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EXPERIMENTAL ARTICLES

Physicochemical and Microbiological Characteristics of Groundwater from Observation Wells of a Deep Radioactive Liquid Waste Repository

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Abstract—A radioactive liquid waste repository was found to be the habitat of a rich microbial community with a high catabolic potential. Groundwater from a depth of 162–189 m contained aerobic saprotrophic and anaerobic fermentative, sulfate-reducing, and denitrifying bacteria. Nitrate-reducing bacteria residing in this groundwater were isolated in pure cultures. Based on the results of their physiological studies, 16S rRNA sequencing, and phylogenetic analysis, the microorganisms isolated were ascribed to one phylogenetic branch, the γ-subclass of gram-negative bacteria. Among six isolates, four belonged to the genus *Acinetobacter*, whereas two others belonged to the genera *Comamonas* and *Aeromonas*. The data obtained indicate that the microflora of the repository can exert a certain effect on the chemical composition of the formation fluids and bearing rocks, as well as on the migration of radionuclides.

Key words: the number of bacteria, groundwater, nitrate reduction, 16S rRNA, phylogeny, radioactive waste.

The proportion of nuclear energy in the energy production of industrialized countries makes up about 70% (France), 35% (Japan), 30% (Germany), and 11–15% (USA and Russia). The development of nuclear industry is closely related to the solution of the problem of nuclear waste disposal.

At present, all concerned countries accepted a conception of the multibarrier storage of nuclear wastes, which are first solidified and then placed in a repository located in a geological formation. To avoid migration of radionuclides, surrounding rocks are usually protected by additional engineering barriers [1–5]. Since subterranean systems are populated with microorganisms, some countries have established special services that exert microbiological control over the integrity of waste reservoirs, including studies of microflora on the surface of reservoirs and in surrounding rocks and simulation of the processes of biofilm formation and biogenic corrosion of the reservoir's metals [1–3, 6–9].

One of the methods of storing nuclear wastes applied in Russia consists in pumping liquid wastes with low and medium radioactivity into deep (400–450 m) porous water-bearing rocks which are reliably isolated from underlying and overlying strata by waterproof clay layers. Wastes, which are injected through an injection well, displace formation water and then percolate through the reservoir bed at a rate equal to that of the formation water (1–10 m/year).

In addition to radionuclides, nuclear wastes (pH 10–12) contain nitrate and acetate anions, whose concentration may reach 200 and 80 g/l, respectively. These components of wastes can interact with surrounding rocks and pollute groundwater. In spite of the high hydrogeological safety of nuclear waste repositories, there exists a hazard of polluting water-bearing strata emerging on the surface with the toxic components of stored wastes. This necessitates control of the motion of toxic wastes, which is implemented by means of local monitoring of groundwater.

The ever-increasing quantity of radioactive wastes calls for the development of new advanced methods for their disposal. Theoretically, acetate and nitrate anions can appropriately be removed from wastes by the microbiological process of denitrification, in which radioresistant microorganisms utilize acetate and reduce nitrates into the ecologically safe molecular nitrogen. It would be expedient to search for such microorganisms directly at the sites of radioactive waste storage.

It should be noted that there are virtually no publications devoted to the microbiological aspects of the disposal of radioactive wastes in Russia. The aim of the

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present work was to study the physicochemical conditions and the composition of microflora in groundwater from the observation wells located on the periphery of a radioactive liquid waste repository. We also attempted to isolate the microorganisms that would be able to transform the main components of wastes (nitrate and acetate anions).

MATERIALS AND METHODS

Groundwater for study was collected from the observation boreholes located on the periphery of a deep repository of low-level (less than 10^{-5} Ci/l) radioactive liquid wastes. The enclosing sandstone stratum was composed of quartz and feldspar (60–70%), the rest being mica and kaolinite clay.

