

## Supplementary Materials

### Biodiversity and potential activity of microorganisms in underground gas storage horizons

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### Supplementary Figures

**Figure S1.** Abundance of microorganisms of the main metabolic groups in water samples from the underground gas storage horizons and from gas collection point (GCP) at the Peschano–Umetskoe, Shchelkovo, Kasimov, and Kaluga underground gas storages. Designations: AOB, aerobic organotrophic bacteria; AMB, aerobic methylotrophic bacteria; FERM, fermenting bacteria; SRB, sulfate-reducing bacteria grown in the medium with lactate; SRB\_H2, sulfate-reducing bacteria grown in the medium with H<sub>2</sub>; MET, methanogens grown in the medium with methanol; MET\_H2, methanogens grown on H<sub>2</sub>/CO<sub>2</sub>.

**Figure S2.** Venn diagrams showing the number and proportion of shared and unique OTUs between the three (a) and five (b) libraries of 16S rRNA genes prokaryotes from UGSs.

**Figure S3.** The relative proportion of the 16S rRNA gene fragment sequences of *Bacteria* and *Archaea* in the libraries from the UGS water samples.

**Figure S4.** Predicted profile of enzymes for part of methane metabolism pathway in prokaryotes from water samples PU\_33 (a) and Kal\_121 (b) according to the KEGG database. The intensity of the color is due to the supposed representation of enzymes. The dominant modules of the methane metabolism pathway are highlighted in red.

**Figure S5.** Predicted profile of enzymes of the sulfur metabolism pathway in water samples PU\_6 (a), PU\_33 (b), and Kal\_121 (c) according to the KEGG database. The intensity of the color is due to the supposed representation of enzymes. The dominant modules of the sulfur metabolism pathway are highlighted in red.

**Figure S6.** Predicted profile of enzymes of the phthalate metabolism (part of the PAH degradation pathway) in prokaryotes from the PU\_6 water sample according to the KEGG database. The dominant modules of the phthalate metabolism pathway are highlighted in red.

**Figure S7.** Predicted profile of enzymes of the benzoate degradation pathway in prokaryotes from the PU\_6 water sample according to the KEGG database. The dominant modules of the benzoate degradation pathway are highlighted in red.

**Figure S8.** Sulfide production by Kal\_SE sulfidogenic enrichment on media with various organic acids, alcohols, or/and H<sub>2</sub>/CO<sub>2</sub> mixture incubated at 25 °C for 85 days.

**Figure S9.** Image of a steel filter fragment with an adhered microbial biofilm (a); elemental composition (b); and maps of spatial distribution of the chemical elements: O (c); C (d); Fe (e); S (f); Na (g); and Cl (h) on the sample surface.

## Supplementary Tables

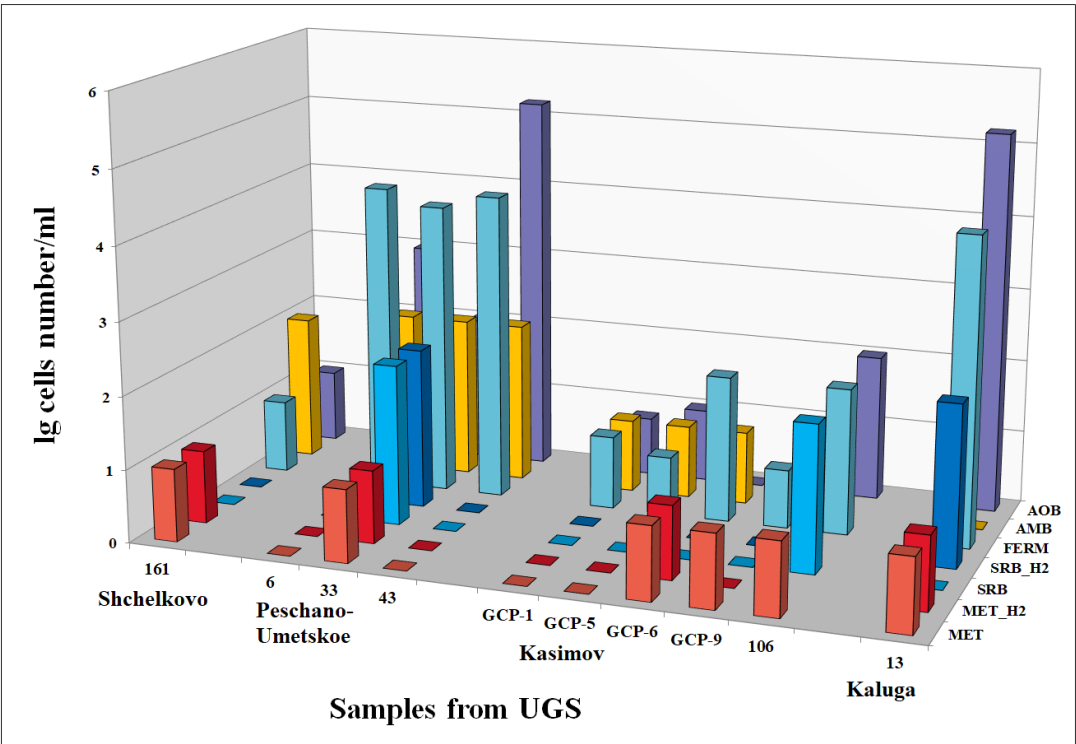
**Table S1.** Characteristics of the UGS horizons used for water sampling.

