

## Article

# Simple and Complex Substrates (Sugar, Acetate and Milk Whey) for In Situ Bioremediation of Groundwater with Nitrate and Actinide Contamination

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**Abstract:** The complex contamination of groundwater near radioactive waste repositories by nitrates and actinides is a common problem for many nuclear fuel cycle facilities. One of the effective methods to remove nitrates and reduce actinide migration activity is bioremediation through the activation of native microbial communities by soluble electron donors and carbon sources. This work evaluated the effectiveness of using simple and complex electron donors to remove nitrate in the microbial community in an aquifer near the B2 storage of the Siberian Chemical Combine (Seversk, Siberia). The addition of sugar and milk whey led to the maximum efficiency of nitrate-ion removal and a decrease in the redox potential of the system, creating optimal conditions for the immobilization of actinide. Special attention was paid to the behavior of uranium, plutonium, neptunium, and americium under conditions simulating groundwater when sugar, acetate, and milk whey were added and when microbial metabolic products were formed. Neither microbial metabolites nor organic solutions were found to have a significant effect on the leaching of neptunium. At the same time, for plutonium, a decrease in yield was observed when rocks were treated with organic solutions were compared to groundwater treatment without them. Plutonium leaching is significantly affected by rock composition. In rocks with a low clay fraction content, its yield can reach 40%. At the same time, microbial metabolites can increase americium (Am) desorption from rocks with a low clay fraction content. Additionally, particle size analysis was performed using a step-by-step filtration approach, aiming to evaluate the risks that are associated with colloidal phase formation. It was shown that microbiological stimulation resulted in particle enlargement, substantially diminishing the presence of actinides in the form of dissolved or sub-50 nm nanoparticles. This outcome significantly reduced the potential for colloidal and pseudocolloidal transfer, thereby lowering associated risks.

**Keywords:** in situ bioremediation; actinides; aquifers; organic substrates; milk whey; acetate; sugar; denitrification; desorption



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## 1. Introduction

The contamination of groundwater with actinides due to accidents and unsafe storage of radioactive waste poses a long-term uncontrolled migration risk and the potential for the entry of such pollutants into water supply systems [1–3]. Low-level radioactive waste repositories with open-type storage are considered to be a significant part of the groundwater contamination problem [4–6]. Such storage facilities were used in the uranium enrichment process and nuclear fuel reprocessing plants in Russia and other countries during the mid-20th century. Currently, these storage facilities are either undergoing preservation or have already been preserved, and the areas have been remediated [7–9]. However, after

more than 50 years of operation, the protective properties of engineering barriers under the influence of technological and natural factors are gradually decreasing, and this results in increased permeability and subsequent contamination of aquifers with soluble waste components. For uranium mining, processing plants, and sublimation plants, the contamination of groundwater with macrocomponents, such as nitrate, sulfate, carbonate, and ammonium ions, as well as microcomponents, including radioactive elements (Ra, Th, and U) and stable elements (As, Cr, Fe, Mn, Ni, V, Zn, etc.), poses a significant issue [4,10,11]. In the case of open-type radioactive waste storage basins, besides nitrates and sulfates, the migration of soluble forms of long-lived radionuclides, such as actinides (U, Np, Pu, and Am) and fission products (Cl, Cs, Se, Sr, and Tc), into groundwater poses a greater risk. It is important to note that the solubility and the migration ability of many elements in higher oxidation states (U, Np, Pu, Se, and Tc) increase in the presence of nitrate and carbonate ions, certain organic complexing agents, iron-bearing clay, and organic colloidal particles [11–17]. The distribution of radioactive and chemical pollutants in groundwater, in addition to their chemical properties, depends on the porosity of aquifers, the mineral composition of surrounding rocks, and the characteristics of hydrodynamic regimes within aquifers. One of the factors influencing the migration of pollutants in groundwater is the biogeochemical one, which is driven by the activity of subsurface microflora capable of being activated by bioavailable elements present in the waste (C, Fe, N, S) [18–22]. In the case of complex contamination, one suitable method for removing nitrogen, sulfur, and actinides is *in situ* bioremediation, which involves the injection of inexpensive soluble substrates in order to activate the microbial community [23–26]. Microbial processes facilitate the removal of nitrate ions through denitrification (i.e., the reduction of nitrate ions to molecular nitrogen) and the removal of ammonium through nitrification (i.e., the two-step oxidation of ammonium in the presence of oxygen). This technology has proven successful, and it has been actively used worldwide for several decades [5,27,28]. A range of inexpensive soluble organic compounds, such as methanol, acetate [29,30], or more complex substrates, including vegetable oil [31,32], are used as substrates for bioremediation. The choice of substrate depends on the microbial composition of the groundwater and hydrogeological parameters. Hydrophobic complex substrates are used when groundwater flow rates are high [33].