Media and enumeration of bacteria. Microorganisms in groundwater were enumerated by the serial tenfold dilution method. Aerobic saprotrophic bacteria were enumerated by plating the dilutions on universal medium often used in ecological studies, namely, plate count agar (PCA) purchased from Difco (United States). Thionic bacteria of the species *Thiobacillus denitrificans* were enumerated using anaerobic Taylor medium [10]. Sulfate-reducing bacteria were detected by the production of hydrogen sulfide in the serial dilutions prepared using Postgate medium B [11] supplemented with sodium lactate and 100 mg/l $Na_2S \cdot 9H_2O$. Denitrifying bacteria were detected by the appearance of molecular nitrogen in Adkins medium [12] supplemented with 2 g/l sodium acetate or 5 g/l sucrose as sources of carbon and energy and 0.85 g/l sodium nitrate as an electron acceptor. Fermentative bacteria were detected by hydrogen production in Adkins medium supplemented with sucrose but not sodium nitrate. Methanogens were detected by the production of methane in media containing 2 g/l acetate or $H_2 + CO_2$ [12]. Anaerobic bacteria were cultivated in Hungate tubes purchased from Bellco Glass Inc. (United States). The gas phase was purified argon, except for methane-producing bacteria cultivated on a mixture of hydrogen and carbon dioxide. Microbial cultures were examined under a Jenaval light microscope equipped with a phase-contrast device (magnification, 2000).

Analytical methods. Molecular hydrogen and nitrogen were analyzed on a model 3700 gas chromatograph equipped with a katharometer and a column (2 m × 2 mm ID) packed with a molecular sieve. The carrier gas was argon at a flow rate of 40 ml/min. The detector and injector were kept at 70 and 40°C, respectively.

Methane was quantified on the same chromatograph equipped with a flame–ionization detector and a SOVKOL prepacked column (1 m × 3 mm ID). The carrier gas was argon at a flow rate of 40 ml/min. The detector and injector were kept at 150 and 140°C, respectively.

Hydrogen sulfide was determined with dimethyl-*p*-phenylenediamine by a modified colorimetric method of Pachmayr as described by Trüper and Schlegel [13].

pH and Eh values were measured using an OP-211/1 digital laboratory pH meter.

Iron, magnesium, calcium, and sodium in groundwater were analyzed on an AAS1N atomic absorption spectrophotometer. Nitrates were analyzed on an Ecotest-01 device fitted with an ELIT-21 ion-selective electrode. Nitrites were determined colorimetrically with sulfanilic acid and α-naphthylamine. Other components in groundwater were analyzed by routine methods [14].

DNA was isolated from cells of pure cultures by the Marmur method [15]. The G+C content of DNA was determined from melting profiles in dilute buffers [16].

The morphological, physiological, and biochemical characteristics of bacteria were studied by routine methods.

Amplification, sequencing, and analysis of 16S rRNA genes. The 16S rRNA genes isolated from cells of pure cultures of nitrate-reducing bacteria were amplified and sequenced using the primers from [17].

Amplification was carried out in 60 mM Tris–HCl buffer (pH 8.8) containing 20 mM $MgCl_2$, 170 mM $(NH_4)_2SO_4$, and 0.01% gelatin. The reaction mixture (500 µl) contained standard concentrations of dNTPs and equimolar amounts of 27f and 1492r primers. Samples were amplified through 30 three-step cycles of DNA denaturation at 94°C for 30 s, primer annealing at 40°C for 1 min, and elongation at 72°C for 2.5 min. 16S rRNA gene fragments were purified on low-melting-point agarose and sequenced in a DNA Sequencer 373A using a kit purchased from Applied Biosystems (N 402080, Ready Reaction Dye Terminator Sequencing Kit with AmpliTaq DNA Polymerase, FS).

The 16S rRNA gene sequences of the strains studied have been deposited in GenBank under the following numbers: A382, AF 188302; A381, AF 188303; A391, AF 188301; A392, AF 188299; D22, AF 188304; and P152, AF 188300.

16S rRNA gene sequences were preliminarily analyzed using the database and software package of the Ribosomal Database Project. Then sequence fragments were aligned manually using the respective sequences of allied reference strains. The rooted phylogenetic trees of bacteria were constructed with the aid of the TREECON software package [18].

RESULTS AND DISCUSSION

Samples of groundwater collected in six observation wells located along the front of the migration of low-level radioactive liquid wastes were investigated with respect to physicochemical characteristics and microbial composition.

