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RUSSIAN EXPERIENCE ON MICROBIOLOGICAL INVESTIGATION OF GROUNDWATER FROM THE ZONE OF LIQUID RADIOACTIVE WASTE DEEP BURIAL

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The article presents the results of investigation of groundwater microorganisms from deep disposal facilities for liquid radioactive waste (RW) of FSUE MCC and JSC SCC carried out in 1998—2016. The microbial community investigated in groundwater was physiologically diverse, but had low geochemical activity. It included aerobic organotrophs and anaerobes (fermenting, denitrifying, sulfate-reducing, and methanogenic microorganisms). Paper shows that injection of RW into aquifers increases population of microorganisms and rates of anaerobic microbial processes resulting from the use of organic and mineral components of the waste. Bacteria isolated from groundwater were found to be capable of nitrate and radionuclide recovery, radionuclide sorption, and affecting the ambient redox potential. Microbiological monitoring of groundwater is required for evaluation of long-term environmental safety of the deep disposal facilities for liquid RW.

Keywords: *microbial ecology, deep disposal sites for liquid radioactive waste, denitrification, radionuclides reduction.*

Introduction

Use of geological environment for RW disposal leads to change of existing conditions for the natural microbial communities in aquifers. Injection of RW components into aquifers leads to stimulation of a number of microbial processes, which need to be taken into account in assessing long-term safety of deep liquid radioactive disposal facilities (DLRWDF). Pumping of waste causes direct contact of major waste components with natural underground microflora, which starts to use the waste as electron donor or acceptor in breathing processes or as a carbon source for cell growth.

Liquid technological and non-technological waste injected to underground aquifers have various composition and activities: alkali and acidic intermediate level waste (ILW), weakly alkali and neutral low-level waste (LLW). Sodium, calcium, iron and ammonium chlorides, bicarbonates, phosphates, sulphates, acetates and oxalates are present in the waste, with overall mineralization reaching up to 200—300 g/dm^3 [1, 2]. One of the main waste macrocomponents is nitrate ion, due to the use of nitric acid in the technological processes.

Microbial populations may affect DLRWDF functioning by influencing geochemical parameters of their underground habitat. Such influence may take many forms, including: 1) microbial impact on the oxidation rate and radionuclide migration

The translation is done by V. I. Shpinkov

(biosorption, bioaccumulation, and dissimilation reduction of metals and metalloids, e.g. U(VI), Se(VI), Cr(VI), Hg(II), Tc(VII), V(V) etc.) [3—6]; 2) accelerated migration of radionuclides with microorganisms or radionuclides concentration by biofilms [7, 8]; 3) biogenic gas (N_2, H_2S, CH_4, CO_2) generation due to the activity of denitrifying, sulphate-reducing and methanogenic microorganisms [8—10]; 4) formation of dominating radionuclide types and mineral phases, including new mineral compounds; 5) production of complexing agents [11]; 6) change of pH and Eh values, and other processes.

Since 1980-s there has been an increase of attention to microbiological studies of territories contaminated with radionuclides. There was a number of well-known works of English, Swedish and Canadian scientists performed in framework of national RW disposal programs, and works of American researchers performed at storage facilities of Hanford, Savannah-River and Oak Ridge [7, 12—15]. The studies covered analysis of microbial biodiversity, identification and investigation of physiology of dominating groups, as well as assessing their potential impact on waste components.

Microorganisms inhabiting geological formations considered as host rocks for designed RW disposal facilities were studied in a number of geomicrobiological research programs. These programs covered granite formations in Canada, Japan, Sweden, Finland, Switzerland and UK, sedimentary rocks in Belgium, Germany, Italy, and Japan, gypsum and salt deposits in Switzerland and Germany and volcanic sinters in the USA. Microorganisms were found in all samples, their number was up to 10^2—10^5 cells/cm^3 [16—20].

Microbiological studies of groundwaters (contaminated with alkali, nitrates, aluminates, chromates, Cs-137 and Tc-99) in the near field of high-level waste storage facility in Hanford (USA) have revealed aerobic heterotrophic bacteria in 11 out of 16 samples, though their number was low (10^4 cells/cm^3). Microbiological studies at Savannah River have identified diverse microflora, including sulphate-reducing bacteria [14, 21].

