

Microbial occurrence and their growth in compacted bentonite in subsurface environments

Can microbes live in highly compacted bentonite?



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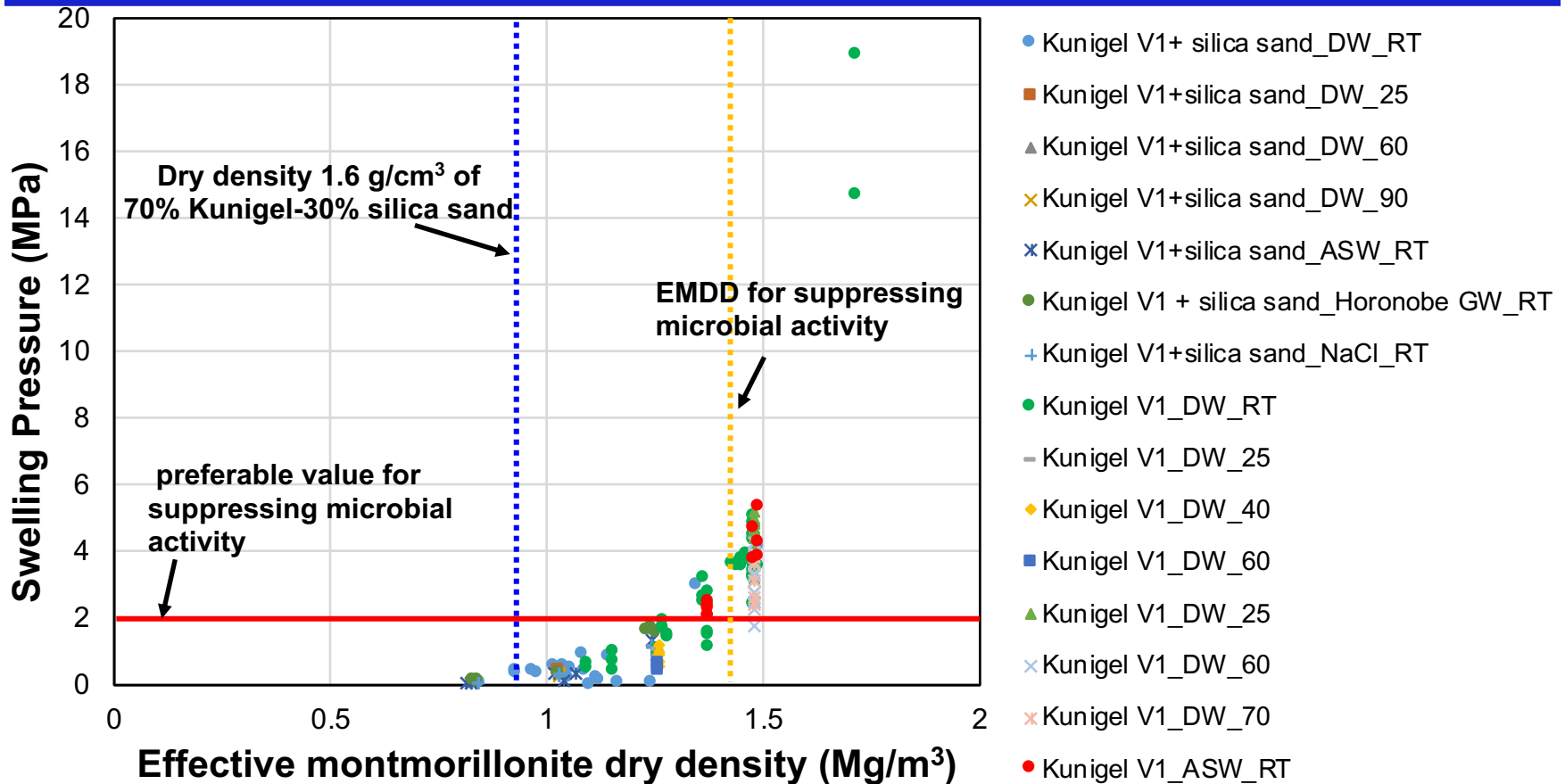
Background

Microbial activity in a HLW repository is of concern for a number of reasons. Their activity may result in microbially influenced corrosion (MIC) which could affect the longevity of the containers.