In the case of bioremediation, an important task is to assess the impact of the substrate on the behavior of actinides. It is known that actinides could form soluble complexes with organic substances, which can increase their migration activity [2,3]. Complexes of uranium with isosaccharinic acid and other products of microbial metabolism have been well studied [34]. Some soluble substrates could lead to the desorption of actinides from rocks due to soluble complex formation. In our previous work, it was determined that bioremediation could reduce the risk of colloidal and pseudocolloidal transport of actinides through the aggregation of colloids by microbial metabolites during *in situ* bioremediation of groundwater near the B2 storage facility at the Siberian Chemical Combine [12]. In this case, milk whey, sugar, and acetate were all used in laboratory and field experiments. Their addition during *in situ* bioremediation led to the removal of nitrate ions and the formation of reducing conditions in the formation [24] that were favorable for the immobilization of actinides.

This study aims to select the most optimal substrate for nitrate removal in groundwater samples from the horizon near the B2 storage facility at the Siberian Chemical Combine in order to assess the possible impact of these substrates on actinide immobilization and remobilization as well as the risk of forming colloidal phases. The obtained results will serve to develop recommendations for industrial stakeholders to enhance the monitoring and the control of radioactive waste repositories and contaminated areas, thereby ensuring better environmental protection and safety measures in handling radioactive materials.

## 2. Materials and Methods

### 2.1. Water Samples

The main flow of groundwater in this aquifer is located at a depth of 10–20 m and depends on the local content of the clay phase of 10–30 m/year. When unloading the aquifer into the nearby river, soluble components of waste are possible. The study used groundwater samples taken from a highly polluted area (sample 1) and from an uncontaminated area (sample 2) near the B2 storage facility at the Siberian Chemical Combine at a 15 m depth (Table 1).

**Table 1.** Physico-chemical parameters of water samples.

Well	1	2
Sampling depth, m	15	15
TOS Salinity, mg/L	3952.0	109.0
pH	6.58	6.41
Eh	65	−30
Oxidizability, mg O/L	13.10	5.11
Fe(total)	0.25	2.38
Na <sup>+</sup>	604.0	3.41
K <sup>+</sup>	3.09	0.59
Ca <sup>2+</sup>	316.60	15.39
Mg <sup>2+</sup>	63.20	2.76
NH <sub>4</sub> <sup>+</sup>	7.64	<0.5
NO <sub>3</sub> <sup>−</sup>	2517.0	0.77
SO <sub>4</sub> <sup>2−</sup>	72.40	0.84
Cl <sup>−</sup>	4.52	2.26
HCO <sub>3</sub> <sup>−</sup>	331.0	67.10
NO <sub>2</sub> <sup>−</sup>	<0.2	<0.2

Sample 1 was taken at a distance of 20 m from the storage in the direction of the groundwater flow. Sample 2 was taken at a distance of 60 m from the other side of the storage away from the direction of the groundwater flow. The technogenic impact was caused by an increase in the concentration of soluble waste components: nitrate ions, hydrocarbonate, and ions Na, K, Ca, Mg, and NH<sub>4</sub>. A decrease in iron content was noted, which was probably due to its oxidation in an oxidizing environment as well as the formation of low-soluble phases. The composition of microbial communities has been described in our previous work [24]. It was found that the samples contained a significant group of bacteria capable of being nitrate reducers (denitrifiers) and sulfate reducers as well as iron reducers.

### 2.2. Sandy-Loam Samples

Rock (sandy loam) samples with different clay fraction contents from contaminated and background areas were used. The loam samples were taken at different distances from the repository at a 15 m depth. Samples s2, s3, and s4 were all taken near the repository, and they have a greater technogenic impact. Sample S2 was taken at the point of water sample 1, and sample s1 was taken at the point of water sample 2 in the uncontaminated zone. Sample s3 was taken at a distance of 30 m from the repository, and sample s4 was taken at a distance of 40 m from the repository. The choice of loam sample selection points was dictated by the importance of evaluating the technogenic factor in changing their sorption parameters in relation to actinides. All of the samples had a typical mineralogical

composition, with a predominance of quartz from 36% (n5) to 53% (n4) and CMP from 10% (n5) to 23% (n2). Up to 25% albite was detected in the sample with a low KSP content. Goethite phase was detected in most of the samples, with a maximum content of up to 4% in sample s1. Samples s1, s3, s4, and s5 contained siderite up to 4% (s1,s5). Sample n2, taken near the repository, contained calcite, which was most likely due to the high calcium content from the repository. It is possible that anthropogenic influences led to a decrease in both goethite and siderite content and to an increase in both smectite and illite content (Tables 2 and 3).

