Reclamation of technogenically damaged territories of a sulfur production enterprise using the method of stimulating microisogenesis in plants

Viktorija Oliferchuk¹, Mariia Samarska¹, Oleg Nagurskyy², Viktor Vasiichuk², Kateryna Vykhivska²

¹ Department of Ecology, Ukrainian National Forestry University, Gen. Chuprynky Str. 134, Lviv, 79057, Ukraine, victorijaoliferchuk@gmail.com

² Viacheslav Chornovil Institute of Sustainable Development, Lviv Polytechnic National University, S. Bandera Str. 12, Lviv, 79013, Ukraine, oleg.a.nahursky@lpnu.ua

Abstract - The possibility of regulating soil fertility by influencing the "bacterium-fungus-plant" system by stimulating mycorrhizal formation in plants has been established. The conducted research makes it possible to develop a model of biological reclamation of disturbed lands using the process of inoculation of plants to maintain the soil ecosystem.

Keywords: reclamation, tailings, sulfur, mycorrhizae.

Introduction

The development of humanity is accompanied by an increase in the area of disturbed lands and a reduction in the number of natural ecosystems, a decrease in their regenerative capacity, and resistance to the action of anthropogenic factors. The placement of mining waste on the surface causes significant damage to natural landscapes.

Mining waste is the products of extraction and processing of mineral raw materials, which are separated from the mass of mined mineral during the development of the deposit, beneficiation, chemical and metallurgical processing of raw materials, which are not used. After the cessation of production at the Yavorivsk SMCE "Sirka" there were tailings deposits, the reclamation of which was not carried out. During the period from the shutdown of the enterprise to the present day, at the tailings storage facilities took place the succession process. As a result of this, an uneven (with a large number of gaps, where dumps protrude to the surface), thin layer of soil was formed on the surface of the basements.

Unreclaimed tailings lead to chemical pollution with sulfur, acidification, geomechanical deformation of the earth's surface, hydrological changes in the form of water flows direction changing and acid drainage. The reclamation of sulfur-bearing areas, especially those which was extracted by Frasch technology, is difficult and complex, since this production leads to significant and multifactorial environmental transformations.

Both disruption of ecosystems and their restoration can be natural or anthropogenic. Natural recovery of disturbed ecosystems occurs according to certain patterns. Among them is a gradual change of species due to the properties of the disturbed landscape and the biological properties of organisms. Such change in groups of organisms are called succession. Natural reclamation of the territory of conserved tailings is not enough to restore disturbed ecosystems. Therefore, these species cannot live there.

In order to engraft plants and their effective growth and fruiting, provided that disturbed areas are covered with a layer of fertile soil, methods of plant inoculation with bacterial preparations are used.

The association of microorganisms, which is proposed to be used for inoculation of the root system of plants and seeds, was declared. Research was conducted to identify the compatibility of the joint action of a number of drugs and their proportions in the created mixture:

"Mykovital" - 3 parts (active agent *Vitasergia svidasoma Oliferchuk* IMB F -100106), "Planriz - Bio" - 10 parts (active agent *Pseudomonas fluorescens*), "Nitrogen fixer" - 30 parts (active agent *Bradyrhizobium japonicum*), "Florabacillin" - 10 parts (active agent *Bacillus subtilis*), Whey - 10 parts (active agent *lactobacillus*), Fertilizer "Agrobolik universal" - 3 parts (used as a corrector of trace element deficiency in plants, as a stimulator of their intensive growth and development and as an immunity booster. Contains an extract of humates from leonardite (a highly oxidized type of lignite), humates from oceanic brown algae and a complex of naturally occurring trace elements in a form easily accessible to plants. The composition is dominated by biologically active components: humic and fulvic acids, which increase the concentration of humus and soil fertility), Fertilizer "Bor Agrobolik" - 3 parts (the same composition as in Fertilizer "Agrobolik Universal" plus molybdenum and boron).

Experiment No.1: The growth of bacterial colonies and the endophyte of the black truffle *Vitasergia svidasoma Oliferchuk* IMB F -100106 was studied on Petri dishes on wort-agar medium, modified VPD medium, medium for bacteria (1% peptone water (pH 7.4), 1% molasses and 1.5% agar. The growth of all bacteria and the endophyte *Vitasergia svidasoma* on the plates had the appearance of a continuous lawn, on liquid media of the same composition, the growth of the cultures corresponded to the logarithmic phase of growth.

Experiment No.2. The compatibility of bacteria with the black truffle endophyte *Vitasergia svidasoma Oliferchuk* IMB F -100106 was studied on the agar media described in experiment No.1. Compatibility was determined by examining the presence of sterile growth zones around paper discs impregnated with cultures of *Pseudomonas fluorescens, Bradyrhizobium japonicum, Bacillus subtilis, Vitasergia svidasoma Oliferchuk* IMB F -100106 and lactobacilli. Sterile zones are not recorded.

