

Zeolites in complex nitrogen biofertilizers

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Abstract

A novel technology for producing complex, ecologically safe biofertilizer, combining advantageous properties of both zeolites and nitrogen-fixing microorganisms has been developed. The resulted biofertilizer is a complex of high-activity nitrogen-fixing strain of *Azotobacter chroococcum*, isolated from the soil in Armenia and the local zeolite modified by the new technology considering the specific features of this microorganism. The new technology for producing the mentioned nitrogen biofertilizer of multiple and prolonged action is conceptually different from known, and has no analogs.

Keywords: biofertilizer, modified zeolite, nitrogen-fixing strain.

1. Introduction

Zeolites are shown to be a group of minerals of volcanic origin can be used in soil improvement and fertilization. Two major properties of zeolites, particularly one- and two-valent cation exchange capability and ability to absorb moisture as well as their adsorbing (cleaning) property, due to which the soil is decontaminated from radioactive nuclides and heavy metals, contribute to their expedient exploitation in agriculture. Zeolites convert the latter into insoluble compounds and reduce the soil acidity. Ecological properties of minerals are supplemented also with a plenty of microelements contained, which play the important role in improving soil quality.

All above noted features of zeolites favor to propagation of useful soil microflora, in particular nitrogen-fixing microorganisms. Zeolitized tuffs are efficient on soil and the plants growth due to their specific peculiarities. Zeolite absorbs moisture in soil, keeping it for a long period and slowly and constantly supplying the plants with water; prevents diseases of plant roots. Zeolite is a source of microelements. It reduces nitrates in soil and fruits in 7-38 %. Zeolites can increase productivity of various agricultural crops by 10-30 %. The productivity rises up to 50 % while combining nitrogen-phosphorous fertilizers and zeolite containing rock [2-6]. In fact, natural zeolites are natural mineral fertilizers. However, nowadays the application of zeolites in agriculture goes beyond by "crude" zeolites. The successful examples of improved preparations are zeolites modified with calcium phosphates or ammonium phosphates [7, 8].

Local zeolites (widespread in the northern Armenia, Noyemberyan region) and their derivatives, specifically modified, i.e. considering the properties of nitrogen-fixing microorganisms, which involved into the zeolite complex, were used in the present research.

Zeolites from the Noyemberyan deposits are predominantly high-silica varieties containing minerals of the zeolite group, particularly clinoptilolite, Ca-heulandite, mordenite, stilbite, phillipsite. Moreover the mineralogical structure of rocks includes montmorillonite, celadonite, chlorite and other rock forming minerals (Table 1)

Table 1.Comparative chemical structure of clinoptilolite containing tuff specimen from various deposits

Mass composition %	Noyemberyan deposit of ARMENIA					"Hektor" California USA*	Island Hokaido Japan*	"Dzegvi" Georgia*	"Aidag" Azerb*.
	"New", Slit Nol8	"Nor Kochb"	"Central"	"Southern" Slit No7	"Nord West"				
SiO_2	68.87	68.75	68.23	69.31	66.80	66.82'	65.27	61.30	65.90
TiO_2	0.29	0.30	0.34	0.32	0.20	-	-	-	0.09
Al_2O_3	12.20	11.92	12.48	12.09	10.58	12.24	14.84	13.00	12.90
Fe_2O_3	1.31	1.63	1.60	1.64	1.27	0.61	0.53	3.60	1.01
FeO	.0.42	0.38	0.55	0.51	0.26	-	-	-	0.18
CaO	3.99	4.37	3.50	3.98	4.42	1.02	2.31	6.30	4.05
MgO	1.12	0.96	0.98	1.07	1.24	0.23	0.88	1.80	0.57
Na_2O	0.97	0.65	2.74	0.40	0.70	4.41	0.88	2.00	2.55
K_2O	1.80	2.20	3.20	3.00	1.78	1.11	1.60	1.18	1.18
H_2O	12.26	13.06	10.87	11.57	11.31	13.2	14.31	13.50	11.45
SiO_2/Al_2O_3	9.6	9.8	9.3	9.8	10.7	9.3	7.5	8.0	8.7

* Specimens from other deposits were used only for the comparative analysis.

2. Experimental

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Natural zeolites were modified by the technique developed by our group [9, 10]. Crushed zeolitic tuff from the Noyemberyan deposit containing 85 % of clinoptilolite and with the particles size of 20-50 microns was treated with the 3-5 % solution of nitrogen containing inorganic or organic compounds (NH_4CL, NH_4NO_3, NH_4NO_3 + NH_4CL (1:1), hydrazine) at pH value 11-12. The solution weight-mass ration was equal to 3-5:1; the exchange temperature was 80-85 ^{0}C; the treatment period was 3 hours with permanent stirring. Afterwards zeolite was separated from the mother liquor by filtration/centrifugation, washed and dried at 120^{0}C.

3. Results and discussion

The idea of combining nitrogen-fixing microorganisms and zeolites in one preparation is based on our earlier experimental data on stimulating action of zeolites upon the metabolic activity of *Az. chroococcum* [11].