**Table 2.** Elemental composition of sandy loams, mass%.

Sample	Na <sub>2</sub> O	MgO	Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	K <sub>2</sub> O	CaO	TiO <sub>2</sub>	MnO	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	S
s1	1.68	1.14	8.64	79.32	1.69	1.46	0.39	0.038	2.75	0.07	0.09
s2	3.18	2.80	7.78	74.20	1.98	4.45	0.01	0.001	2.15	0.05	2.78
s3	1.11	0.98	11.81	77.51	2.82	0.44	0.600	0.11	4.41	0.10	<0.02
s4	1.20	0.96	11.69	79.03	3.00	0.46	0.570	0.06	2.84	0.08	<0.02
s5	1.2	1.4	13.4	75.7	2.9	1.5	0.78	0.11	2.9	0.1	<0.02

**Table 3.** Mineral Composition of sandy loams, %.

Mineral Phase	Samples				
	s1	s2	s3	s4	s5
Quarz	50	42	49	53	36
Siderite	4	-	3	1	4
Calcite	-	4	-	-	-
Potassium feldspar	22	20	20	20	10
Albite	3	6	-	-	25
Amphibole	-	-	-	-	2
Goethite	4	2	3	1	-
Smectite	8	13	5	5	10
Kaolinite	3	3	5	5	5
Illite	5	10	5	5	5
Chlorite	-	-	-	-	3

At the same time, the total iron content in the sample was insignificantly different from the others. This could be a consequence of the dissolution of crystalline phases and the formation of both X-ray amorphous phases of iron oxide and the penetration of this element into the interlayer space of clay minerals. In the remaining samples, the clay mineral content ranged from 15% to 20%.

### 2.3. Methods

Substrate screening was carried out using 20 mL of water samples in 30 mL hermetically sealed penicillin vials, where dissolved sterilized organic additives were added at a concentration of 2–4 g/L, depending on the sample. Sterilized nitrate ions solution was added to sample 1 to a final concentration of 1000 mg/L. The experiment was carried out for 28 days by measuring the content of nitrate and nitrite ions in the water phase at a temperature of 12 °C, which was close to natural conditions. The gas phase was represented by argon. The rate of nitrate removal and nitrite formation was defined as the difference in their concentrations at the start and the end point, divided for an experimental time.

Sorption experiments were carried out with in sealed penicillin vials, which were preliminarily purged with argon. Radionuclide solutions ( $10^{-7}$  M of  $^{233}\text{U}$ ,  $^{237}\text{Np}$ , and  $^{239}\text{Pu}$ ) were injected with an insulin syringe. The phase ratio was 0.1 g/mL. Radionuclides were added to air-dried rock samples (1 g weight) in nitrogen-oxide form with a maximum concentration in a minimum volume (0.01 mL) of solution. After addition, samples were moistened and stirred thoroughly for 60 days [14,35]. The samples were then air-dried and stored for 6 months in sealed tubes in a refrigerator. Solutions of a mixture of sugar, acetate, and whey (2 g/L) were used as the leaching aqueous phase. The volume of the aqueous phase was 5 mL. The establishment of equilibrium was monitored by conducting measurements on 1 mL aliquots of the solution at specific time intervals, including 15 min, 75 min, 6 days, and 34 days.

Desorption experiments. Radioisotopes  $^{239}\text{Pu}$ ,  $^{237}\text{Np}$ , and  $^{152}\text{Eu}$  in the form of nitric acid were introduced into 1 g air-dried rock samples, with the maximum concentration in a minimal volume (0.01 mL) of solution. After the introduction, the samples were moistened and subjected to thorough agitation for a duration of 60 days.

Subsequently, the samples were dried to an air-dried state and stored for 6 months in sealed vials in a refrigerator. A solution composed of a mixture of sugar, acetate, and whey (at a specific concentration) was utilized as the leaching aqueous phase. The volume of the aqueous phase was 5 mL. After removing the supernatant liquid, the rocks were treated with a 0.04 M  $\text{NH}_2\text{OH}\cdot\text{Cl}$  solution in 25%  $\text{CH}_3\text{COOH}$  with a volume of 5 mL, followed by intensive agitation, and they were then left in contact for 10 days. Subsequently, the aqueous phase of the rocks was opened with a mixture of hydrofluoric and sulfuric acid in order to calculate the balance of the radioisotope content after radiochemical analysis.

The size of particles in the samples was determined by step-by-step filtration with syringe-mounted Vladipor (Russia) filters that were 2.4, 1.2, 0.8, 0.4, 0.22, 0.1, and 0.05  $\mu\text{m}$  in diameter [36]. The concentration of the ions and radionuclides was determined in each filtrate.

Analysis of the loam samples composition was carried out by X-ray phase analysis on a Panalytical Aeris powder X-ray diffractometer with Cu  $K\alpha$  anode at 40 kV and 15 mA at the Shared Use Center, Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences. For XRD analysis after drying, the samples were ground in a corundum mortar to a powder state. The survey was carried out on a PANalytical AERIS device (Malvern, United Kingdom). The sample was taken from  $2^\circ$  to  $65^\circ$   $2\theta$  in 0.002 increments and a  $\frac{1}{4}$  rad slit. The obtained results were interpreted using the HighScore Plus program, PDF2 database.

Anion and cation concentrations were measured using a CGE capillary electrophoresis system (Capel-105M, LUMEX Instruments, Sankt Peterburg, Russia).

The determination of Eh and pH values were carried out using an ANION-4100 pH meter/ionomer (Novosibirsk, Russia) with an electrode combination.