Table 1. Physicochemical characteristics of samples of formation water taken from the observation wells of a subterranean radioactive liquid waste repository

Well	Depth, m	pH	Eh, mV	Fe_{total}, mg/l	Mg^{2+}, mg/l	Ca^{2+}, mg/l	NO_3^-, mg/l	HCO_3^-, mg/l	$Na^+ + K^+$, mg/l	Cl^-, mg/l
A-38	180	7.9	280	6.5	13.1	23.0	4.09	158.6	25.7	<2
A-39	168	7.8	290	6.65	14.25	31.0	1.87	335.5	64.2	<2
D-1	189	9.1	210	16.3	9.5	17.5	1.25	305	71.3	<2
D-2	162	7.9	295	29.5	23.0	45.3	2.0	347.7	18.7	<2
D-4	171	7.5	310	237.5	15.5	38.5	0.63	280.6	31.97	<2
P-15	163	7.5	300	23.0	15.3	64.0	0.35	311.1	19.0	<2

Physicochemical characteristics of groundwater. Samples of groundwater were collected from a depth of 162–189 m; they exhibited neutral and slightly alkaline pH values (7.5–9.1), a redox potential of +210 to +310 mV, low mineralization, and radioactivity at a maximum permissible concentration level, which indicated that these observation wells were located outside the line of radioactive contamination (Table 1). The predominant anion in groundwater was bicarbonate, whose concentration ranged from 158.6 to 347.7 mg/l. The concentration of nitrates did not exceed 4 mg/l. The contents of chlorides (< 2 mg/l), sulfates (< 4 mg/l), and nitrites (< 0.5 mg/l) in groundwater were at the level of their detectability. The major cations were $Na^+ + K^+$ (19–71.3 mg/l), Ca^{2+} (17.5–64 mg/l), Mg^{2+} (9.5–23.5 mg/l), and Fe (6.65–29.5 mg/l). At the same time, iron concentration in the groundwater sample taken from the well D-4 was as high as 237 mg/l. The temperature of groundwater was 12–14°C.

Microbial composition of groundwater. Samples of groundwater were analyzed for some physiological groups of microorganisms which could theoretically reside in this habitat. The microbial population of samples was analyzed by cultural methods. The taxonomical position of pure cultures was determined by the analysis of 16S rRNA gene sequences.

The microbial populations of groundwater samples were different (Table 2). The lowest number of bacteria was found in sample D-4. The number of aerobic saprotrophic bacteria in other samples ranged from 2.5×10^2 to 7.2×10^4 cells/ml. Anaerobic fermentative bacteria producing hydrogen from sucrose were most abundant in samples P15 and A39 (1.3×10^5 cells/ml).

In spite of the low content of sulfates (< 4 mg/l), groundwater samples contained sulfate-reducing bacteria, although their number was small (no more than 250 cells/ml). It should be noted that the occurrence of sulfate-reducing bacteria in the subterranean habitats lacking sulfates is not surprising, since these bacteria can obtain energy for growth not only from sulfate reduction, but also from the reduction of other oxidized sulfur compounds and oxides of nitrogen, iron, and other metals. Furthermore, they can ferment various organic substrates and implement the interspecies transfer of hydrogen to microbial acceptors [11].

The number of methanogenic bacteria was very small (several cells/ml), which could be explained by a relatively high redox potential of groundwater. The samples investigated contained heterotrophic denitrifying bacteria capable of growth on acetate and nitrates (1.3×10^3–6×10^5 cells/ml) and sucrose and nitrates (from 6 cells/ml to 1.3×10^6 cells/ml).

Thus, the microflora of groundwater had a high catabolic potential and was able to efficiently mineralize various organic substrates in the processes of aerobic oxidation and anaerobic fermentation, denitrification, sulfate reduction, and methanogenesis.

Isolation of nitrate-reducing bacteria. Pure cultures of nitrate-reducing bacteria were obtained, via enrichment cultures, from the highest dilutions of groundwater in which dinitrogen could still be detected by gas chromatography. We mainly searched for the nitrate-reducing bacteria that could utilize acetate, one of the major components of radioactive wastes. During the isolation of nitrate-reducing bacteria, we also used sucrose, which is the major component (up to 50%) of molasses, an affordable waste of the sugar industry widely used in biotechnology as a growth substrate.

Growth of the enrichment cultures of nitrate-reducing bacteria in media with acetate, sucrose, and nitrates was accompanied by a considerable decrease in the concentration of nitrates. Among the products of nitrate reduction, we detected nitrite and molecular nitrogen.

Pure cultures of nitrate-reducing bacteria were obtained by plating enrichments on agar media with nitrates and sodium acetate and incubating the plates at 30°C in anaerobic jars filled with purified argon. All the pure cultures (we succeeded in the isolation of six cultures) were able to grow anaerobically on sodium nitrate as an electron acceptor and on sodium acetate as the sole source of carbon and energy. Four of these cultures reduced nitrates to nitrites, and two cultures turned out to be true denitrifying bacteria capable of reducing nitrate to molecular nitrogen (Table 3).