Previous studies in Japan

- **Sulfate reducer would not be able to survive in the buffer materials. Therefore, microbologically influenced corrosion (MIC) would not affect the longevity of the containers (Nishimura et al., 1999, JNC, 1999).**
- Isolated sulfate reducing bacteria or iron-oxidizing bacteria, and indigenous microorganisms in the bentonite were used for the corrosion experiments.
- The incubation periods for the laboratory experiments were from a few weeks to 3 months.
- Culture-dependent methods were used to detect microorganisms, although **most of the microorganisms (>99%) are not be able to culture.**
- A dry density of ≥ 1.6 g/cm³ will be significant to suppress microbial activity (in case of 100% MX-80).

Swelling Pressure as a function of effective montmorillonite dry density in Kunigel V1 bentonite

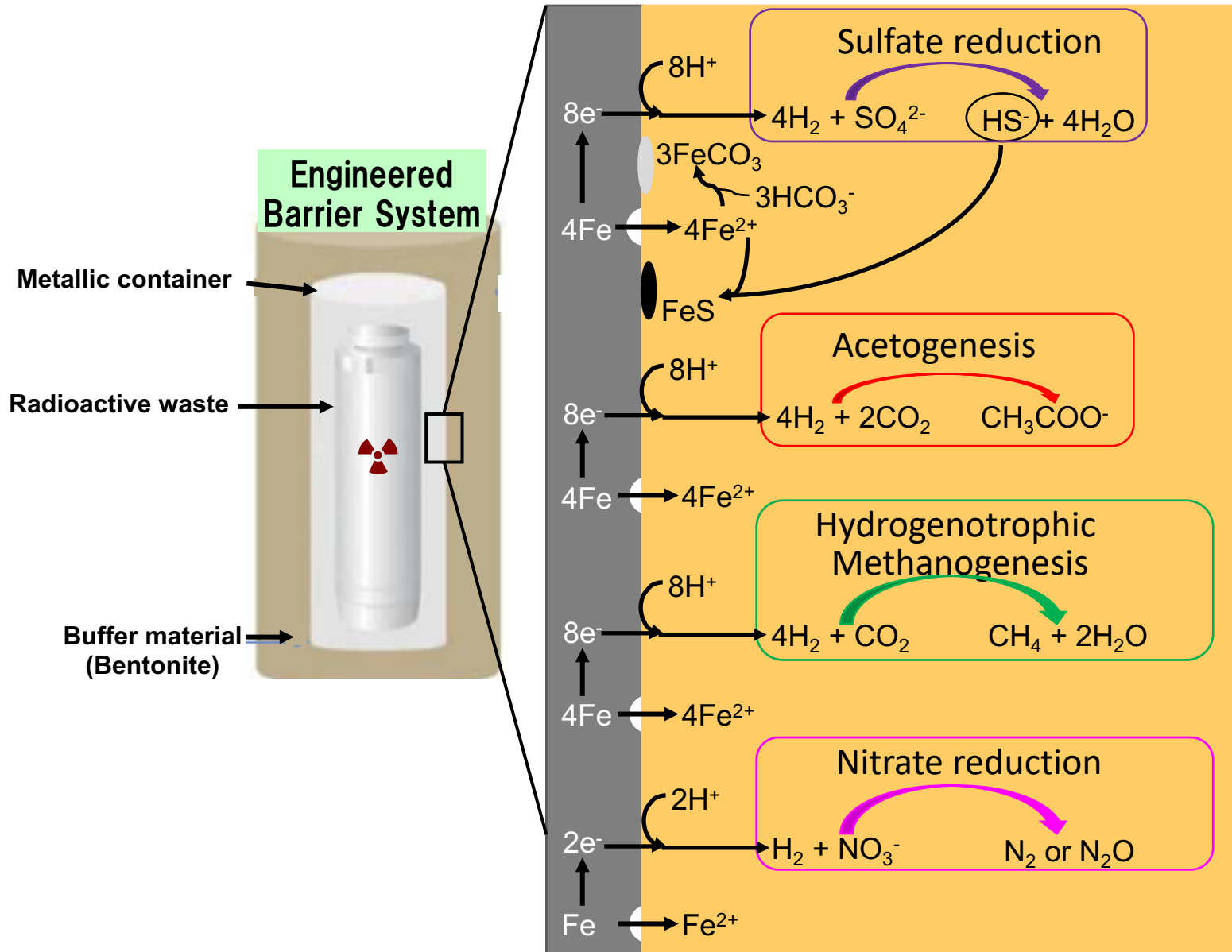


The data was referred from JAEA Buffer Material Database: <https://bufferdb.jaea.go.jp/bmdb/>

Condition for reducing microbial activity in buffer materials

- Dry density of bentonite: $\geq 1.6 \text{ g/cm}^3$ (in case of MX-80)
with a corresponding Effective montmorillonite dry density: $\geq 1.4 \text{ g/cm}^3$
- Swelling pressure: $\geq 2 \text{ MPa}$