Radiochemical analytic. The equilibrium for neptunium was achieved at the slowest rate, taking 6 days, whereas europium and plutonium reached equilibrium within 75 min. Based on these observations, all desorption experiments were conducted with a desorbent solution incubation period of 6 days. The plutonium content was determined by alpha spectrometry, neptunium was determined by the luminescent method [36,37], and europium was determined by gamma spectrometry. In this study, europium was used as a model for studying americium. Therefore, the results will be provided as americium studies.

### 3. Results

#### 3.1. Screening of Substrates for Nitrate Removal (Accumulation of Nitrite Ions) in Samples of Groundwater

Table 4 presents the results of the substrate screening for denitrifier activation in groundwater samples from wells 1 and 2. A sample from well 2, which had the lowest contamination, was supplemented with sodium nitrate at a concentration of 1 g/L. It was found that milk whey (MW), glucose (Gl), and sodium acetate (Ac) based on nitrate

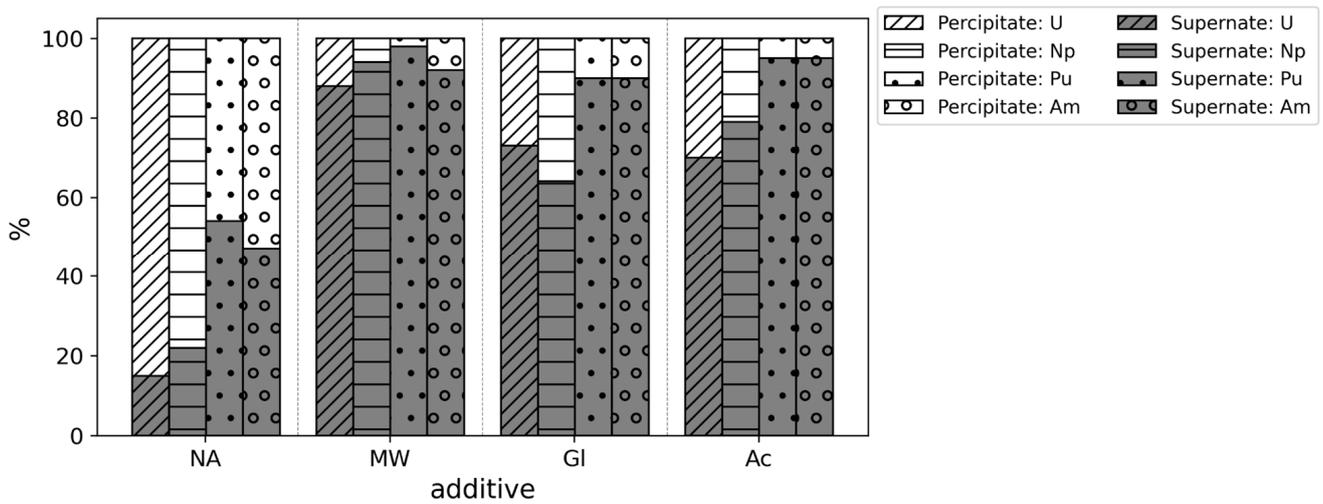
removal rates were the most optimal substrates for activating denitrification processes in both samples. These substrates resulted in low nitrite accumulation rates (columns 3, 5). Previously, using milk whey at various concentrations in field experiments showed high effectivity of nitrate removal in situ in our research [24] and in other research [37,38] due to its complex composition and the presence of bioavailable elements, including potassium, phosphorus, etc.

**Table 4.** Rates of nitrate removal/nitrite accumulation in groundwater samples 1 and 2, mg/L/day.

Substrates	Sample 1		Sample 2	
	NO <sub>3</sub> <sup>-</sup> Removal	NO <sub>2</sub> <sup>-</sup> Accumulation	NO <sub>3</sub> <sup>-</sup> Removal	NO <sub>2</sub> <sup>-</sup> Accumulation
Hydrogen	89.1	0.2	102.6	0.5
Methanol	106.2	5.0	41.8	7.9
Ethanol	96.5	2.9	92.2	8.4
Glycerin	45.7	2.9	55.8	3.9
Oxalate	33.4	8.6	32.4	11.4
Acetate	115.1	4.3	160.6	6.8
Lactate	86.5	4.3	39.5	3.9
Glucose	96.5	2.1	104.1	6.7
Sucrose	136.1	5.0	106.5	7.5
Milk whey	232.8	0.2	136.7	0.15
Brewing waste	222.1	16.7	94.0	0.56
Sunflower oil	78.6	16.0	56.8	1.1