**Table S2.** Physicochemical characteristics of the water sampled in 2021 from aquifers and gas collection points (GCP) at the Peschano–Umetskoe, Shchelkovo, Kasimov, and Kaluga underground gas storages.

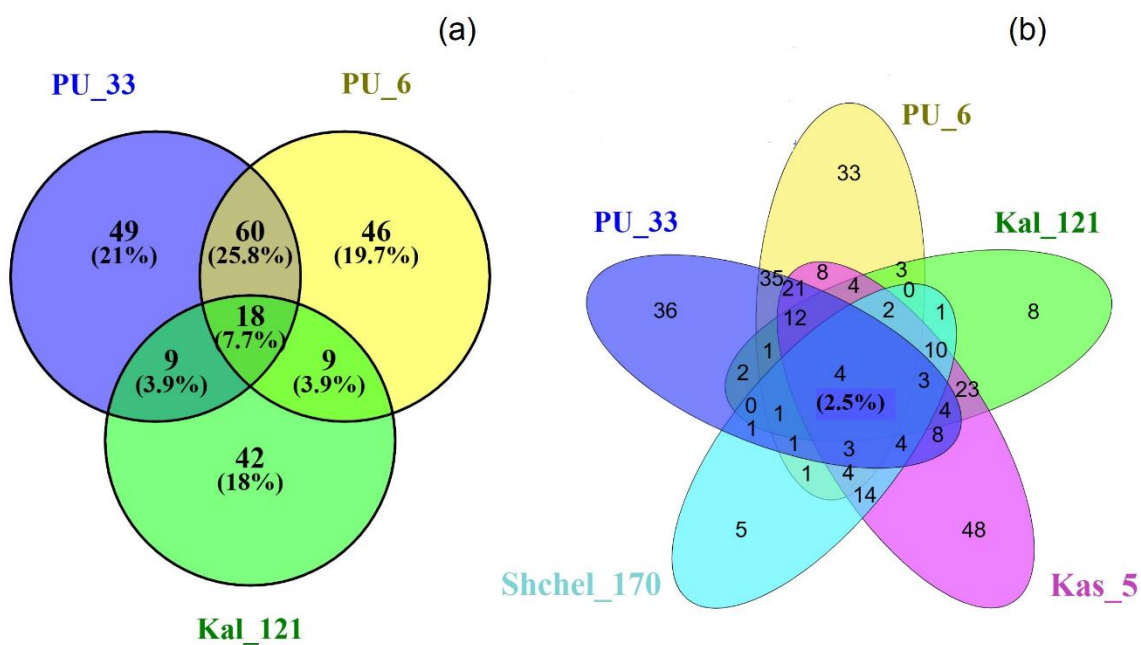
**Table S3.** Diversity indices of the 16S rRNA gene sequences of prokaryotes in the libraries from UGS samples.

**Table S4.** Potential contribution of microorganisms from UGS water samples in nitrogen, sulfur, and methane metabolism pathways and benzoate and polycyclic aromatic hydrocarbon (PAH) degradation according to the KEGG database.

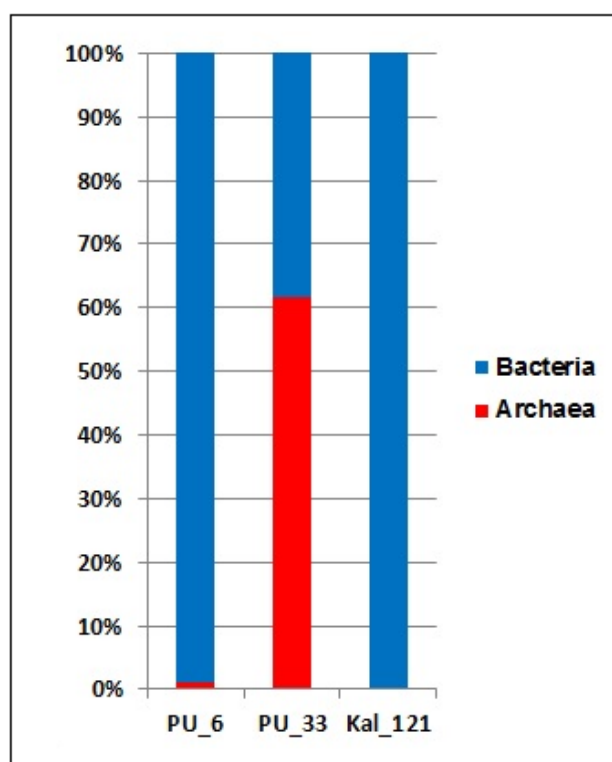
**Table S5.** Potential contribution of microorganisms from UGS water samples in hydrogen metabolism according to the BRENDA database [66].