- Water activity: $a_w < 0.96$

Conceptual scheme of microbial effects on corrosion of metallic container



Tasks for microbiology

To evaluate the long-term stability of engineered barrier system,

- **Necessary to evaluate MIC effects using Japanese bentonite, e.g. Kunigel V1.**
- **Anaerobic microbial consortium (including SRB, Acetogen, Methanogen, etc.) should be evaluated for metal corrosion.**
- **Long-term behavior of microbial activity in bentonite should be evaluated.**
- **Culture-independent methods to analyze microorganisms should be used.**

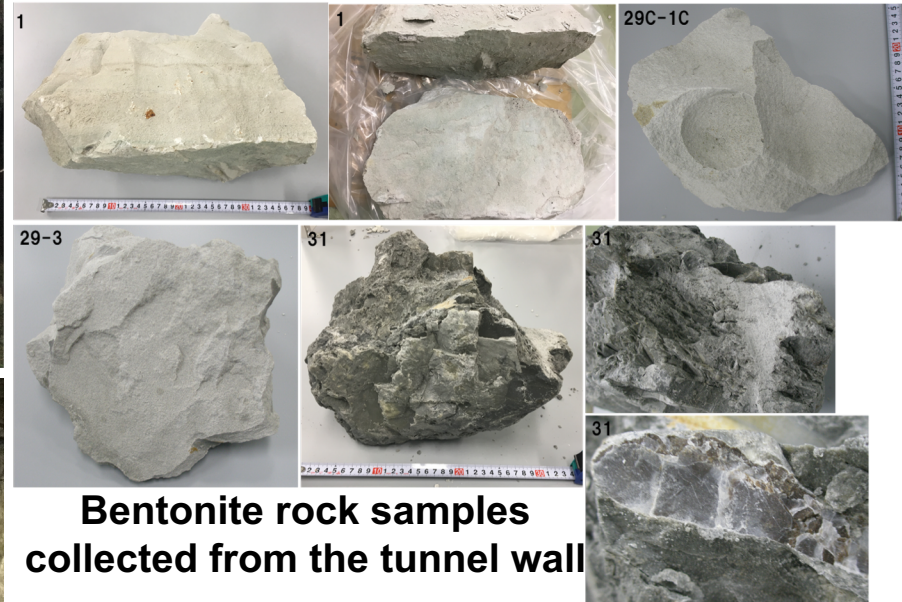
Today's topics

- 1. Field investigation of ancient bentonite rocks at Tsukinunno bentonite deposit in Japan.**
 - To evaluate long-term behavior of microbial activity in bentonite rocks at subsurface environments.**

- 2. An in-situ experiment for corrosion of carbon steel at 350 m depth of the Horonobe Underground Research Laboratory.**
 - To evaluate microbial effects on corrosion of carbon steel in bentonite buffer materials with Kunigel V1 and anaerobic microbial consortium.**

Field investigation of ancient bentonite rock at Tsukinuno deposit in Japan

Umenokida deposit at Tsukinuno bentonite mine (Kunimine Industries)