The bacteria isolated were represented by different morphotypes: straight and curved rods and cocci arranged singly or in pairs. Spores were not produced.

Table 2. Abundance of aerobic and anaerobic microorganisms in samples taken from the subterranean radioactive liquid waste repository (cells/ml)

Sample	Aerobic saprotrophs	Thionic bacteria	Fermenters	Denitrifiers		Sulfate-reducers	Methanogens	
	PCA*	NO_3^-	Sucrose	Acetate + NO_3^-	Sucrose + NO_3^-	Lactate + SO_4^{2-}	$H_2 + CO_2$	Acetate
A-38	5×10^3	0**	$>10^4$	$>1.3 \times 10^3$	$>2.5 \times 10^5$	25	0	25
A-39	2.5×10^2	0	1.3×10^5	6×10^5	$>1.3 \times 10^6$	2.5×10^2	0	2.5
D-1	9.2×10^3	0	1.3×10	6×10^4	1.3×10^2	25	0	0
D-2	7.2×10^4	0	$\geq 10^4$	$>1.3 \times 10^3$	6	25	0	0
D-4	5×10	0	1.3	6×10^4	$>1.3 \times 10^2$	2.5	25	0
P-15	2×10^3	0	1.3×10^5	1.3×10^4	1.3×10^6	25	0	0

* Plate count agar.

** Stands for the absence of colonies in the case of plating of 1 ml of formation water.

All the isolated bacteria were typical saprotrophs capable of aerobic utilization of volatile fatty acids (acetate, propionate, and butyrate) and lower alcohols. Under aerobic conditions, the bacteria could grow on potato agar, nutrient agar, and wort agar. Under anaerobic conditions, they could grow on medium with nitrate and acetate.

Of interest was the study of the adaptation of the bacteria isolated to the physicochemical conditions of their habitat (redox potential, pH, temperature, medium mineralization, and content of available organic matter). We found that the strains that we isolated could grow at neutral and slightly alkaline pH values, at temperatures from 6 to 40°C, and with mineralization not exceeding 20 g/l NaCl (with the exception of strain A382, which tolerated up to 100 g/l NaCl).

In separate experiments, we tested the radioresistance of the bacteria. To this end, bacterial suspensions containing 5×10^9 cells/ml were irradiated at 20–24°C with γ-radiation from a ^{60}Co source in a dose of 1.3 Mrad at a dose rate of 325 krad/h. The bacteria turned out to be resistant to such irradiation.

The phylogenetic analysis of nitrate-reducing bacteria. The phylogenetic position of the strains isolated was determined on the basis of analysis of 16S rRNA gene sequences. For strains A382 and D22, we determined almost complete sequences (about 1450 nucleotides); for other strains, we determined about 350 nucleotides with positions from 30 to 380 according to *E. coli* numbering. As can be seen from the constructed phylogenetic tree (see figure), the nitrate-reducing bacteria isolated belong to different phylogenetic groups of the γ-subclass of proteobacteria. Most strains are close to representatives of the genus *Acinetobacter*: the degree of homology of strain A392 with the type strain of *A. calcoaceticus* is 99.7%, the degree of homology of strains A382 and A391 with the type strain of *A. lwoffii* is 99.2–99.7%, and the degree of homology of strain P152 with the type strain of *A. johnsonii* is 99.7%. These values correspond to the homology values recorded among strains within the aforementioned species (99.5–99.9%). As shown earlier, analysis of 16S rRNA gene sequences allows one to quite reliably identify representatives of the genus *Acinetobacter* [19]; therefore, strains A392, A382, A391, and P152 can be referred to the aforementioned species. None of these strains was close to the radioresistant species *A. radioresistens* [20], which may indicate that radioresistance is peculiar to various representatives of the genus *Acinetobacter*.

Strain A381 can be affiliated to the genus *Aeromonas* (the most close species is *A. media* with a 99.4% homology). It should be noted that the genus *Aeromonas* is genetically homogeneous with a degree of interspecies homology of 16S rRNA gene sequences as high as 97.3–99.9%. The 16S rRNA gene sequences of some species of this genus turned out to be virtually identical, which raised doubts about their taxonomical status [21]. In this connection, it is difficult to decide whether strain A381 is a new species of the genus *Aeromonas*.

