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Alvaro Rocha
Ekaterina Isaeva *Editors*

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Methodological Basis of Ground Composition and Traits Formation by Biotechnological Techniques

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Abstract. Microorganisms are widespread in grounds of different composition and genesis. Theoretical and experimental studies demonstrated that metabolic activity of microorganisms led to changes in solid, liquid, and gas components of ground. This capability of microorganisms could be used to form grounds with a given composition and traits. The proposed article describes the innovative methodology for the formation of specified ground traits, based on activation of ground microorganisms. The methodology includes following stages: the assessment of solid, liquid, and gas components of a ground; the identification of ground microorganisms; the choice of microorganisms to use; the development of technologies to activate chosen microorganisms; and the development of technologies to form specified ground traits. The practical implementation of the methodology was patented.

Keywords: Ground traits · Microorganisms · Innovative methodology

1 Introduction

Microorganisms are the numerous living organisms of our planet inhabiting almost all elements of the geological environment [1–5]. Microorganisms are widespread in grounds of different genesis and are the part of their biotic component. The number of microorganisms depends on the type of grounds and their chemical composition, and varies greatly, however, the direct dependence of their number on the content of organic substance is established [6, 7]. Microbial populations of grounds are able to decompose various substances, but the optimal development of microorganisms demands certain conditions [2].

The metabolic products of microorganisms are their biomass, various oxides that easily return to geochemical cycles, and gases. The exclusive role of microorganisms in biogeochemical processes was noted by Vinogradsky [1], Isachenko [8], Kuznetsov [9], and Vernadsky [10]. The physiology of microorganisms and their influence on minerals, organic chemicals, and gases give the reason to expect that they will have a substantial effect on the traits of ground components, thereby changing the mineral composition of the ground, its structure, and physical and mechanical traits.

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A limited number of papers describes the influence of microorganisms on the composition and traits of grounds [11–17]. Dashko [18] examined natural and technogenic factors affecting the microbiota in the underground space of the metropolis, and showed that the activity of microorganisms induced the irreversible transformation of ground traits and contributed to corrosion of industrial materials. She studied also the biochemical gas generation in grounds and the effect of microorganisms on mechanical traits of water-saturated sands [19].

However, the influence of microorganisms on the composition and traits of the solid, liquid, and gas components of grounds has not been sufficiently studied yet. The purpose of this paper is to develop a methodology for the formation of ground composition and traits by biotechnological techniques. This methodology includes several stages, sequential execution of which should give necessary results. It should be noted, that each stage of the methodology could be performed with numerous methods or techniques preferred by the researcher.

2 Formation of Ground Solid Component's Traits by Biotechnological Techniques

2.1 Assessment of the Ground Traits, Mineral Content, and Particle Size Distribution

The studies were carried out on the territory of Perm on the right side of the river Kama, where alluvial sands of quaternary age lied under a thin turf-soil layer – fine-grained dense sand occured to the depth of 0.2 m, and medium-grained medium-dense sand underlied. Fine-grained dense sand contained (mass %): quartz – 84.2, feldspar – 12.3, hydromica – 3.5. Neither calcite nor gypsum were detected [20]. Another traits of ground are thorough described in [20], some of them are presented below in comparison with ones after microbial treatment.

2.2 Assessment of the Microbiological Content of the Ground

The analysis of the microbial component of the ground was done using corresponding media and 10-fold dilutions with 3-tube most probable number technique as described [20]. The analysis revealed the presence of organotrophic aerobic microorganisms in it. Their number was several millions cells per gram of the ground. Ammonifying microorganisms were also detected. The number of this group of microorganisms reached up to 1 million cells per gram of the ground. All of ammonifying microorganisms were capable to metabolize urea. Microorganisms capable of denitrification and iron reduction were also revealed in the ground. The number of each of these groups was several thousands cells per gram of the ground [20]. This identified groups of microorganisms form carbon dioxide during their growth on organic compounds. The rise of carbon dioxide content could lead to precipitation of calcite cement in ground pore space. Calcite cement precipitation is an ubiquitous process which plays an important role in natural systems [21]. The influence of identified groups of microorganisms on ground components' traits is presented in Table 1. To choose the group of microorganisms suitable to the formation of specified traits of the ground it is need to consider the conditions of the environment and the requirements of microorganisms to proliferate in it.