Major attention was paid to investigation of sulphate-reducing, methanogenic and denitrifying microorganisms capable of reducing RW macrocomponents (sulphate, bicarbonate, nitrate) with generation of gas (hydrogen sulphide, methane, nitrogen, carbon oxides). For example, reaction of nitrate reduction by denitrifying bacteria in an acetate-containing environment leads to generation of carbon dioxides in addition to nitrogen (or other gaseous nitrogen oxides):

$$8NO_3^- + 5CH_3COO^- \rightarrow 4N_2 + 10CO_2. \qquad (1)$$

Sulpahte-reducing prokaryotes reduce sulphate ions to hydrogen sulphide in presence of molecular hydrogen or organic substrates in the following reaction:

$$8[H]^+ + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-. \qquad (2)$$

Methanogens are capable of generating methane from hydrogen and carbon dioxide in reaction (3), and also out of simple organic substrates (acetate, methanol, etc):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O. \qquad (3)$$

Bacteria reducing iron and radionuclides with variable oxidation rates impact the geochemical conditions of the environment leading to generation of insoluble or low-soluble phases and colloids, thus, in some cases, increasing the mobility of radionuclides.

Microbiologic studies of DLRWDF in Russia were started in 1998 at DLRWDF "Severny" site, where disposal of liquid RW produced by FSUE "Mining and Chemical Combine" took place. The studies were triggered by the need for investigation of a number of processes connected to DLRWDF environmental safety, first of all enhanced gas generation and non-uniform and poorly predictable propagation boundary of nitrate ions.

In 2003 the studies were continued at DLRWDF "Seversky" used to inject liquid RW produced by JSC SCC.

Microbiological studies of ground water at DLRWDF "Severny" site, Zheleznogorsk

Microbiological studies of aquifer liquids taken from observation boreholes of I and II operational layers of DLRWDF "Severny" site were carried out in the period between 1998 and 2006 [8—10, 22—24]. The quantity of microorganisms of main physiological groups, and the rates of sulphate reduction and methane generation processes were determined; accumulating and pure cultures of dentrifying bacteria were obtained for laboratory modeling of biogenic gas generation. Main attention was paid to microbiological studies of the II operational layer, which was used for injection of low-active waste with nitrate-ion content of 10 g per 1 dm^3.

It was demonstrated that the aquifers outside the contamination area were a fresh water ecosystem with limited quantity of organic matter and biogenic elements (nitrogen and phosphorus). Therefore, the quantity of microorganisms of the main physiological groups (aerobic organotrophs and anaerobic fermentation, denitrifying, sulphate- and iron-reducing and methanogenic) (Table 1) and the rates of sulphate reduction and methane generation (Table 2) were low.

Table 1. Quantity of microorganisms (cells/cm³) in aquifer waters of I and II layers of DLRWDF "Severny" site in 2005

Bore-hole	Aerobic organotrophs	Denitrifying	Fermentation	Sulphate-reducing	Methanogenic H_2+CO_2	Methanogenic Acetate
			I aquifer			
A-5	10^6	10^6	10^6	10^4	10	0
A-19	10^8	10^5	10^8	10^3	10	<10
A-22	10^6	10^5	10^8	10^3	0	0
A-26	10^6	10^7	10^8	10^2	<10	<10
<-2	10^8	<10	10^5	10	0	0
C-15	10^3	10^2	10^7	10^2	0	0
P-6	10^7	10^2	10^7	<10	0	0
			II aquifer			
D-4	10^7	10^5	10^8	10	0	0
A-36	10^7	10	10^4	10	0	0
A-38	10^8	<10	10^8	10^2	0	0
A-39	10^8	10^2	10^4	10^2	0	0
AH-34	10^6	0	10^8	<10	<10	0

Table 2. Rates of sulphate reduction and methane generation in aquifer waters of I and II layers of DLRWDF "Severny" site in 2004