Strain D22 can be ascribed to the genus *Comamonas* (the closest species is the type species *C. terrigena* with a 97.5% homology of 16S rRNA gene sequences). Such a degree of homology is less than the intraspecies homology characteristic of the known species *C. terrigena* (98.9%) and *C. testosteroni* (99.9–100%); therefore, strain D22 may represent a new species of the genus *Comamonas*.

Thus, the nitrate-reducing bacteria isolated from the subterranean habitat turned out to be taxonomically diverse, and some of them may represent new species.

It is known that acinetobacters are very abundant in soils, water, wastewater, and subterranean rocks [4, 22]: their proportion in the aerobic microbial population of soils and water can reach 0.001% [22]. The representatives of the genus *Comamonas* are also usual inhabitants of soils and water bodies. The occurrence of these groups of bacteria in the groundwater samples

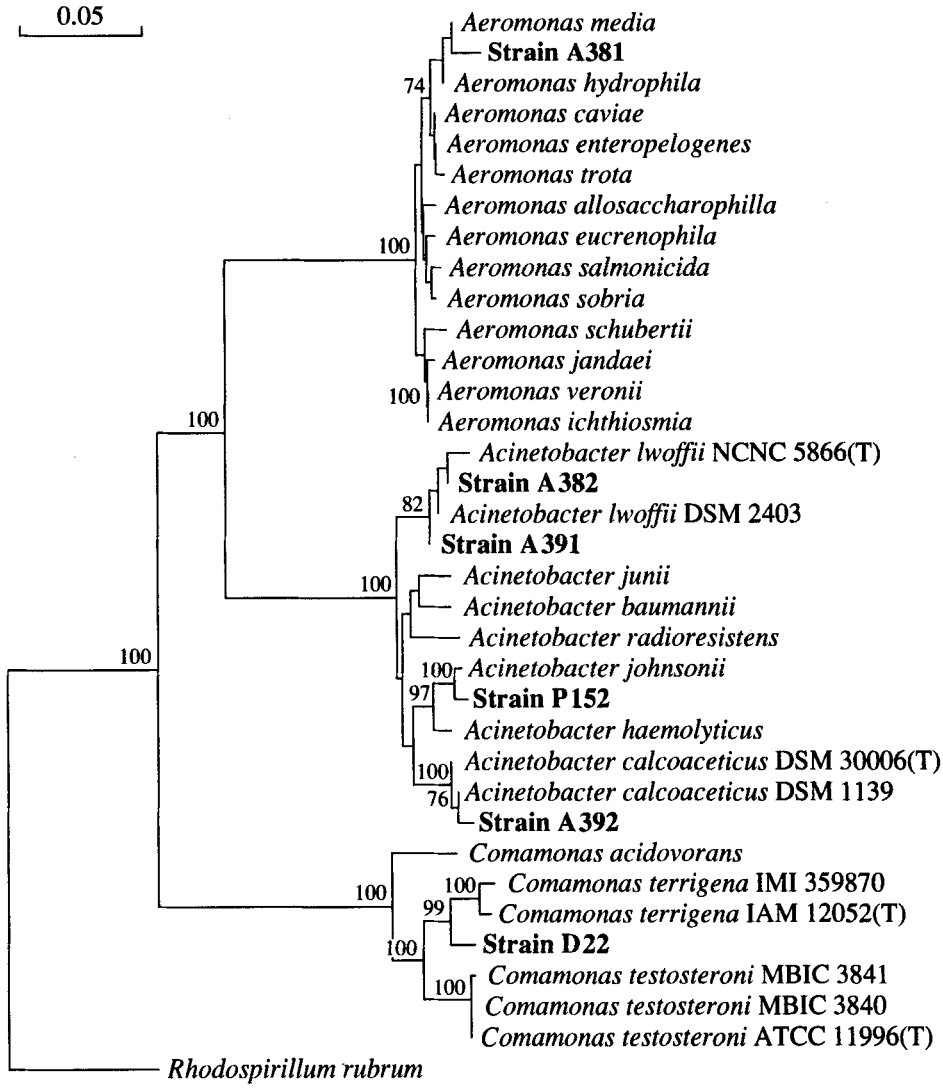
Table 3. Some differentiation features of nitrate-reducing bacteria isolated from formation water