Bentonite rock samples collected from the tunnel wall

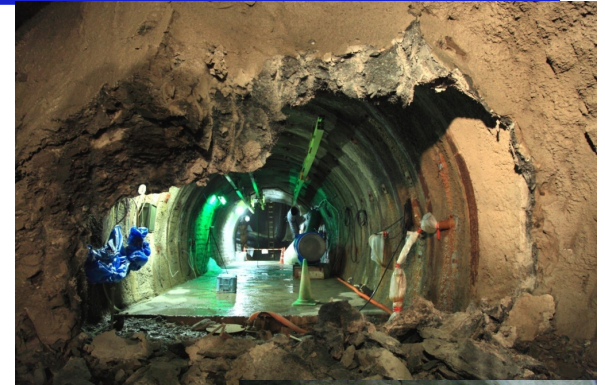
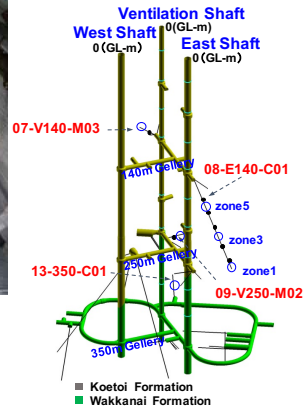
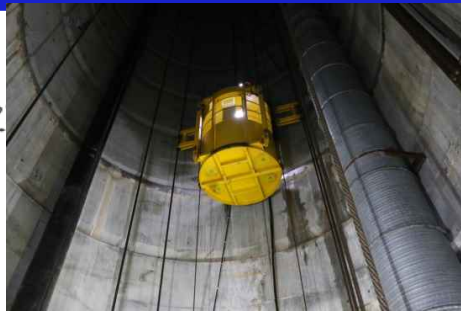
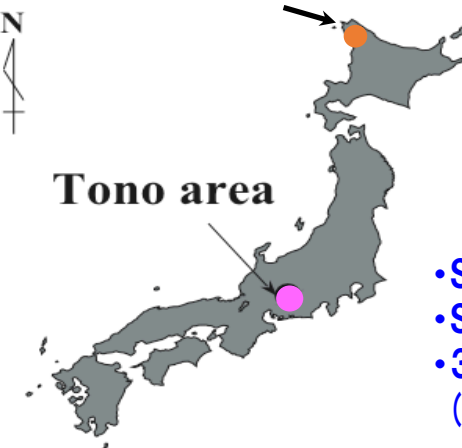
Sample layer: 3 (different contents of montmorillonite)
Sample number: 4



sample name	# of layer	location in the mine	type of bentonite	year of the tunnel excavation	period after tunnel excavation	montmorillonite content (%)	
1	1	middle	Na	2000	19 years	28	low-montmorillonite content
29-1C	29	north	Na	2013, Oct.	6 years & 6 months	34-36	raw materials for Kunigel V1
29-3	29	south	Na	2019, Apr.	1 month	34-36	raw materials for Kunigel V1
31	31	north	Na	2018, Aug.	8 month	38-48	raw materials for Kunigel V1

Horonobe URL

Tono area

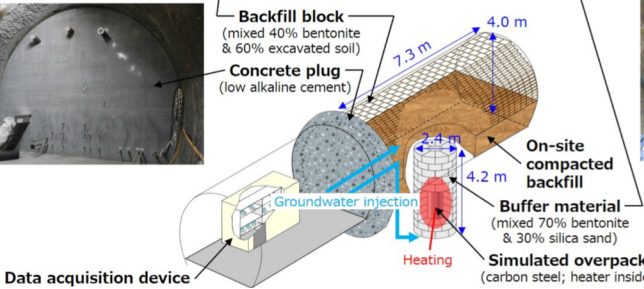
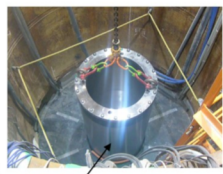
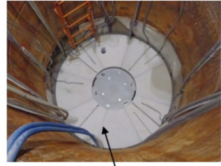


- Sedimentary rocks
- Saline water
- 380 m depth
(will be excavated down to 500 m depth in the future)



The HIP was set up in February 2023 as an NEA* joint project with the participation of currently 9 organisations from Germany, Korea, Romania, Taiwan and United Kingdom

- Task A: Solute transport experiment with model testing;
- Task B: Systematic integration of repository technology options; and
- Task C: Full-scale EBS dismantling experiment.