### 3.2. Distribution of Radionuclide Forms in the Solution/Sediment System in A Sample of Natural Water

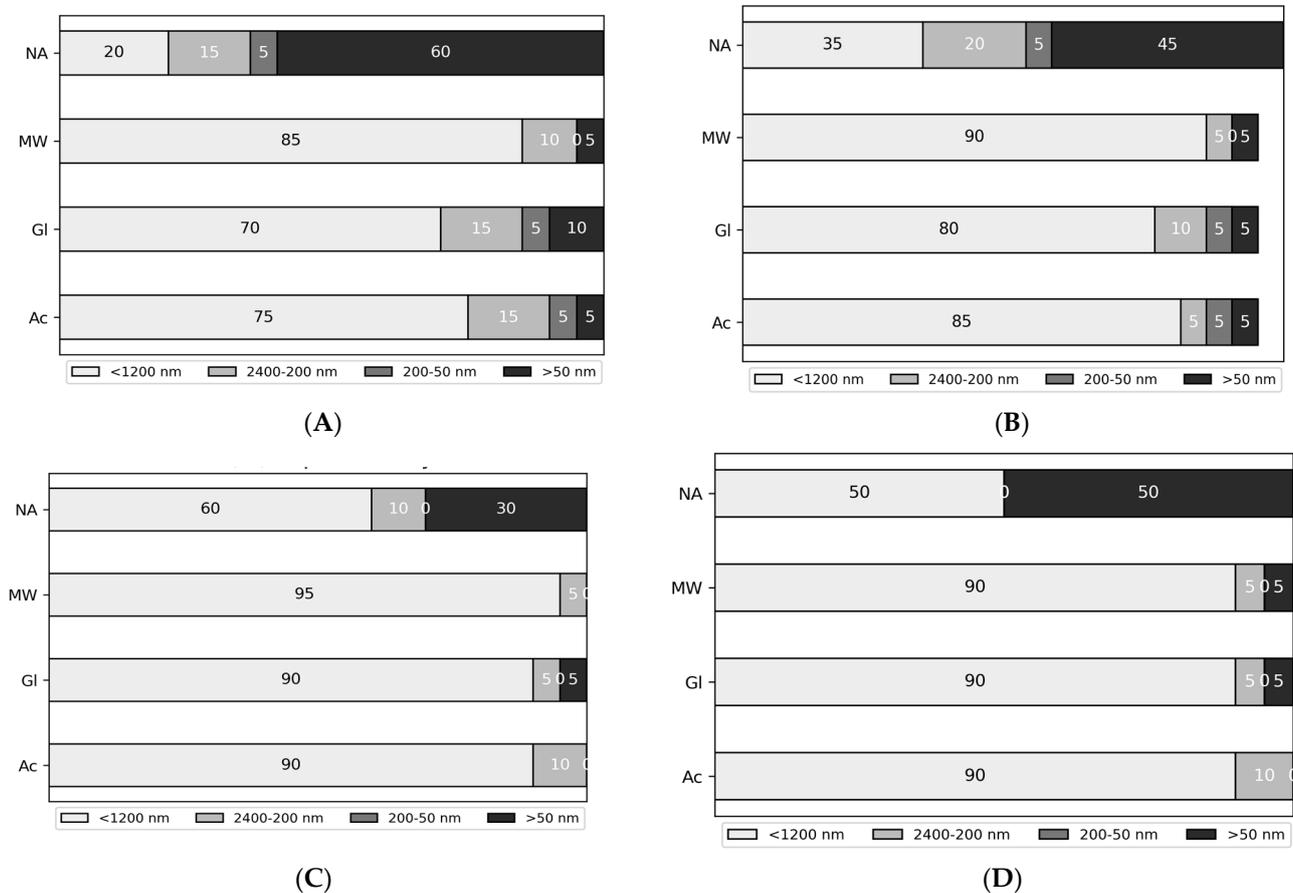
The distribution of actinides between the solid and liquid phases in samples before and after microbial processes are presented in Figure 1. At the beginning of the experiments, more than 85% of uranium was in the liquid phase, and the maximum removal effect was achieved by milk whey (MW), with a removal efficiency of over 85% from the solution. This can be attributed to the high intensity of the microbial processes and the presence of phosphates in MW, which could create low-solubility precipitates with uranium. The removal efficiency of uranium using acetate and sucrose reached 70–75%. For the other actinides, the maximum removal efficiency from the solution was also observed when MW was added, specifically 95% for neptunium, 98% for plutonium, and 92% for americium. The removal efficiency of americium and plutonium through microbial activation using acetate and sucrose was above 90%, while for neptunium, the removal efficiency was over 65% when sucrose was added, and was around 78% when acetate was added. It is important to note that in sample 2 with a nitrate content of 3.2 g/L, before microbial processes, more than 85% of uranium, 78% of neptunium, 55% of americium, and 45% of plutonium were present in the solution. This could be related to hydrolysis processes of actinides and their binding with clay and iron colloidal particles in water. Previously, we described the binding process of natural colloids during the flow of microbial processes in a sample of groundwater with the stimulation of microbial processes by molecular hydrogen [12].



**Figure 1.** Actinide distribution between solid and liquid phase before and after microbial processes in sample 2 stimulated by milk whey (MW), glucose (GI), acetate (Ac), and without stimulation (NA).