Strain	A382	A381	A391	A392	D22	P152
Cell shape	Ovoid	Ovoid	Ovoid	Ovoid	Curved rods	Ovoid
Motility	–	–	–	–	+	–
Spores	–	–	–	–	–	–
Aerobic growth on:						
formate	–	–	–	–	–	–
acetate	+	+	+	+	+	+
propionate	+	–	+	+	+	+
butyrate	+	–	+	+	+	–
ethanol	–		+	+	–	
citrate	–	+	–	+	–	+
pyruvate	+	+	+	+	+	+
lactate	+	–	+	+	+	+
fumarate	+	+	+	+	+	+
glucose	–	–	–	–	–	–
sucrose	–	–	–	–	–	–
arabinose	–	–	–	–	–	–
maltose	–	–	–	–	–	–
lactose	–	–	–	–	–	–
fructose	–	–	–	–	–	–
peptone	–	–	+	+	–	–
potato agar	+	+	+	+	+	+
Anaerobic growth on:						
NO_3^- + acetate	+	+	+	+	+	+
$NO_3^- \longrightarrow N_2$	–	+	–	–	+	–
$NO_3^- \longrightarrow NO_2^-$	+	+	+	+	+	+
Optimum temperature, °C	37	30	30	30	30	30
Optimum concentration of NaCl, %	2	0.5	0.5	0	0	0
G+C content, mol %	43.8	59.6	45.0	40.5	66.0	45.2
S_{AB}	0.938	0.952	0.944	1.000	0.823	0.835
The closest species according to RDP	*Acinetobacter lwoffii*	*Aeromonas media*	*Acinetobacter lwoffii*	*Acinetobacter calcoaceticus*	*Comamonas terrigena*	*Acinetobacter johnsonii*

that we investigated confirms knowledge about the abundance of these bacteria in nature.

The results presented in this paper show that radioactive liquid wastes stored in the subterranean repository contain viable microorganisms of different physiological groups. Under laboratory conditions, these microorganisms carried out various catabolic reactions, which indicates that they can potentially affect the geochemistry of groundwater and, therefore, the migration of radionuclides. Of great importance is the presence of denitrifying bacteria in groundwater, since they can contribute to the removal of acetate and nitrate anions from radioactive liquid wastes.

Along the front of the migration of underground radioactive liquid wastes, where they are diluted with formation water, denitrifying, fermentative, and methanogenic bacteria can drive the processes of formation of nitrogen, methane, and carbon dioxide. Substrates for these microbiological processes can arrive with wastes (acetate and nitrates), are present in the subterranean system used for waste storage (bicarbonate and organic matter), or can be formed as a result of water



The phylogenetic tree of the nitrate-reducing bacteria isolated from the observation boreholes of a subterranean radioactive liquid waste repository. The tree is constructed based on the results of 16S rDNA sequencing. The scale bar represents 5 nucleotide substitutions per 100 nucleotides. Numbers near internal branches refer to the bootstrap replications (out of 100 resampling) confirming the grouping of the species to the right of the branch. Bootstrap values of less than 70% are not shown.

radiolysis (hydrogen) [7]. In any case, the results of this study show the necessity of investigating the microflora of subterranean repositories and provide evidence for the possibility of applying some microorganisms revealed in situ to the removal of acetate and nitrate anions from radioactive liquid wastes.

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REFERENCES

1. West, J.M., McKinley, I.G., and Chapman, N.A., Microbes in Deep Geological Systems and Their Possible Influence on Radioactive Waste Disposal, *Rad. Waste Management Nuclear Fuel Cycle*, 1982, vol. 3, pp. 1–15.
2. West, J.M., Christofi, N., and McKinley, I.G., An Overview of Recent Microbiological Research Relevant to the Geological Disposal of Nuclear Waste, *Radioact. Waste Manage. Nucl. Fuel Cycle*, 1985, vol. 6, no. 1, pp. 79–95.
3. West, J.M., McKinley, I.G., Grogan, H.A., and Arme, S.C., Laboratory and Modelling Studies of Microbial Activity in the Near Field of the HLW Repository, *Mater. Res. Soc. Symp. Proc.*, 1985, vol. 50, pp. 533–538.
4. Pedersen, K., Arlinger, J., Ekendahl, S., and Hallbeck, L., 16S rRNA Diversity of Attached and Unattached Bacteria in Boreholes along the Access Tunnel to the Aspo Hard Rock Laboratory, Sweden, *FEMS Microbiol. Ecol.*, 1996, vol. 19, pp. 249–262.
5. Stroes-Gascoyne, S. and West, J.M., Microbial Studies in the Canadian Nuclear Fuel Waste Management Program, *FEMS Microbiol. Rev.*, 1997, vol. 20, pp. 573–590.