In-situ corrosion experiment at 350 m depth of Horonobe URL

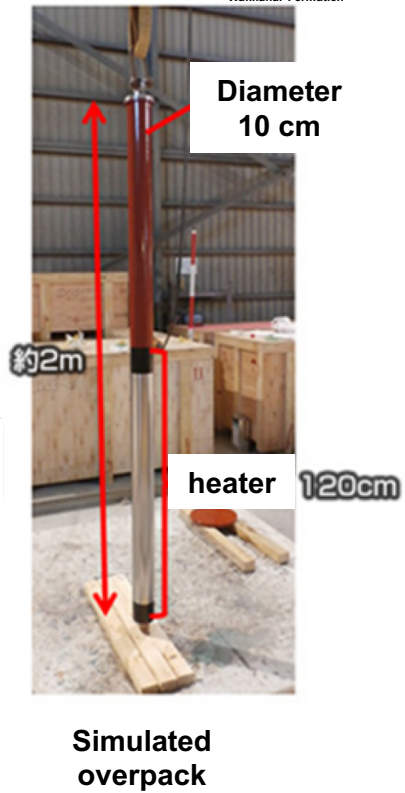
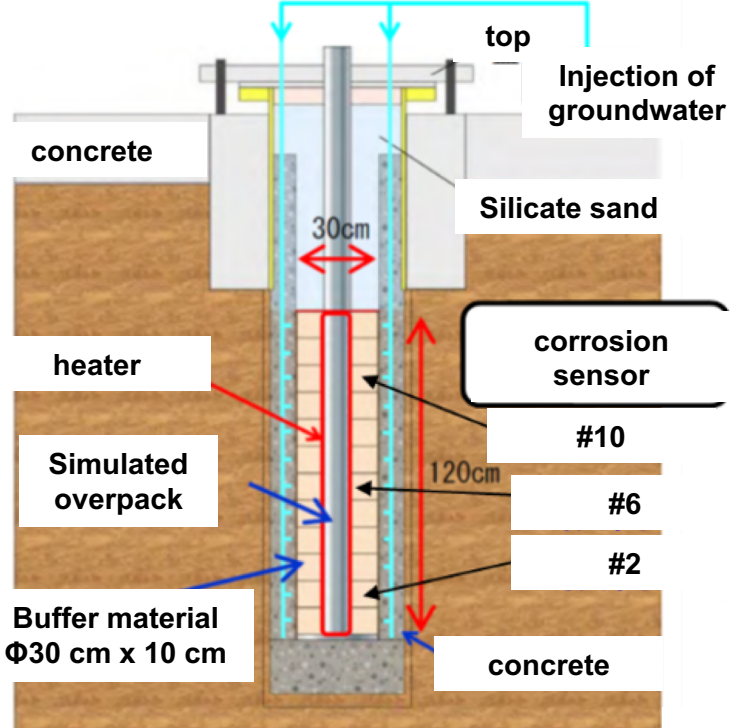
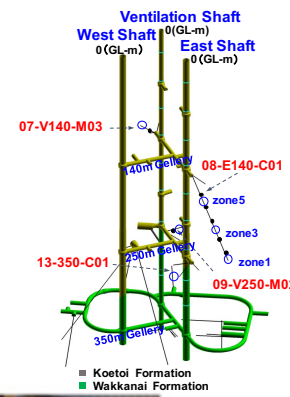
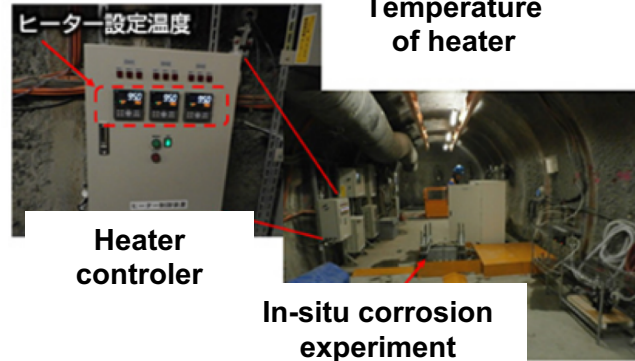
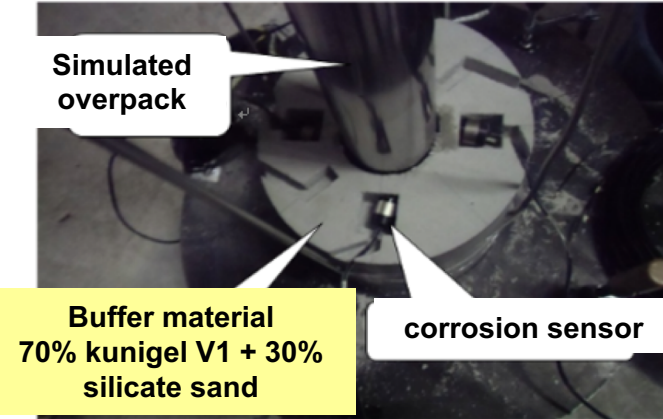
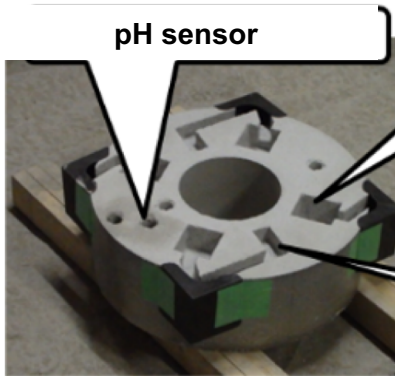
Depth: 350 m (Sedimentary rocks, siliceous shales)