Sample	SO_4^{2-}, mg/dm³	HCO_3^-, mg/dm³	Acetate, mg/dm³	Rate of sulphate reduction, μg S^{2-}/(dm³·day)	Rate of methane generation, μg CH_4/(dm³·day)
			I aquifer		
A-3	45.2	277.5	3.1	0.171	0.023
A-19	30.8	330.0	4.3	0.09	0.19
A-26	79.8	236.9	6.5	0.026	0.034
			II aquifer		
A-38	8.5	253.1	3.9	0.06	0.006
A-39	6.4	349.3	10.7	0.052	0.637
D-1	10.1	261.9	6.8	0.034	0.032
D-2	11.8	378.2	8.6	0.122	0.0512

Liquid RW localized in these aquifers contains organic matter which can be used as a substrate and electron donor for microorganisms as demonstrated for the samples taken from the II operational aquifer. The quantity of denitrifying, sulphate-reducing and aerobic microorganisms in the waste localization area at the distance of 40—60 m from the injection wells and in waste dispersion area (200—300 m) was higher than similar parameters in the non-contaminated sections of the aquifer. Analysis

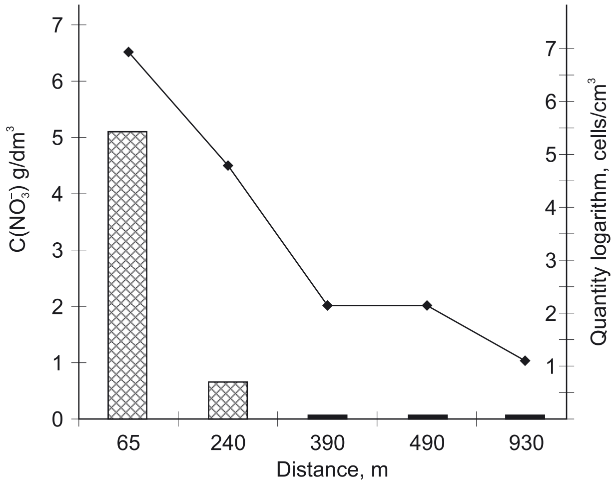

Fig. 1. Nitrate ion concentration (bars) and quantity of denitrifying bacteria (curve 2) in samples taken at various distances from the injection wells (2004)

of the results of eight-year monitoring performed in 2006 demonstrated correlation of the number of denitrifying bacteria and concentration of nitrate-ion in aquifer liquid (Fig. 1). Thus, the number of denitrifying microorganisms in the aquifer liquid may be used as an additional parameter to identify the distance of waste propagation [9, 10, 23, 24].

Denitrifying bacteria are activated by injection of nitrate-ions and organic matter with waste, leading to gas generation. This was confirmed by the results of laboratory modeling of biogenic gas generation using samples of underground liquid enriched in nitrate and acetate taken from boreholes of I and II operational aquifers (Fig. 2). Ground water enrichment in acetate and nitrate stimulated gas generation. Gas chromatographic studies showed that for concentrations of $NaNO_3$ up to 4 g/dm³, molecular nitrogen dominated in the gas phase, whilst for higher concentrations CO_2 was detected. Denitrification process was observed for environments containing up to 10 g of sodium nitrate per 1 dm³, i. e.

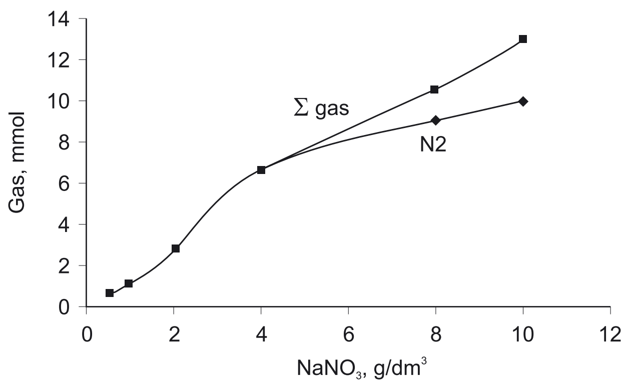

Fig. 2. Biogenic gas generation by microorganisms in aquifer liquid of borehole A-38, enriched in nitrate and sodium acetate (1:2 weight/weight) in various concentrations

in environments virtually corresponding in terms of sodium nitrate content to low level waste [23, 24].