The aerobic environment conditions does not favour to the development of denitrifying and iron reducing microorganisms in the ground. Moreover, an activation of these groups of microorganisms would require not only the generation of anaerobic conditions in the ground, but the introduction of biomass of these groups of microorganisms into the ground due to their low abundance. An activation of denitrifying microorganisms requires also the addition of nitrate salts to the ground. It should be noted that the activity of the iron-reducing microorganisms is usually accompanied by the destruction of the ferrous cement, that binds the ground particles, and a subsequent decrease in the strength characteristics of the ground. We suppose, this decrease will be difficult to compensate for the precipitation of calcite cement. Calcite precipitation is favored by alkaline conditions, so the use of aerobic organotrophic microorganisms will require the addition of organic substances and alkaline supplements to the ground. The metabolism of urea by ammonifying microorganisms is accompanied not only by the formation of carbon dioxide, but the formation of alkaline conditions due to the release of ammonia. These reasons predetermined the choice of ammonifying microorganisms for their subsequent use in the formation of ground solid component's traits.

Thus, microorganisms influencing on the ground composition and traits were identified in the studied ground. To form the specified ground traits, it is necessary to develop a technology for microorganisms activation, which is discussed below.

2.3 Microorganism Activation Technologies for Specified Ground Traits Formation

To give the ground specified traits, it is necessary to activate corresponding groups of microorganisms in the ground. Methods of microorganisms activation include a biomass addition and a the selection of nutrient medium content, as well as the generation of environment conditions (ground temperature, humidity, etc.) suitable for microorganisms optimal vital activity. Laboratory and field investigations were carried out to study this items.

Laboratory Investigations. The effect of the amount of biomass of ammonifying microorganisms on the ground traits was studied [22]. Ground traits were analyzed according to government prescribed methods. The duration of laboratory experiments was 15 days.

The increase of biomass of ammonifying microorganisms was accompanied with the increase of the deformation modulus of fine-grained sand of 35–80% versus abiotic control. The study of activation of ammonifying microorganisms with urea demonstrated that the volume of released gas in the sand increased. The effect of ammonifying microorganisms on another ground traits were described also [22].

According to results of the study, to stimulate the growth of ammonifying microorganisms in the ground, the nutrient medium had to contain glucose and nutrient broth; to activate vital activity of this microorganisms medium had to contain urea.

Table 1. The influence of identified groups of microorganisms on ground components' traits

Changes in ground traits			Group of microorganisms	Processes in ground solid component
Solid component	Liquid component	Gas component		
Decrease of porosity; Increase of strength	Increase of pH in pore solution	No changes	Aerobic organotrophs	Precipitation of $CaCO_3$ in pore space of a ground
Decrease of porosity; Increase of strength	Increase of pH in pore solution	No changes	Ammonifying microorganisms	Precipitation of $CaCO_3$ in pore space of a ground
Decrease of porosity; Increase of strength	Increase of pH in pore solution; Decrease of moisture	Increase of gas presence; Increase of gas content	Denitrifying microorganisms	Precipitation of $CaCO_3$ in pore space of a ground
Decrease of porosity; Increase of strength	Increase of pH in pore solution	No changes	Iron-reducing microorganisms	Precipitation of $CaCO_3$ in pore space of a ground; Dissolution of ferrous cement

Field Investigations. Four field plots, 1.0 × 1.0 m in dimensions, were prepared and investigations were carried out as described [20]. The upper topsoil layer from the plots was removed completely, and surface of the plots was leveled. Two plots were experimental, two plots were used as a blank control.

Each of the test plots was initially treated with 40 L of a solution contained 200 g of urea, 40 g of glucose, and 60 g of dry nutrient broth. In addition, 4 g of biomass of ammonifying microorganisms was added to the one of the test plot. Each control plot was treated with 40 L of water.

The second treatment of the plots was performed 10 days after. Forty liters of a solution contained 1480 g of $CaCl_2$, 800 g of urea, 40 g of glucose, and 4 g of dry nutrient broth were used to treat each experimental plot. Forty liters of water were used to treat each control plot. The treatment was repeated twice more at 10 days intervals between treatments, after that physical and mechanical traits (*i.e.* density, porosity, moisture, particle size distribution, deformation modulus, filtration rate) of ground samples of experimental and control plots were analyzed according to government prescribed methods, The Russian Federation State Standards, as described [20]. The duration of field experiment was 30 days. The differences in the traits of experimental and control plots' samples was assumed as microbial effects.