Buffer materials: 70%Kunigel V1, 30% silica sand

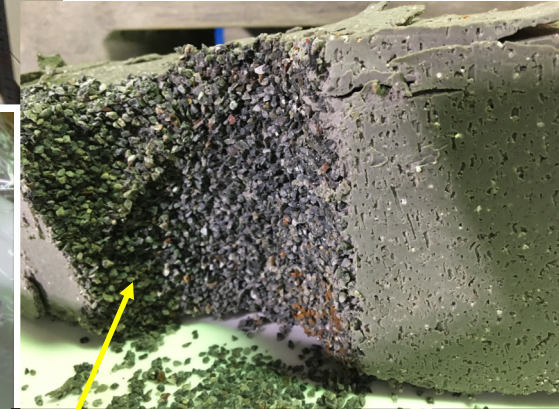
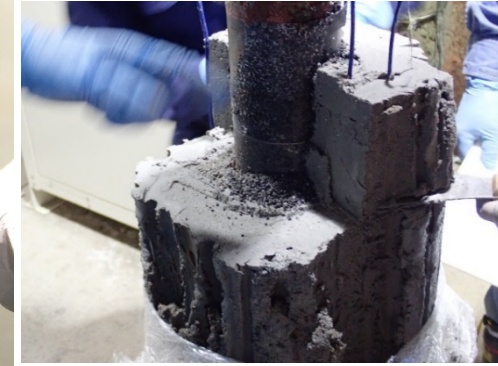
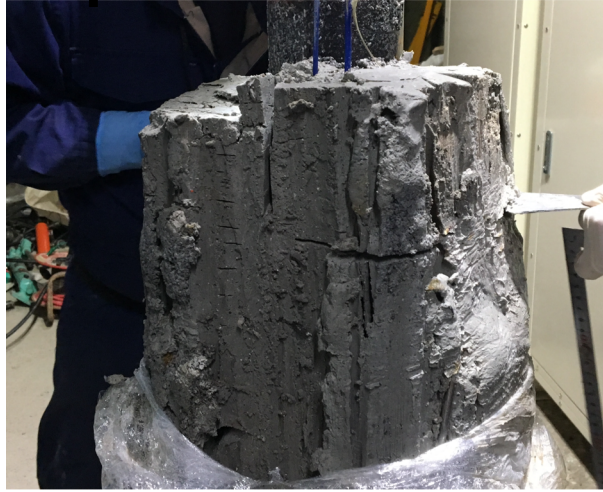
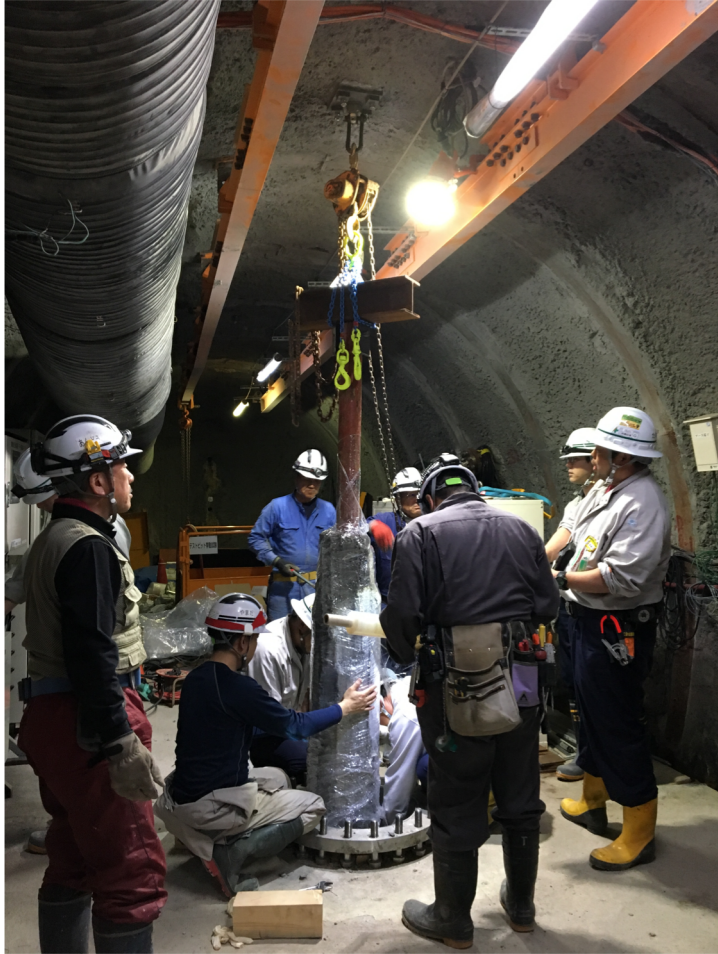
Dry density of buffer materials: 1.6 Mg/m³