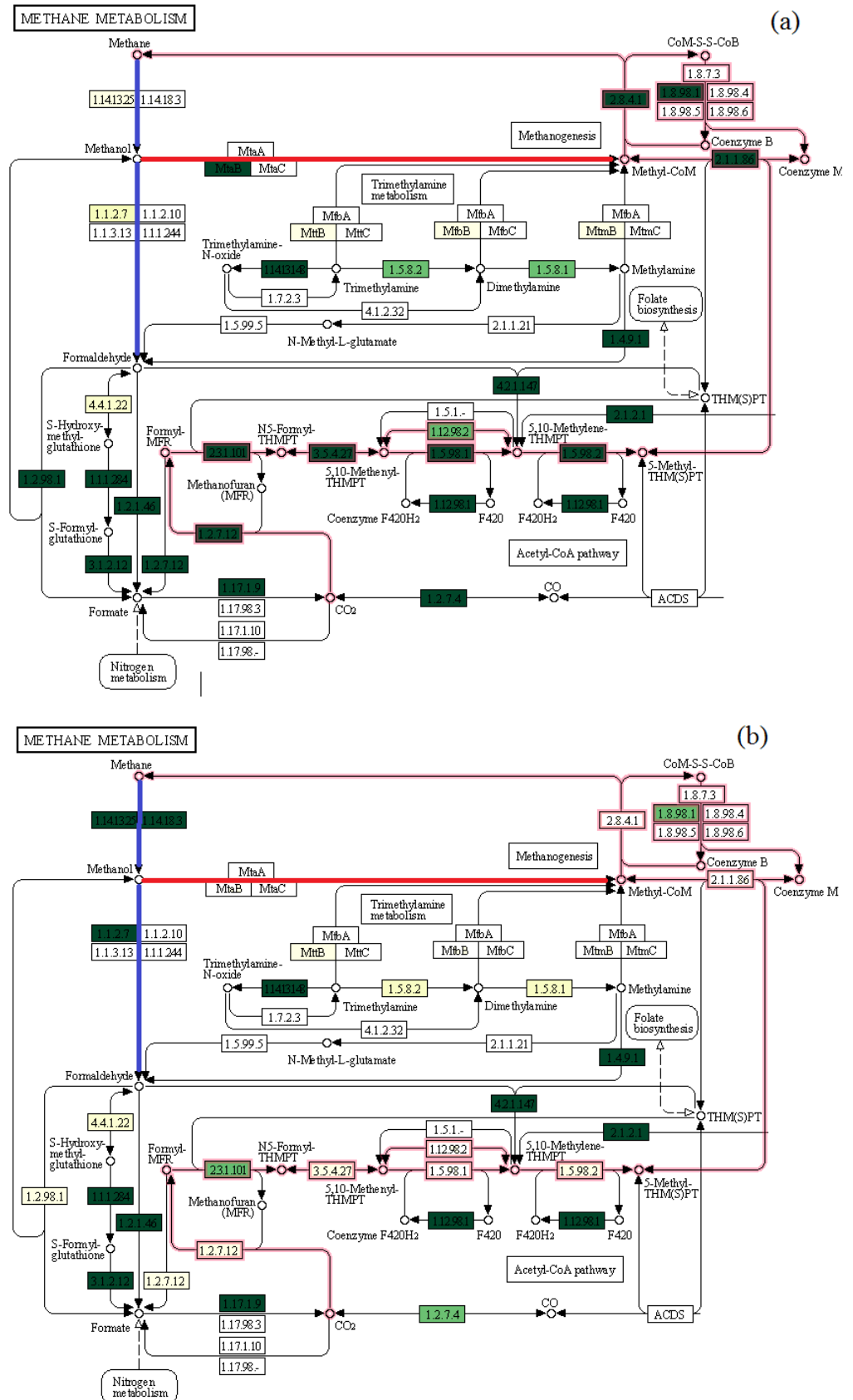
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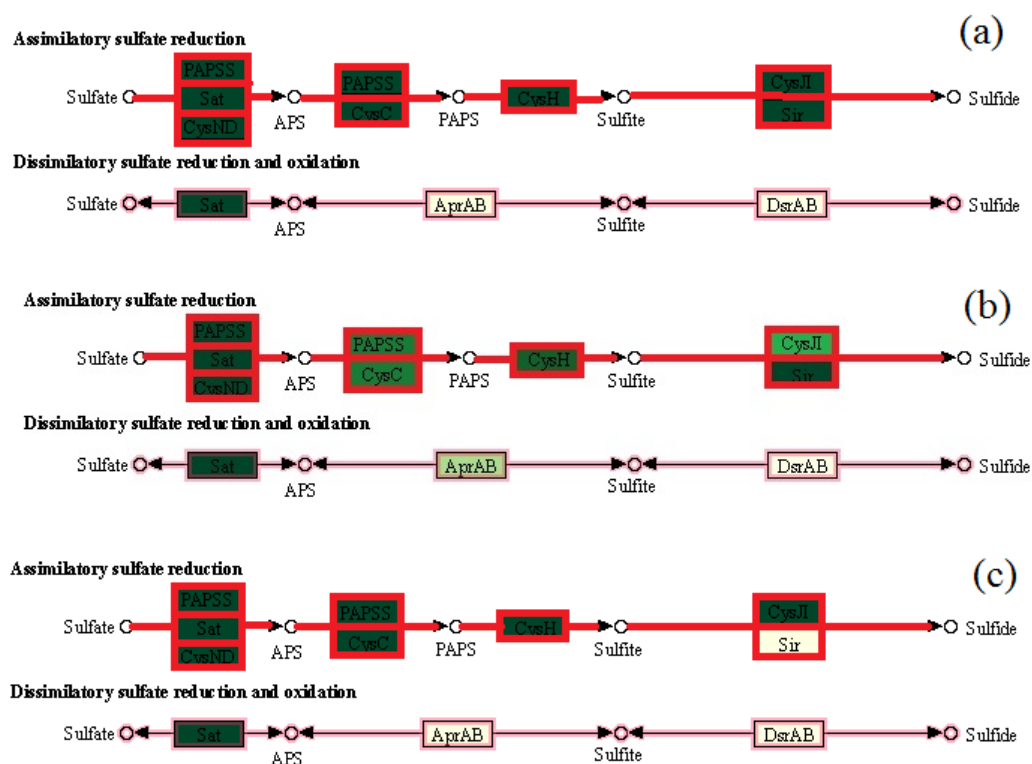