Molecular nitrogen is a chemically inert gas, it dissolves in underground waters, and forms gas phase in the zone of maximum activity of denitrifying bacteria. At the same time, propagation of carbon dioxide with water solutions may lead to reduction of environment pH and dissolution of calcium, magnesium, and iron carbonate salts [24].

Microbiological studies of ground water at DLRWDF "Seversky", Seversk

Microbiologic studies at DLRWDF "Seversky" were carried out in 2003—2006 and in 2016 using the same methodical approaches as in studying the samples taken from DLRWDF "Severny" site. Minor microbial communities have been found in natural underground waters, including aerobic organotroph bacteria and anaerobic fermenting, denitrifying, iron and sulphate reducing, and methanogenic microorganisms. The number of microorganisms and rates of sulphate reduction and methane generation increased in the area of waste dispersion and were comparable to the values observed for ground waters of DLRWDF "Severny" site" [25—28].

Denitrifying bacteria of underground aquifers at DLRWDF "Seversky". High nitrate content in the liquid waste called for special attention to be paid to the group of denitrifying bacteria. This was connected to the fact that liquids taken from the surface storage facilities and III operational aquifer of DLRWDF (used for disposal of non-technological LLW) contained not more than 10^3 cells/cm^3 of denitrifying bacteria, whilst in the II operational aquifer their quantity was an order of magnitude higher (10^4 cells/cm^3) (Fig. 3). Content of denitrifying bacteria in the underground waters of the II operational aquifer used to dispose technological waste with high nitrate-ion content, reached 10^5 —10^7 cells/cm^3.

Potential denitrification rate was evaluated for isolated samples of groundwater taken from aquifers used for LLW disposal, in addition to evaluation of the impact of introduction of $NaNO_3$ (0.85 g/dm^3) and sodium acetate (2 g/dm^3) and molecular hydrogen on molecular nitrogen generation. Ground water samples were incubated at 18—20 °C prior to measurement of dynamic content of molecular nitrogen in the gas phase by gas chromatography. Content of the rest of gaseous denitrification products (NO and NO_2) was not analyzed, therefore it is likely that denitrification rate values obtained in the experiments were underestimated.

Denitrifying rate in the liquid samples taken from the surface storage facilities in the absence of stimulating compounds was in the range of 0 to

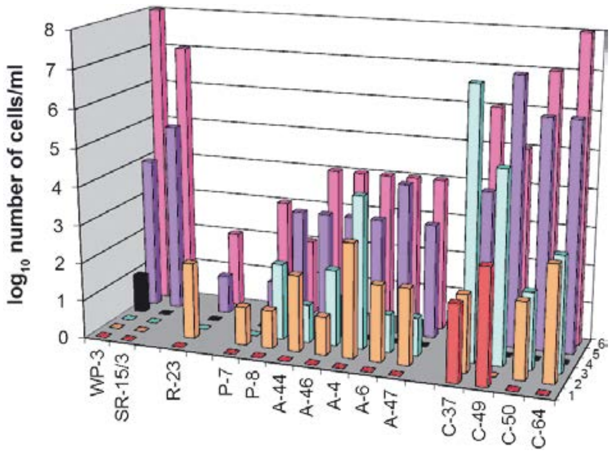

Fig. 3. Number of microorganisms in the liquids taken from surface storage facilities (water reservoir and sludge storage), natural aquifer waters (borehole R-23) and liquids taken from the disposal area for low-level (II and III aquifers, boreholes P-7 and A-47) and intermediate level waste (II aquifer, boreholes C-37-S-64) of DLRWDF "Seversky". Key: methanogenic in acetate environments (1) or H_2/CO_2 environment (2), denitrifying (3), sulphate-reducing (4) and fermentation (5) bacteria and aerobic organotroph bacteria (6) [26]

0.08 mg N_2/dm^3·day, the rate for operational aquifer water was in the range of 0 to 0.1 N_2/dm^3·day (Table 3) [26]. Addition of molecular hydrogen or acetate in combination with nitrate to isolated samples had stimulated molecular nitrogen generation in ten of eleven studied underground water samples.