6. Pedersen, K., Investigation of Subterranean Bacteria in Deep Crystalline Bedrock and Their Importance for the Disposal of Nuclear Waste, *Can. J. Microbiol.*, 1996, vol. 42, pp. 382–391.
7. Pedersen, K., Microbial Life in Deep Granite Rock, *FEMS Microbiol. Rev.*, 1997, vol. 20, no. 3/4, pp. 399–414.
8. McKinley, I.G., Hagenlocher, I., Alexander, W.R., and Schwyn, B., Microbiology, in Nuclear Waste Disposal: Interfaces and Reaction Fronts, *FEMS Microbiol. Rev.*, 1997, vol. 20, no. 3/4, pp. 545–556.
9. Humphreys, P., McGarry, R., Hoffmann, A., and Binks, P., DRINK: A Biogeochemical Source Term Model for Low Level Radioactive Waste Disposal Sites, *FEMS Microbiol. Rev.*, 1997, vol. 20, no. 3/4, pp. 557–572.
10. Kuznetsov, S.I. and Dubinina, G.A., *Metody izucheniya vodnykh mikroorganizmov* (Methods for Studying Aquatic Microorganisms), Moscow: Nauka, 1989, pp. 146–148.
11. Postgate, J.R., *Sulfate-Reducing Bacteria*, Cambridge: Cambridge Univ. Press, 1984, 2nd edition.
12. Adkins, J.P., Cornell, L.A., and Tanner, R.S., Microbial Composition of Carbonate Petroleum Reservoir Fluids, *Geomicrobiol. J.*, 1992, vol. 10, no. 2, pp. 87–97.
13. Trüper, H.G. and Schlegel, H.G., Sulphur Metabolism in *Thiorhodaceae*: I. Quantitative Measurements on Growing Cells of *Chromatium okenii, Antonie van Leeuwenhoek*, 1964, vol. 30, no. 3, p. 225.
14. Reznikov, A.A., Mulikovskaya, E.P. and Sokolov, I.Yu., *Metody analiza prirodnykh vod* (Analytical Methods for Natural Waters), Moscow: Nedra, 1970.
15. Marmur, J., A Procedure for the Isolation of DNA from Microorganisms, *J. Mol. Biol.*, 1961, vol. 3, pp. 208–218.
16. Owen, R.J., Hill, L.R., and Lapage, S.P., Determination of DNA Base Compositions from Melting Profiles in Dilute Buffers, *Biopolymers*, 1969, vol. 7, p. 16.
17. Edwards, U., Rogall, T., Bloeker, H., Ende, M.D., and Boeettge, E.C., Isolation and Direct Complete Nucleotide Determination of Entire Genes, Characterization of Gene Coding for 16S Ribosomal RNA, *Nucleic Acids Res.*, 1989, vol. 17, pp. 7843–7853.
18. Van de Peer, Y. and De Wachter, R., TREECON for Windows: A Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Appl. Biosci.*, 1994, vol. 10, pp. 569–570.
19. Rainey, F.A., Lang, E., and Stackebrandt, E., The Phylogenetic Structure of the Genus *Acinetobacter, FEMS Microbiol. Lett.*, 1994, vol. 124, pp. 349–353.
20. Nishimura, Y., Ino, T., and Iizuka, H., *Acinetobacter radioresistens* sp. nov. Isolated from Cotton and Soil, *Int. J. Syst. Bacteriol*, 1988, vol. 38, no. 2, pp. 209–211.
21. Collins, M.D., Martinez-Murcia, A.J., and Cai, J., *Aeromonas enteropelogenes* and *Aeromonas ichthiosmia* Are Identical to *Aeromonas trota* and *Aeromonas veronii*, Respectively, as Revealed by Small-Subunit rRNA Sequence Analysis, *Int. J. Syst. Bacteriol.*, 1993, vol. 43, pp. 855–856.
22. Towner, K.J., The Genus *Acinetobacter, The Prokaryotes*, Balows, A. *et al.*, Eds., New York: Springer, 1999, pp. 3137–3143.