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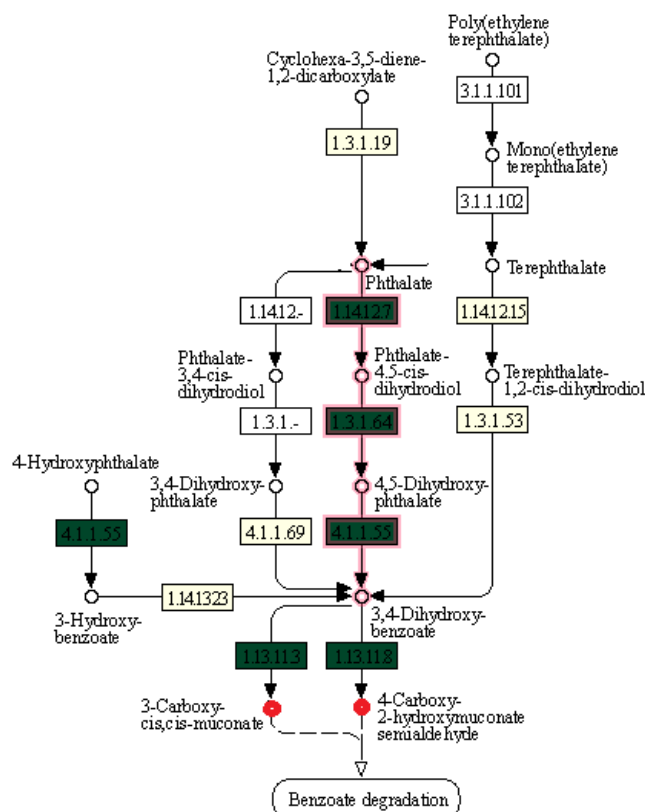
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