An activation of aboriginal microorganisms of the ground increased the density of ground from 1.64 g cm^{-3} to 1.72 g cm^{-3}, and reduced it porosity from 0.725 to 0.682 and filtration rate from 5.76 m day^{-1} to 1.04 m day^{-1}. Similar changes were characteristics of the ground samples from the depth of 0.2 m. Activation of aboriginal microorganisms of the ground affected on particle size distribution and mineral content of the ground also. Results of XRD analysis and scanning electron microscopy analysis revealed the formation of calcite particles in pore space of the ground as the result of ammonifying microorganisms activation. This particles cemented quartz and feldspar grains. Calcite was not detected in the samples of control plots [20].

Thus, laboratory and field investigations made it possible to identify the general patterns of change in the composition and traits of ground contained activated microorganisms, and to determine environment conditions and composition and amount of microbial biomass needed for the work of microorganisms in optimal mode. The results of the investigations were patented (patent no. RU 2646279). A logical continuation of theoretical and experimental research was the development of a methodology for the formation of specified ground traits by activation of microorganisms.

3 Methodology of the Formation of Specified Ground Traits by Biotechnological Techniques

The results of theoretical and experimental studies [1–3, 7, 9, 21] showed, there was a sufficient number of diverse microorganisms, capable of both decomposing and producing various compounds, in microbial populations of grounds. The formation of specified ground traits using microorganisms should take into account physiological characteristics of microorganisms. Different environment conditions contribute to development of different groups of microorganisms, and a change of environment conditions entails corresponding change in ground microflora [23].

The proposed methodology is based on the generation of necessary external and internal conditions in a ground (redox potential, pH of pore solution, chemical composition, etc.) by activation of chosen microorganisms, which accomplish certain physical and biochemical processes, resulted in specified traits of a ground. The methodology of the formation of ground composition and traits by biotechnological techniques should include following stages:

- assessment of mineral content and particle size distribution of a ground;
- evaluation of ground pore solution pH, ionic composition of film water on ground particles, and groundwater pH and ionic composition;
- assessment of composition of gas component of a ground;
- assessment of microbiological component of a ground;
- grouping of microorganisms according to the type and the intensity of their effect on the formation of specified traits of grounds and rocks;
- choise of appropriate group of microorganisms, and development of technologies to activate it;
- development of technologies to form specified composition and traits of a ground.

Some groups of microorganisms and methods of their activation for the formation of specified traits of dispersed grounds are presented in Table 2.

Table 2. Group of microorganisms, which could be used for the formation of specified traits of dispersed ground

Changes in ground traits			Group of microorganisms	Processes in ground solid component
Solid component	Liquid component	Gas component		
Decrease of porosity; Increase of strength	Decrease of pH	Not changed	Nitrifying microorganisms	Addition of ammonium or nitrite salts; Generation of aerobic conditions
Increase of porosity; Decrease of strength	Decrease of pH	Not changed	Sulfur- and sulfide-oxidizing acidophilic microorganisms	Addition of sulfur or sulfidic salts; Generation of aerobic conditions
Decrease of porosity; Increase of strength	Increase of pH	Not changed	Ammonifying microorganisms	Addition of aminoacids; Addition of urea
Decrease of porosity; Increase of strength	Increase of pH; Decrease of moisture	Increase of gas presence; Increase of gas content	Denitrifying microorganisms	Addition of nitrate salts and organic compounds; Generation of anaerobic conditions
Decrease of porosity; Increase of strength	Increase of pH	Not changed	Iron-reducing microorganisms	Addition of ferric salts and organic compounds; Generation of anaerobic conditions
Decrease of porosity; Increase of strength	Increase of pH	Not changed	Sulfate-reducing microorganisms	Addition of sulfate salts and organic compounds; Generation of anaerobic conditions
Increase of porosity; Decrease of strength	Not changed	Increase of gas presence; Increase of gas content	Methanogenic microorganisms	Addition of formate or acetate or methanole; Generation of anaerobic conditions

4 Conclusion

Theoretical and experimental studies of the influence of ground microbiota on ground components' traits demonstrated that certain man-made changes in environment conditions could activate metabolism of definite groups of microorganisms resulted in changes of ground traits. These findings made possible to develop the innovative methodology for the formation of specified ground traits, based on the activation of ground microorganisms. The methodology includes following stages: the assessment of solid, liquid, and gas components of a ground; the identification of ground microorganisms; the choice of microorganisms to use; the development of technologies to activate chosen microorganisms; and the development of technologies to form specified ground traits. The practical implementation of the methodology *in situ* was patented for dispersed grounds. We suppose this methodology has no limitations being implemented to any ground *ex situ.*

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