Period of Ex.: ~3.5 years

Temperature: ~95°C



Sampling of the bentonite buffer and corrosion products

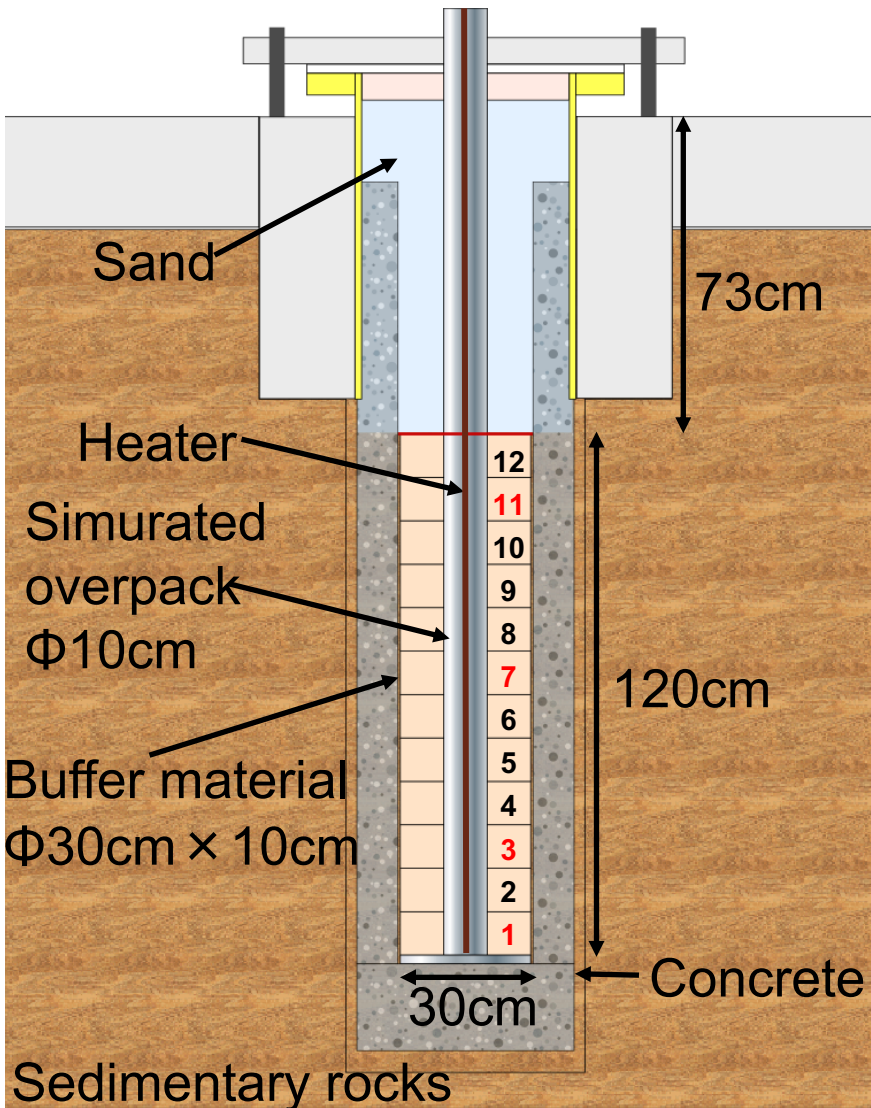


Silicate sand got into a space between the carbon steel and the buffer during the process of swelling.