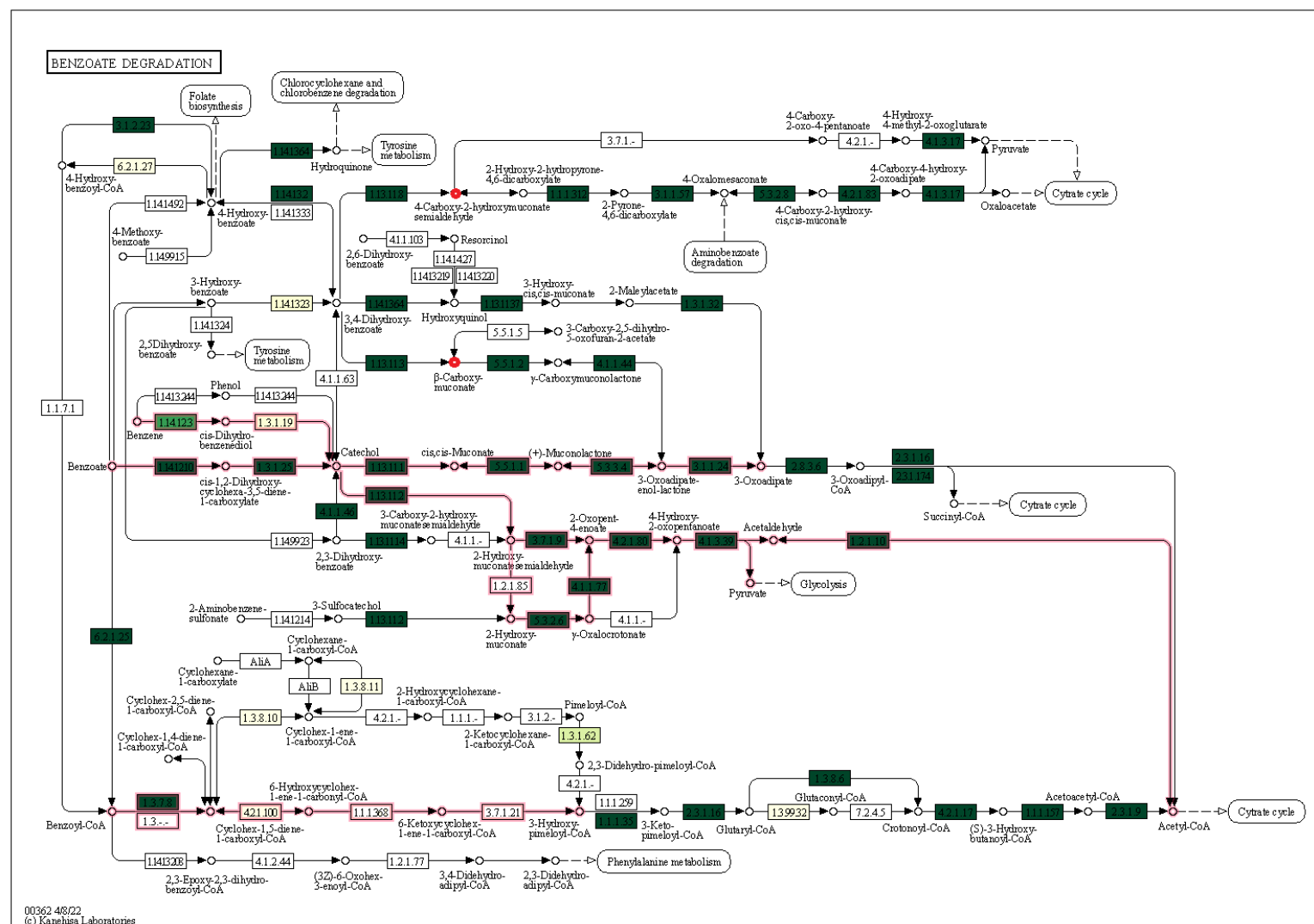
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