The obtained results shows that denitrifying microcosms of underground waters are capable of nitrate reduction, however, at a slow rate due to the absence of carbon and energy.

Table 3. Denitrification rates in isolated ground water samples taken in the vicinity of surface storage facilities and from operational aquifers of DLRWDF "Seversky" used for LLW disposal

Borehole number, sample	NO_3^-, mg/dm^3	NH_4^+, mg/dm^3	SO_4^{2-}, mg/dm^3	Rate of denitrification, mg N_2/(dm^3·day)		
				No additives	In presence of	
					$H_2+NO_3^-$	Acetate+NO_3^-
P-15/3	21000	750	160	0	0	0
P-15/8	3960	21	51	0.06	1.93	0.09
P-15/10	0.2	4.4	42.4	0.08	2.93	0.32
<-7	2157	0.53	392.5	0.05	2.0	0.22
<-8	135.9	0.2	96.8	0	0.32	2.62
P-23	0.25	0.9	7.5	0	2.25	0.35
A-44	1348	0.1	246	0.05	0.56	2.68
A-46	0.25	0.55	60.7	0.1	0.87	0.2
A-4	856.9	1.26	94.8	0	4.5	0.3
A-6	0.25	1.69	20.9	0	4.0	0.8
A-47	39.4	6.79	63.7	0.05	3.37	3.5

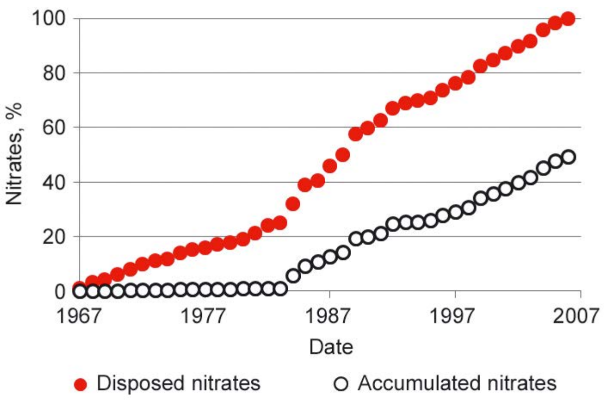

Fig. 4. Contemporary content of nitrate disposed with waste in the sands of operational aquifers of DLRWDF "Seversky"

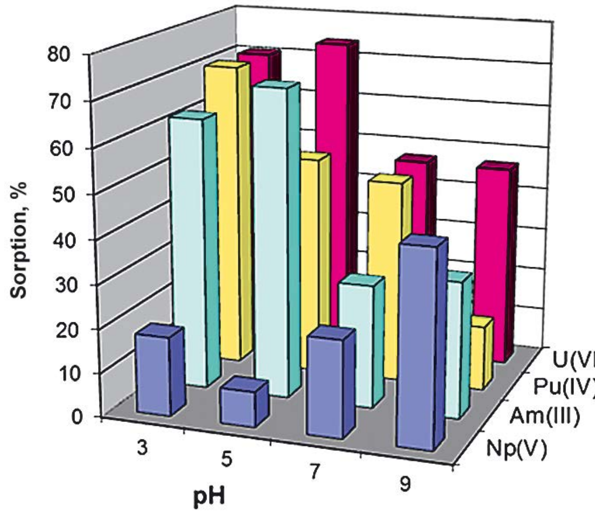

Fig. 5. Sorption of 237Np(V), 241Am(III), 238Pu(IV) and 233U(VI) by biomass of Pseudomonas fluorescens C64-1 bacteria for various solution pH [26]

Results of laboratory evaluations of denitrifying rate in the underground water samples were used at DLRWDF "Seversky" to draw up balances of nitrate-ions disposed of at III operational aquifer from the start of DLRWDF operation, and taking into account the current content and distribution in the underground waters of the aquifer.

The known dynamics of nitrate-containing waste disposal at DLRWDF "Seversky" and denitrification rate allows assessing the nitrate concentration due to denitrification process. For 2007 assessments, 49% of disposed nitrate was found in the sands of operational aquifers (Fig. 4).