Experiment No. 3 We studied the effect on the growth and reproduction of bacteria of the endophyte *Vitasergia svidasoma*. *Oliferchuk* IMB F -100106. *Vitasergia svidasoma Oliferchuk* IMB F -100106 was added to liquid media with *Pseudomonas fluorescens, Bradyrhizobium japonicum, Bacillus subtilis*, and lactobacilli during cultivation. Flasks with bacteria without the use of endophytes served as controls. After 3 days of cultivation, the number of bacterial cells in the flasks with the addition of *Vitasergia svidasoma Oliferchuk* IMB F -100106 increased, in the case of *Pseudomonas fluorescens* - by 32% compared to the control, *Bradyrhizobium japonicum* - by 37% compared to the control, *Bacillus subtilis and lactobacilli* - by 35 % compared to the control.

Experiment No.4. "Agrobolik universal" fertilizer was added to each of the cultivated microorganisms. In the growth curve of the periodic culture, stimulation of the exponential phase of growth and a decrease in the time of the lag phase were recorded. (In the lag phase, the culture adapts to the new cultivation conditions, synthesis of mRNA, inducible enzymes or an increase in the activity of enzymes necessary for assimilation of new components of the environment occurs, the number of cells practically does not change).

Experiment No.5. Fertilizer "Bor Agrobolik" with molybdenum and boron was added to each of the cultivated microorganisms. In the growth curve of the periodic culture, an increase in the time of the lag phase by 18 hours was recorded, and therefore the drug has a stressful effect on microorganisms. In the exponential phase of growth, which begins after adaptation of the cells to the cultivation conditions, the natural increase in the geometric progression of bacterial cells did not occur.

Conclusions

The association of bacteria *Pseudomonas fluorescens, Bradyrhizobium japonicum, Bacillus subtilis* and lactobacilli with the addition of the endophyte *Vitasergia svidasoma Oliferchuk* IMB F -100106 can become an effective inoculant for plant roots and their seeds, and promote better rooting and increase plant immunity, for agricultural plants can increase yield.

Creation of an association is possible immediately before inoculation. Each type of bacteria and endophyte requires certain cultivation conditions and appropriate environments.

The addition of "Agrobolik universal" fertilizer to the association of microorganisms will contribute to additional nutrition of microorganisms at the beginning of their settlement after the inoculated plant or its seeds have entered the soil and will contribute to better adaptation of microorganisms. We also recommend adding "Liposam" or "Agrolip" for additional nutrition and adhesion of cells to the roots of plants and seeds.

Addition of "Boron Agrobolik" fertilizer plus molybdenum and boron to the association of microorganisms must be done very carefully and only in soils with a strong deficiency of these trace elements. It is better to apply this fertilizer when needed, separately from the declared association.

References

1. Biletskoho, V. S. (2013). Mala hirnycha entsyklopediia [Small mining encyclopedia]. (Vol. 3). Donetsk. : Skhidnyi vydavnychyi dim, 644p. (in Ukrainian).

2. Kent, J.A (2007). Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology. 1. p. 1171. ISBN 978-0-387-27842-1.

3.Schreiner, B. (2008). Der Claus-Prozess. Reich an Jahren und bedeutender denn je. *Chemie in unserer Zeit.* 42 (6), 378–392.

4.Eow, J. S. (2002). Recovery of sulfur from sour acid gas: A review of the technology. *Environmental Progress*. 21 (3): 143–162.

5. Hyndman, A. W., Liu, J. K., & Denney, D. W. (1982). Sulfur Recovery from Oil Sands. *Sulfur: New Sources and Uses. ACS Symposium Series.* 183, 69–82. doi:10.1021/bk-1982-0183.ch005. ISBN 978-0-8412-0713-4.

6.Oliferchuk V., Kendzora N., Shukel I., Samarska M., & Olejniuk-Puchniak O. (2023). The role of V-strategist endophytes in stimulating the formation of mycorrhizal interactions and soil regeneration, *Symbiosis in Nature*, 269–2023. DOI: 10.5772/intechopen.109912

7.Oliferchuk V., Fedorovych D., Samarska M., Bunetsky V., Samborskyy M., Kachor A., . . . & Hotsii N. (2022) Changes in the structure of myco- and microbiocenosis of soil when using immobilized on biochar strains of fungi and bacteria as an example of ecosystem maintenance services. *Ecological Engineering & Environmental Technology*. 6, 442–452. DOI: https://doi.org/10.12912/27197050/152522

8.Oliferchuk V., Fedorovych D., Kopiy L., Kravtsov D., Kendzora N., Krynytskyy H., ... & Ahiy V. (2023). Structure of Microscopic Fungal Species in Soils at Amber Mining Territories and during the use of New Technology of Pine Plantation Formation. *The Open Agriculture Journal*. 17. DOI: <u>10.2174/18743315-v17-e230120-2022-12</u>

9.Oliferchuk V., Kendzora N., Hotsii N., Shukel I., Olejnyuk-Pukhnyak O., Samarska M., Nahurskyi O., & Vasilchuk V. (2023) Changes in the Structure of Soil Microscopic Fungi in the Territories of Yavoriv and Podorozhenie Sulfur Quarries. *Ecological Engineering & Environmental Technology*. 4(3), 120–134. DOI: 10.12912/27197050/159629