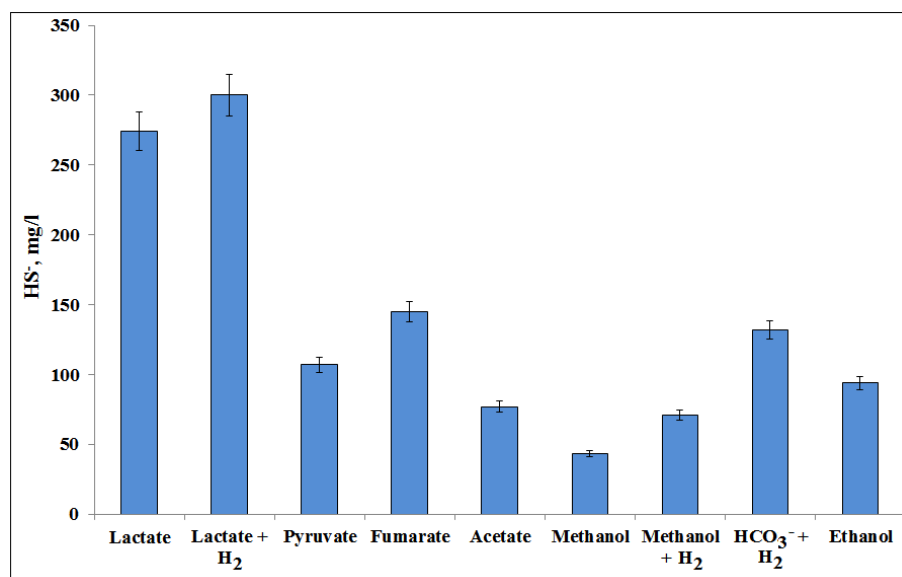
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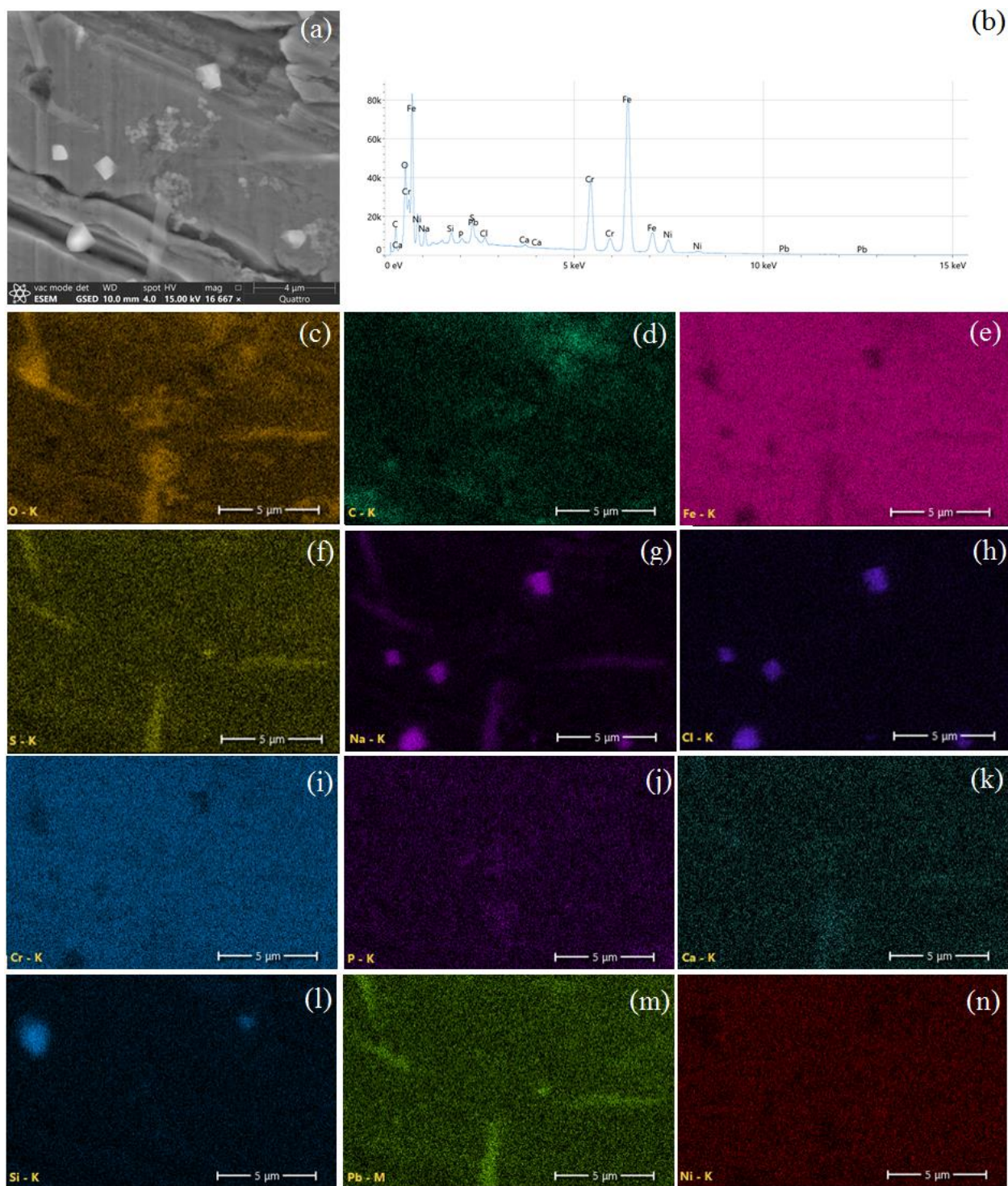