In case only denitrification processes were present, the figure should have been 62%. The nitrate disbalance with account for denitrification was 13%, including 5% of nitrate deposited in clay layers of operational aquifers and unreachable for sampling from monitoring boreholes. The eight percent disbalance may be explained taking into account the scope of waste disposed, non-uniform mineralogic composition of rocks with depth and the dimensions of host aquifer. Calculations show that if the volume of contaminated water in the operational aquifer remains at the same level (cease of waste disposal), dilution and denitrification processes will lead to complete reduction of nitrates to molecular nitrogen within 26 years.

These results demonstrate that denitrifying bacteria are capable of reducing nitrate-ions in the condition of DLRWDF "Seversky". Passive denitrification in operational aquifers may be stimulated by injection of cheap organic substrates.

Bacterial effect on radionuclide transformation. Radionuclide reduction and biosorption was studied for more than 50 strains of bacteria taken from underground water samples [25, 26]. Some of the aerobic bacteria found adsorbed (accumulated) actinides and other trans-uranium elements present in the waste (^{238}Pu(IV), ^{237}Np(V), ^{233}U(VI), ^{241}Am(III) и ^{90}Sr(II)), and did not adsorb ^{137}Cs and ^{99}Tc (Fig. 5) [25—27].

Radionuclide biosorption is unlikely to be strongly manifested in underground waters of operational aquifers with low microbial content. However, microbial populations grow up to 107 cells/cm^3 in the waste dispersion areas containing both radionuclides and organic matter, thus potentially leading to biosorption of metals (Fig. 5, 6).

Detection of microorganisms capable of metal reduction and reduction of sulphates to sulphides is an environmental safety relevant issue for RW disposal [25, 26, 28]. Such microbial organisms produce new weakly soluble mineral phases, causing changes of environment geochemical properties and potentially immobilizing the radionuclides. A number of sterns of sulphate- and iron (III)-reducing bacteria (*Shewanella* genus) obtained from operational aquifers [26] were shown to be capable of reducing Fe (III), U(VI) and Np(V) in presence of various organic substrates. Experiments carried out in accumulating substrates showed higher effectiveness of radionuclide reduction.

Second stage of investigation of samples taken from operational aquifers of DLRWDF "Seversky" took place in 2016. A number of samples demonstrated increase of the number of denitrifying and aerobic organotroph bacteria compared to previous results, potentially connected to the propagation of the front of nitrate ions and other waste components away from the injection boreholes. It was demonstrated that there was a capability of combining the nitrogen and sulphur cycles in underground waters due to functioning of autotrophic bacteria reducing sulphur compounds to sulphate with simultaneous reduction of nitrate-ion to