3.3. Actinides Size Distribution in a Sample of Natural Water after Substrates Addition

At Figure 2A–D, the distribution of actinides between the solid and liquid phases before and after microbial processes in sample 2 are shown. In the samples without microbial activation (NA), more than 60% of uranium (Figure 2A), 45% of neptunium (Figure 2B), 30% of plutonium (Figure 2C), and 50% of americium (Figure 2D) were dissolved or in the form of nanoparticles smaller than 50 nm.



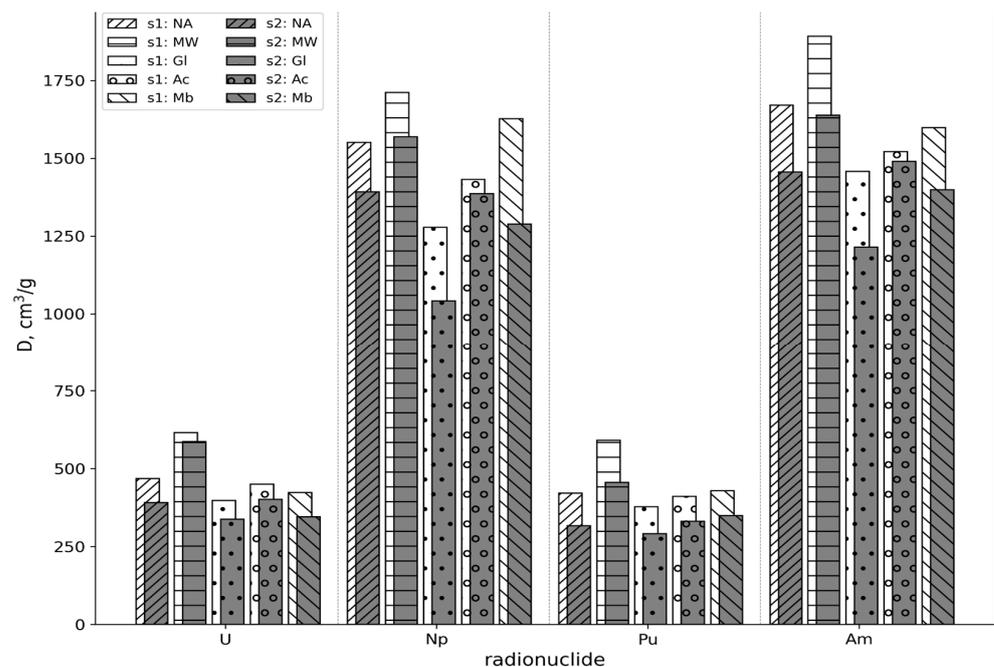
**Figure 2.** (A) Uranium content in particles by size distribution in sample 2 stimulated by milk whey (MW), glucose (GI), acetate (Ac), and without stimulation (NA). (B) Neptunium content in particles

by size distribution in sample 2 stimulated by milk whey (MW), glucose (Gl), acetate (Ac), and without stimulation (NA). (C) Plutonium content in particles by size distribution in sample 2 stimulated by milk whey (MW), glucose (Gl), acetate (Ac), and without stimulation (NA). (D) Americium content in particles by size distribution in sample 2 stimulated by milk whey (MW), glucose (Gl), acetate (Ac), and without stimulation (NA).

It was found that when MW was added, more than 85% of uranium was present in particles larger than 1200 nm. When acetate and glucose were added, the content of particles larger than 1200 nm was 70–75%. Moreover, the content of dissolved and colloidal forms smaller than 50 nm was no more than 5% when MW and acetate were added, and it did not exceed 10% when glucose was added (Figure 2A). After microbiological stimulation in the case of neptunium, the formation of particles larger than 1200 nm was observed, contributing to over 80% of the total. The contribution of dissolved and small colloidal forms was below 5% (Figure 2B). In the case of plutonium and americium, the proportion of particles larger than 1200 nm was more than 90%, while particles smaller than 50 nm accounted for less than 50% (Figure 2C,D).