**Figure S7.** Predicted profile of enzymes of the benzoate degradation pathway in prokaryotes from the PU\_6 water sample according to the KEGG database. The dominant modules of the benzoate degradation pathway are highlighted in red.



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**Figure S9.** Image of a steel filter fragment with an adhered microbial biofilm (a); elemental composition (b); and maps of spatial distribution of the chemical elements: O (c); C (d); Fe (e); S (f); Na (g); Cl (h); Cr (i); P (j); Ca (k); Si (l); Pb (m), and Ni (n) on the sample surface.



**Table S1.** Characteristics of the UGS horizons used for water sampling.

Parameters	Peschano–Umetskoe UGS	Shchelkovo UGS	Kasimov UGS	Kaluga UGS
Geographic coordinates	51°53'71.35 "N, 45°67'25.00" E	55°89'85.73" N 37°98'97.62" E	54°90'53.37" N, 41°23'59.81" E	54°55'80.86" N, 36°10'63.80" E
Horizon	Tulskiy	Shchigrovsky	Nizhne Shchigrovsky	Gdovsky
Lithology	Carboniferous deposits represented by interlayers of quartz sandstones, slightly micaceous with calcareous cement	Weakly cemented sandstones and siltstones of the Fran stage	Terrigenous deposits of the Fran stage, represented by sand and clay bundles	Upper Proterozoic sandstones in the raised part of the structure
Depth, m	1025–1127	925–980	760–820	650–980
Total salinity, g/L	110–145.6	129–131	137.4–219.4	0.83–198.4
Water density, g/cm <sup>3</sup>	1.090–1.110	1.090	1.099–1.110	0.998–1.302
pH	4–4.5	6.4–7.0	6.1–8.8	5.4–9.7
Composition of natural water-soluble gas (v/v), %	N <sub>2</sub> (7.9), HC* (87.5), CO <sub>2</sub> (0.5), H <sub>2</sub> (3.2); O <sub>2</sub> (0.9)	N <sub>2</sub> (10–90), HC (0–86), CO <sub>2</sub> (0–4), H <sub>2</sub> (1–40)	N <sub>2</sub> (70–94), HC (0.05–14), CO <sub>2</sub> (0.2–22), H <sub>2</sub> (0.3–24)	N <sub>2</sub> (0.8–95), HC (0.2–97), CO <sub>2</sub> (0–6), H <sub>2</sub> (0.1–31)
Formation temperature, °C	33	24	22	17–22

\*, HC, hydrocarbons: Sum of CH<sub>4</sub>, C<sub>2</sub>H<sub>6</sub>, C<sub>3</sub>H<sub>8</sub>, i-C<sub>4</sub>H<sub>10</sub>, n-C<sub>4</sub>H<sub>10</sub>, C<sub>4</sub>H<sub>8</sub>, C<sub>5</sub>H<sub>12</sub>.

**Table S2.** Physicochemical characteristics of the water sampled in 2021 from aquifers and gas collection points (GCP) at the Peschano–Umetskoe, Shchelkovo, Kasimov, and Kaluga underground gas storages.

Sample	Total salinity*, g/L	pH	Concentration, mg/L			
			Methanol	Σ(C <sub>2</sub> –C <sub>4</sub> alcohols)	Acetate	Σ(C <sub>3</sub> –C <sub>5</sub> VFA)
Peschano-Umetskoe UGS						
6	163.86	5.85	0	0.3	267.3	23.8
33	12.96	5.09	1.5	0.7	82.7	11.9
43	146.13	5.85	0	0.5	61.9	6.3
Shchelkovo UGS						
161	77.14	5.93	1.9	1.6	18.8	3.5
Kasimov UGS						
GCP-1	74.20	5.67	3153.1	6.1	50.1	3.2
GCP-5	105.59	5.69	2264.2	4.7	34.3	3.3
GCP-6	122.50	5.67	10296.4	24.6	67.4	4.8
GCP-9	77.14	5.65	2873.5	6.4	72.5	6.4
Kaluga UGS						
13	<2.00	8.34	230.7	1.5	42.3	41.4
121	14.00	10.92	0	0	34.3	10.5

\*, Total salinity calculated as NaCl concentration, g/L

**Table S3.** Diversity indices of the 16S rRNA gene sequences of prokaryotes in the libraries from UGS samples.

Parameter	Libraries of 16S rRNA gene fragments from wells:		
	PU_6	PU_33	Kal_121*
Number of sequences in the library	15126	22716	9534
Number of OTUs	144	182	96
Good's coverage, %	98	99	91
Chao1	145	183	122
Shannon-Weaver diversity index (H)	2.9	2.1	0.7
Berger-Parker Dominance index (D)	39.4	61.6	88.6

\*Data for Kal\_121 library from Nazina et al. [30].

**Table S4.** Potential contribution of microorganisms from UGS water samples in the pathways of nitrogen, sulfur, and methane metabolism and in benzoate and polycyclic aromatic hydrocarbon (PAH) degradation according to the KEGG database.