The selection of the high-activity *Az. chroococcum* strain have been carried out via screening of natural isolates by their nitrogenase activity and increased ability to fix atmosphere nitrogen, by antibiotic activity towards microorganisms-phytopathogens, as well as by their high viability in soil.

The microscope investigation of *Az. chroococcum A1* cells, cultivated in the medium containing natural or modified zeolites, testified about morpho-physiological changes in cells, particularly they were noticeably larger and mobile than cells cultivated in the medium without zeolite.

Evaluation of the titer and biomass of *Az. chroococcum A1,* cultivated in the presence of zeolites, both natural and modified, testified to essential influence of zeolites upon the growth and propagation.

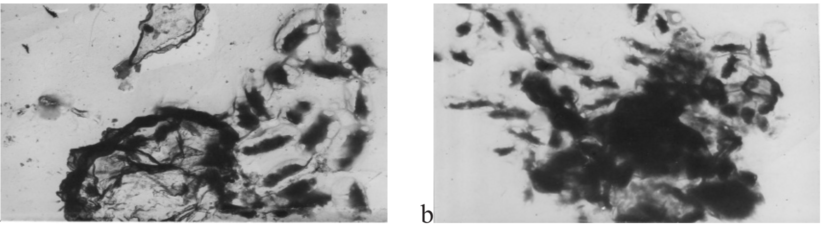
Test of various concentration of zeolites within 0,1-3,0 % limits has shown that the higher stimulating effect was observed at 2 % concentration of zeolite.
The average results of 7-10 experiments summarized in Table 2 demonstrate the higher culture titer (cell/ml) in comparison with control values while cultivating identical quantity of the *A1* strain cells in synthetic media with 2 % of modified zeolite, for 48 hours. The difference was expressed more in case of the modified zeolite. The stimulating action of zeolites was especially evident (500 times higher) in the Eshby synthetic [12] medium containing the modified zeolite. The special experiments have shown 2 times higher culture biomass (39,6 g/l against 21,7 g/l) in the medium containing zeolite. This finding also confirmed the stimulating effect of zeolites upon the growth and propagation of *Az. chroococcum*.

Table 2. The growth *of Az. chroococcum A1* in various cultivating media

Cultivating medium	Culture titer, cell/ml			
	Without zeolite	+ Natural zeolite	+ Modified zeolite AZ 1**	+ Modified zeolite AZ 2***
Synthetic Eshby medium	$2{,}4 \cdot 10^{8}$	$9{,}0 \cdot 10^{9}$	$9{,}5 \cdot 10^{10}$	$1{,}2 \cdot 10^{11}$
Semisynthetic Eshby medium*	$3{,}4 \cdot 10^{10}$	$5{,}4 \cdot 10^{10}$	$8{,}9 \cdot 10^{10}$	$1{,}2 \cdot 10^{11}$

* containing sucrose and molasses as a carbon source; ** AZ 1 – modification with NH_4NO_3 + NH_4CL (1:1);
*** AZ 2 – modification with hydrazine solution

The X-ray testing results showed that the culture cultivation in the presence of zeolites modified with nitrogen compounds intensified zeolite-microbe interaction and changed the culture-zeolite complex combination, Fig.1.



a b

Figure 1 X-ray testing of the biofertilizer with natural (a) and modified (b) zeolite

The crystals of the modified zeolite (b) illustrated in Fig.1 are almost covered by microbial cells whereas the crystals of the natural zeolite (a) are only framed.
Considering the experimental results on interaction of the selected nitrogen fixing strain and zeolites our group has designed the conceptually different from the known method of soil enrichment, which have no analogs: the azotobacter strain cultivation in the presence of natural or modified zeolites resulted in a new efficient nitrogen fertilizer.

The biopreparation has been successfully tested with different cultures both in hothouses and under field conditions (Test-protocols, performed by Yerevan State University, ‘Armenia Tree Project’, ‘Green Lane’ Agricultural Assistance NGO). Fig. 2

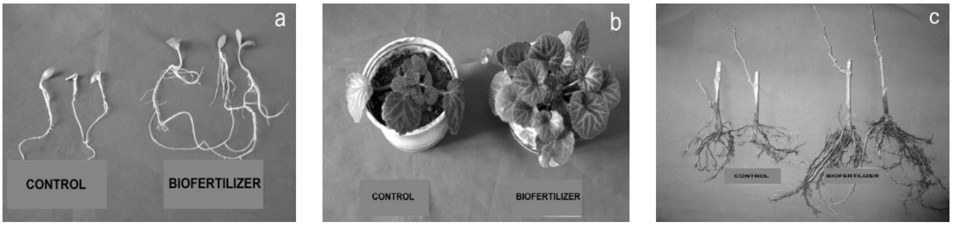


Figure 2 The effect of the biofertilizer upon:
a) seed germination; b) the rootage growth; c) the growth of plant land part

The test results demonstrated the stimulating effect of the preparation upon the seed germination, the growth of rootage and land part of plants and proved the designed technology and the new preparation to be advantageous from the point of ecological biosafety, efficiency and cost.

The preliminary data testify to the universality of the designed technology, which could be efficient in producing similar preparations based on other microorganisms, essential for soil enrichment, particularly phosphorous bacteria, actinomycetes, yeasts, and lacto bacteria, natural and modified zeolites.

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