#### 3.4. Actinides Sorption on Rock Samples in the Presence of Milk Whey, Sugar, Acetate, and Metabolites

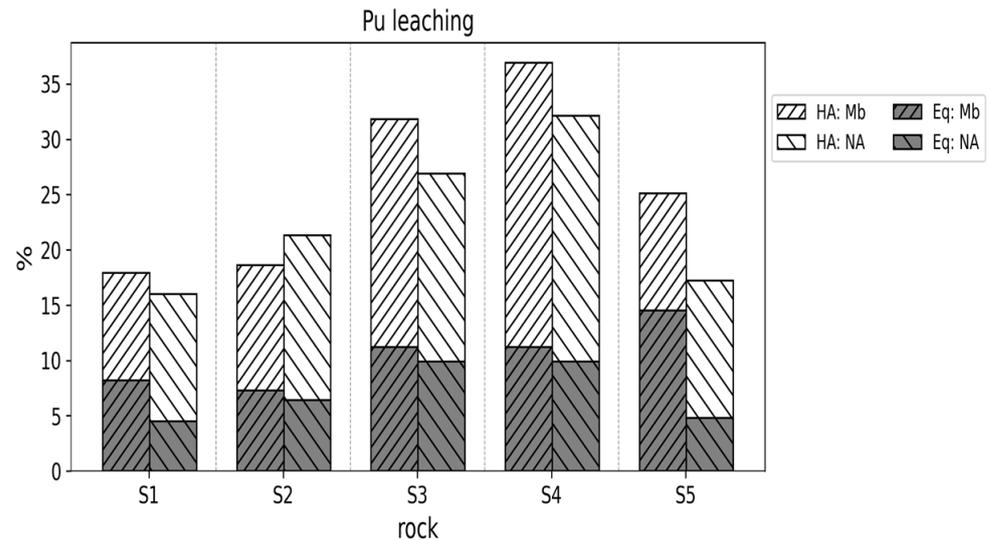
Figure 3 presents the distribution coefficients of U, Np, Pu, and Am in rocks obtained from wells B2-32 and B2-38 in the presence of milk whey (MW), glucose (Gl), acetate (Ac), metabolites (Mb), and without additives (NA). For all of the radionuclides in each media, a slightly higher distribution coefficient was observed for sample 1 compared to sample 2. It should be mentioned that sample 1 contained a higher concentration of iron-bearing minerals, such as siderite and goethite (see Table 2), and this could contribute to better retention of radionuclides by the rock. Furthermore, the highest distribution coefficient was consistently found in the presence of milk whey, while the lowest was observed for the glucose. The highest distribution coefficient was noted for americium, with a comparable value for neptunium, while the coefficients for uranium and plutonium exceeded them by nearly threefold.



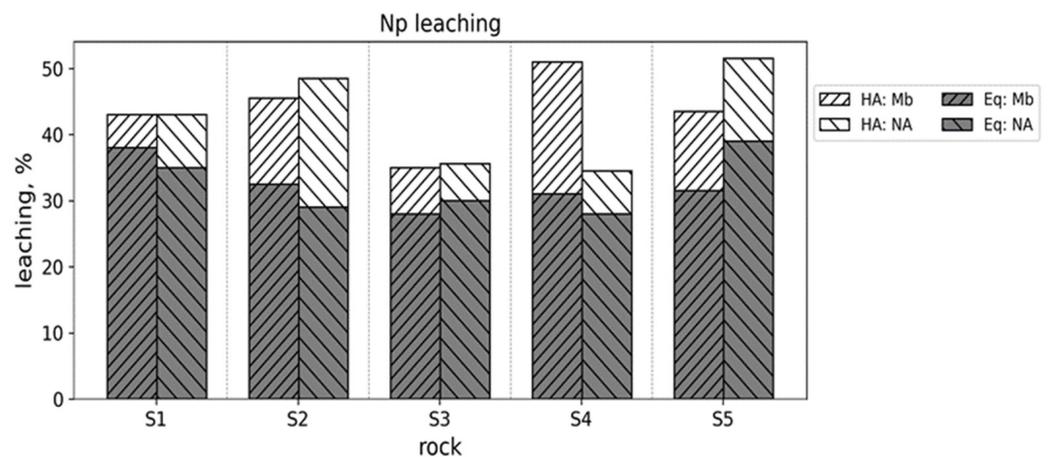
**Figure 3.** The distribution coefficient of Np, Pu, and Am in soils in the presence of organic additives and metabolites, cm<sup>3</sup>/g NA, no additives, milk whey MW, glucose Gl, sodium acetate, Ac, metabolites Mb.

### 3.5. Desorption of Radionuclides from Rock Samples in the Presence of Substrates

The leaching was measured (Eq) into two different medium water and metabolites, and, after that, leaching by hydroxylamine (HA) was carried out. Figures 4–6 represent the leaching results of Pu, Np, and Am, respectively, into metabolites (right-hatch pattern) and water (left-hatch pattern), followed by the subsequent leaching in the presence of hydroxylamine (white-colored bars).



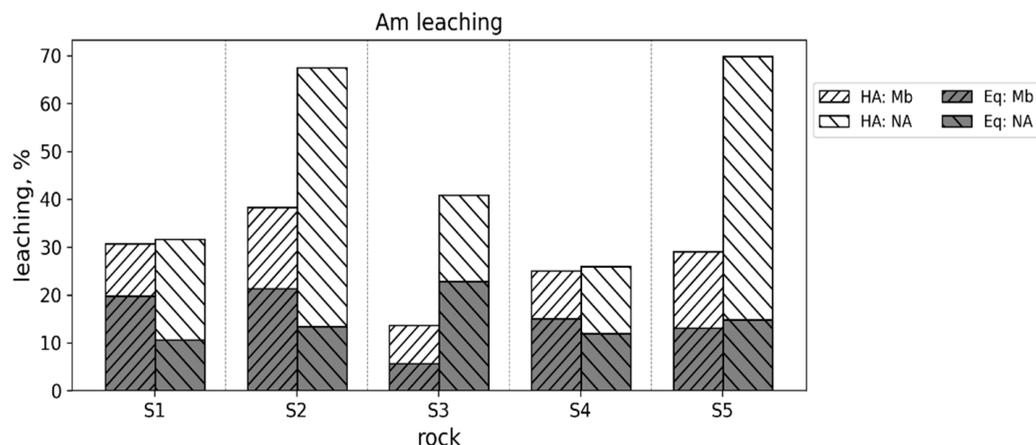
**Figure 4.** Total amount of plutonium desorption from the rock in the presence of metabolites (right-hatch pattern, white and grey bars) and without additives (left-hatch pattern, white and grey)—white bar's for natural water (Eq) and by hydroxylamine (left-hatch pattern).