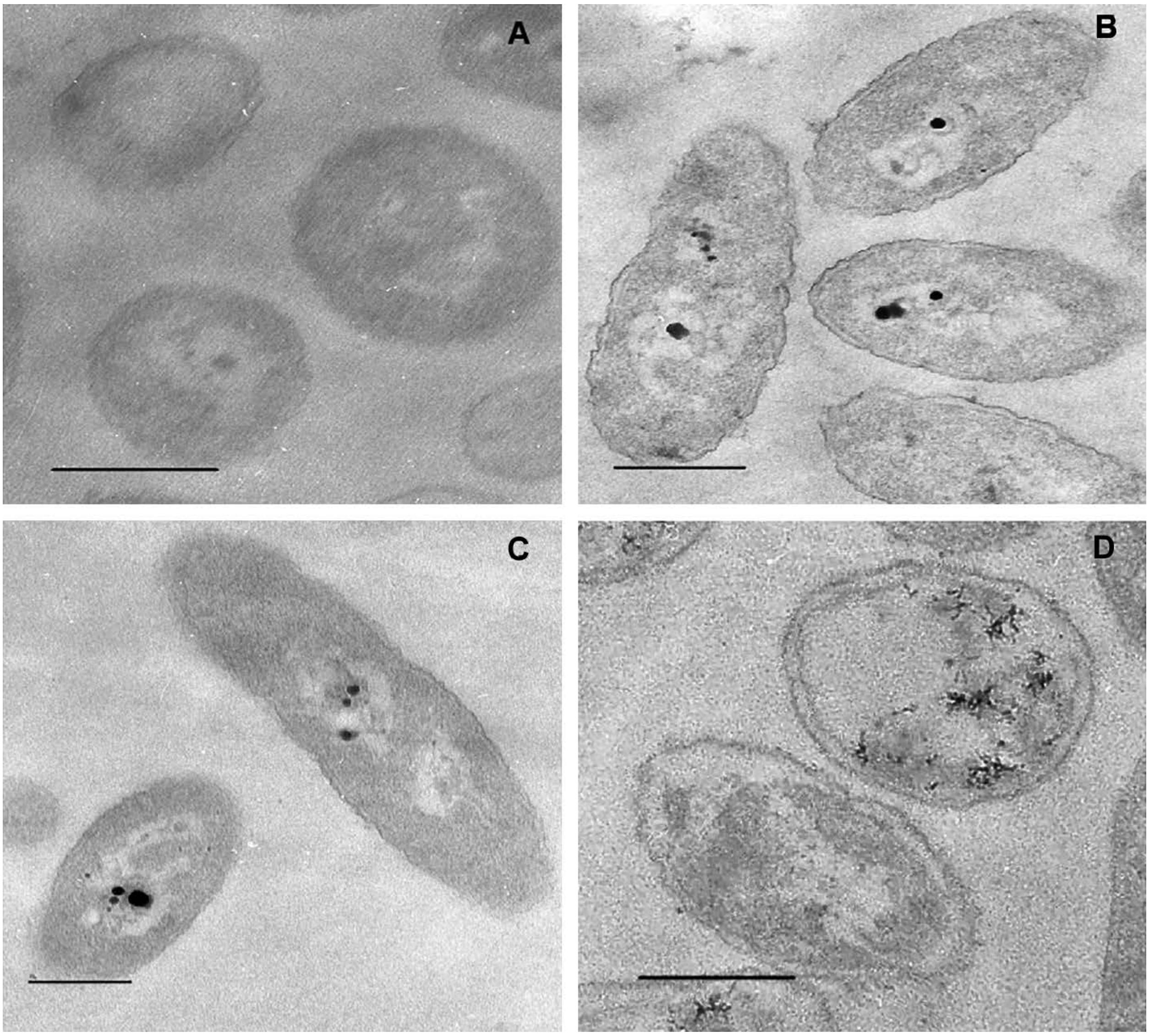

Fig. 6. Submicroscopic organization of Pseudomonas grimontii C-61-1 cells in the absence of radionuclide (A) and in presence of 233U(VI) (B), 241Am(III) (C) and 237Np(V) (D). Ruler size corresponds to 1 µm.

nitrate. Tionic, nitrifying and ANANNOX bacteria were identified for the first time. The latter ones are capable of anaerobic reduction of nitrate ions to molecular nitrogen using ammonium as an electron donor.

The presence of viable, but geochemically low active sulphate-reducing and methanogenic microorganisms was found in underground waters. Low content of sulphates, organic matter and high oxidation-reduction potential of underground waters in the area of nitrate ion injection prevents the growth of sulphate-reducing bacteria and methane-generating archaeons. Injection of organic substrates, preliminarily those stimulating growth of denitrifying bacteria may lead to reduction of nitrate ion concentration and oxidation-reduction potential of underground waters and production of an environment favorable for growth of sulphate-reducing bacteria. This group of bacteria is most promising from the point of view of use for formation of local biogeochemical barrier with respect to radionuclide migration, as the produced hydrogen sulphide will promote further transition of oxidized radionuclides to a reduced form and their deposition in rocks in form of low-soluble sulphides.

Composition of microbial community of contaminated underground waters was investigated for the first time by high throughput gene sequencing 16S pRNK in process of studying deep RW disposal facilities in Russia.

