnolipids and trehalose lipids increase the phytoextraction effectiveness of copper(II) ions significantly (2-3 times).
- Alfalfa with trehalose lipids are usable for cleaning soils contaminated with copper(II) ions, because effective phytoextraction of heavy metal from soil is achieved, and main part of removed copper(II) ions is translocated in upper ground parts of plant.

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