Genus	Sulfur			Benzoate			Methane			PAH		
	PU_33	PU_6	Kal_121	PU_33	PU_6	Kal_121	PU_33	PU_6	Kal_121	PU_33	PU_6	Kal_121
<i>Marinobacter</i>	0.4	0.2	<b>94.2</b>	0.6	0.3	<b>97.8</b>	0.1	0.2	<b>90.6</b>	1.0	0.2	<b>79.0</b>
<i>Halomonas</i>	0.6	<b>50.7</b>	0.3	0.8	<b>46.7</b>	0.2	0.1	<b>53.6</b>	0.3	2.3	<b>56.2</b>	0.3
<i>Methanobacterium</i>	<b>63.8</b>	0.3	0.0	<b>57.6</b>	0.2	0.0	<b>92.6</b>	2.3	0.0	<b>26.9</b>	0.0	0.0
<i>Pseudomonas</i>	3.6	<b>19.5</b>	0.6	4.3	<b>16.6</b>	0.5	0.5	<b>13.3</b>	0.4	<b>10.8</b>	<b>17.0</b>	0.6
<i>Rhodococcus</i>	<b>11.0</b>	<b>13.6</b>	0.0	<b>23.9</b>	<b>21.0</b>	0.0	2.0	<b>12.9</b>	0.0	<b>43.4</b>	<b>15.4</b>	0.0
<i>Methylococcus</i>	0.0	0.0	3.5	0.0	0.0	0.3	0.0	0.0	5.7	0.0	0.0	0.0
<i>Acetobacterium</i>	6.2	0.7	0.0	1.3	0.1	0.0	2.3	1.4	0.0	2.0	0.1	0.0
<i>Syntrophobotulus</i>	3.3	0.0	0.0	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
<i>Acidovorax</i>	0.3	2.1	0.1	0.3	2.0	0.1	0.0	1.4	0.1	0.5	1.1	0.1
<i>Spirochaeta</i>	5.7	0.0	0.0	1.3	0.0	0.0	0.9	0.0	0.0	3.0	0.0	0.0
<i>Brevundimonas</i>	0.6	2.8	0.0	0.8	2.5	0.0	0.1	2.3	0.0	0.9	1.1	0.0
<i>Ralstonia</i>	0.7	1.3	0.2	1.5	1.9	0.3	0.1	1.3	0.2	2.8	1.5	0.3
<i>Sphingobium</i>	0.2	2.2	0.0	0.6	3.7	0.0	0.1	2.4	0.0	1.6	4.0	0.0
<i>Aurantimonas</i>	0.8	1.9	0.0	1.0	1.7	0.0	0.2	1.9	0.0	2.5	1.7	0.0
<i>Clostridium</i>	0.2	1.1	0.0	0.1	0.4	0.0	0.1	1.5	0.0	0.0	0.1	0.0
<i>Dietzia</i>	0.1	0.2	0.0	1.6	1.8	0.0	0.3	2.5	0.0	2.3	1.1	0.0
<i>Desulfotignum</i>	0.0	0.0	1.1	0.0	0.0	0.8	0.0	0.0	2.6	0.0	0.0	0.3
<i>Halolactibacillus</i>	0.2	1.7	0.0	0.1	0.3	0.0	0.0	1.5	0.0	0.0	0.0	0.0
<i>Shewanella</i>	0.1	1.5	0.0	0.0	0.8	0.0	0.0	1.3	0.0	0.1	0.4	0.0
<i>Dethiosulfatibacter</i>	2.1	0.0	0.0	3.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0

**Table S5.** Potential contribution of microorganisms from UGS water samples to hydrogen metabolism according to the BRENDA database [66].

<b>Genus</b>	<b>PU_33</b>	<b>PU_6</b>	<b>Kal_121</b>
<i>Methanobacterium</i>	<b>61.613</b>	0.707	0
<i>Pseudomonas</i>	0.779	<b>10.320</b>	0.398
<i>Rhodococcus</i>	<b>2.104</b>	<b>6.260</b>	0.010
<i>Methylococcus</i>	0	0	<b>3.734</b>
<i>Acidovorax</i>	0.066	<b>1.441</b>	0.073
<i>Clostridium</i>	0.097	<b>1.397</b>	0.011
<i>Shewanella</i>	0.018	<b>1.249</b>	0
<i>Halanaerobium</i>	0.003	0.006	0.001
<i>Mycobacterium</i>	0.001	0.009	0
<i>Azotobacter</i>	0.000	0.006	0.000
<i>Cupriavidus</i>	0.000	0.001	0.003
<i>Pelomonas</i>	0.000	0.002	0.001
<i>Rhizobium</i>	0.000	0.002	0
<i>Brachybacterium</i>	0.001	0.001	0
<i>Hydrogenophaga</i>	0.000	0.002	0
<i>Paracoccus</i>	0.000	0.001	0
<i>Desulfomicrobium</i>	0.000	0	0.001
<i>Megasphaera</i>	0	0.001	0
<i>Escherichia/Shigella</i>	0	0	0.001
<i>Pseudonocardia</i>	0	0.001	0