**Figure 5.** Total amount of neptunium desorption from the rock in the presence of metabolites (right-hatch pattern, white and grey bars) and without additives (left-hatch pattern, white and grey bars)—white bars for natural water (Eq) and by hydroxylamine (left-hatch pattern).

For Pu, Np, and Am, leaching percentages ranged from 16% to 37%, 14% to 70%, and 35% to 51.5% for all of the samples. The lower contribution of hydroxylamine to overall leaching in the case of Np was observed. Plutonium demonstrated the highest overall desorption in samples 3 and 4, while europium showed this trend in samples 2 and 5. Notably, plutonium exhibited comparable desorption values with both metabolites and water. In the case of americium, leaching with HA was generally higher, particularly in samples 2 and 5, which displayed maximum leaching. The leaching percentages of radionuclides into metabolites indicated a preferential interaction between the radionuclides and organic components that were present in the samples. As previously mentioned, minerals such as

siderite and goethite could be crucial to the affinity of the rock with radionuclides. Sample 1 contained the highest fraction of siderite and goethite, while sample 3 had a value close to this but still lower. Furthermore, sample 5 showed a high concentration of siderite similar to sample 1, though it did not contain goethite. In addition, sample 4 had the lowest presence of siderite and goethite, while sample 2 did not contain siderite but did have a slightly higher amount of goethite.



**Figure 6.** Total amount of americium desorption from the rock in the presence of metabolites (right-hatch pattern, white and grey bars) and without additives (left-hatch pattern, white and grey bars)—white bars for natural water (Eq) and by hydroxylamine (left-hatch pattern).

#### 4. Conclusions

The comparison of various substrates for nitrate removal from groundwater and the evaluation of the impact of these substrates on americium, plutonium, and neptunium mobility, as well as the risks associated with colloidal phase formation, was conducted in this study.

The highest efficiency in activation of microbial communities was shown by complex substrates (milk whey) and monosubstrates (glucose, sugar, acetate). In laboratory experiments, these substrates do not affect the degree of leaching of all radionuclides. Moreover, the formation of large actinide-containing particles observed in the solution can lead to the sedimentation of clay and iron-containing particles, as we have previously shown in [12]. This can reduce the risk of its colloidal and pseudocolloidal transfer.

Based on the results of laboratory experiments, it was found that:

1. The mobility of neptunium was more significant than americium and plutonium, and the leaching by natural water was in a range from 28 to 39%.
2. For samples with high aluminum and sulfur content, a significant reduction in americium leaching was observed after pre-treatment of microbial activation. In the presence of hydroxylamine (HA), this difference reached up to 40% of the total.
3. In the case of the plutonium in the samples with a high aluminum content, a minor increase in leaching with pre-treatment was observed in the presence of HA. The proportion of plutonium leached without pre-treatment in this case constituted 70% to 80% of the total.
4. The occurrence of active microbial processes on rocks may be associated with the reduction of iron and the conversion of highly crystalline ferruginous phases into less crystalline ones with a higher sorption capacity with respect to actinides.

These results emphasize the importance of considering rock composition when developing strategies to modify the mobility of actinides using bioremediation techniques.

The interactions between hydroxylamine and rocks, specifically with iron-containing minerals as well as manganese oxides due to their solvation, reduces the effectiveness of the immobilization of actinides. Moreover, the specific phase of the mineral, whether crystalline or amorphous, during the interactions is important [2,11,20,39,40]. Additionally, to effectively retain plutonium and americium, the capacity of aluminosilicates is expected to be more important. Furthermore, it is worth mentioning that the impact of metabolites on the retention of americium also depends on the rock type. In some rocks, the use of metabolites leads to an increased retention of americium. Thus, it is shown that the method of in situ groundwater bioremediation can be safely applicable in case of its complex pollution with nitrogen compounds and actinides. Examples of such cases are aquifers near the surface of liquid waste storages in radiochemical plants at a stage of their conservation or in a stage of long-term protection in the post-conservation period. Moving forward, we intend to assess actinide behavior under varying pH conditions, focusing on the impact of alkaline and acidic risks during bioremediation. Additionally, we hope to study the role of microbial iron mineral transformation in actinide immobilization. The goal of these studies is to enhance our understanding of actinide interactions, which will lead to more effective bioremediation strategies for contaminated sites.

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