The method allows identifying microbial biodiversity in samples, including non-cultivated forms, which may be present in quantities several orders of magnitude above that of cultivated prokaryotes. Genes 16S pRNK of aerobic nitrifying bacteria of the first phase, oxidizing ammonia to nitrate, were found. Genes of 2nd stage nitrifcators oxidizing nitrites to nitrates belonged to *Candidatus Nitrotoga*,

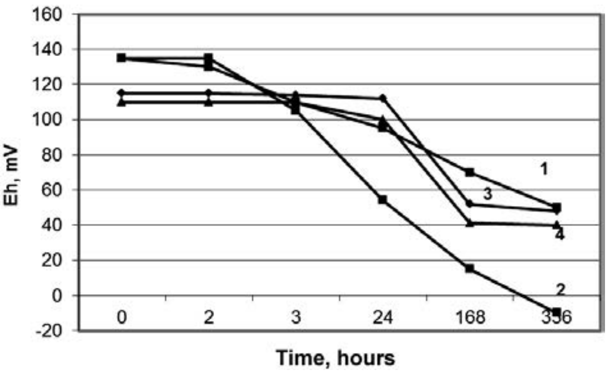

Fig. 7. Reduction of Eh (mV) of acetate- and nitrate-containing environment in process of growth of denitrifying bacteria Thermomonas fusca SHC-3-19 (1), Microbacterium oxydans SHC 3-5 (2), Pseudomonas veronii SHC-8-1 (3) and Ensifer adhaerens SHC-2-14 (4), recovered from operational aquifers of DLRWDF "Seversky"

Nitrobacter, Nitrospina, Nitrospira genus bacteria and to non-cultivated bacteria of *Nitrospiraceae* family. Genes 16S pRNK of denitrifying bacteria belonged to representatives of species Pseudomonas, Denitratisoma, etc., which are capable of participation in reduction of uranium and technetium. Process of redox-potential change by denitrifying microorganisms was studied in laboratory experiments (Fig. 7). It was demonstrated that for the temperature of 150 °C addition of acetate and lactoserum to underground water lead to reduction of Eh values from the oxidizing range of +200…+250 to the reduction range of −100… −150 mV due to nitrate reduction to N_2. Consumption of oxidizers in the system offers conditions for transition of redox-sensitive metals (uranium, plutonium, neptunium), into low-valence less mobile forms. Such an approach may be used for establishment of a geochemical barrier for radionuclides in the contaminated aquifer in process of DLRWDF closure.

Conclusion

Summary of the results of microbiologic studies of DLRWDF shows that subterranean biota of the studied contaminated sections of operational aquifers has low quantity, but is diverse in terms of physiological parameters and is capable of activation in case of waste components injection to the aquifer. Therefore, the following needs to be taken into account:

- the significance of denitrification process in migration of nitrate ions. Without due account of this factor, simulation of nitrate propagation does not correspond to the actual aureole. Nitrate-ion is one of the main waste components and one of the most toxic macrocomponents;
- possibility of microbial participation in radionuclide migration. Biogeochemical processes may lead to reduction of mobile radionuclides — technetium ($Tc^{7+} \rightarrow Tc^{4+}$), neptunium ($Np^{5+} \rightarrow Np^{4+}$) and uranium ($U^{6+} \rightarrow U^{4+}$) thus limiting their propagation. There should be confirmed the capability for their bioreduction in operational aquifers, first of all at the waste propagation front, where the pH-Eh conditions are favourable for such reactions;
- possibility of microbial participation in radionuclide (Am, Pu, U) transport in biocolloid form. Such data are required for a reliable forecast of liquid RW disposal consequences.

Thus, it is evident that microorganisms present in subterranean aquifers used for RW disposal take part in processes connected both to transformation of waste macrocomponents and to radionuclide migration. The currently available experimental data are insufficient to identify the extent of microbial processes effect on DLRWDF environmental safety and the potential of their use in closure of DLRWDF (establishment of biogeochemical barriers). Insufficiency of experimental data is connected to the lack of long-term comprehensive studies (microbiological monitoring) of the whole area of DLRWDF. Obtaining numerical parameters for forecasting calculations would be possible only for a system approach to comprehensive study of biogeochemical processes.

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