Sampling deposits on the surface of heater (carbon steel)

Schematic drawing of the in-situ OP corrosion experiment at the Horonobe URL

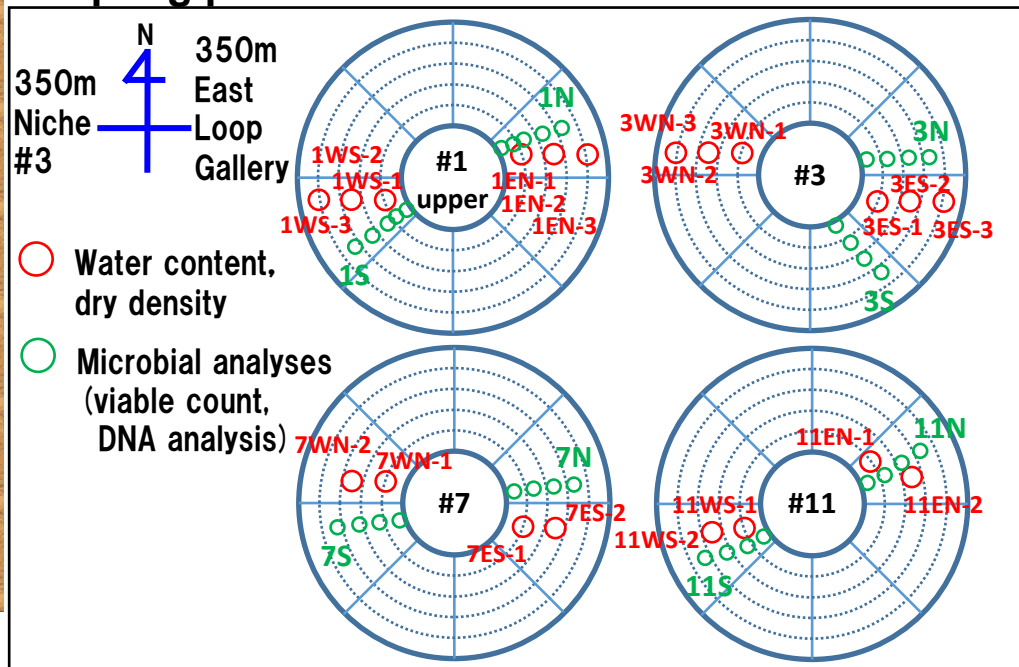
Configuration of the OP experiment



Sampling image

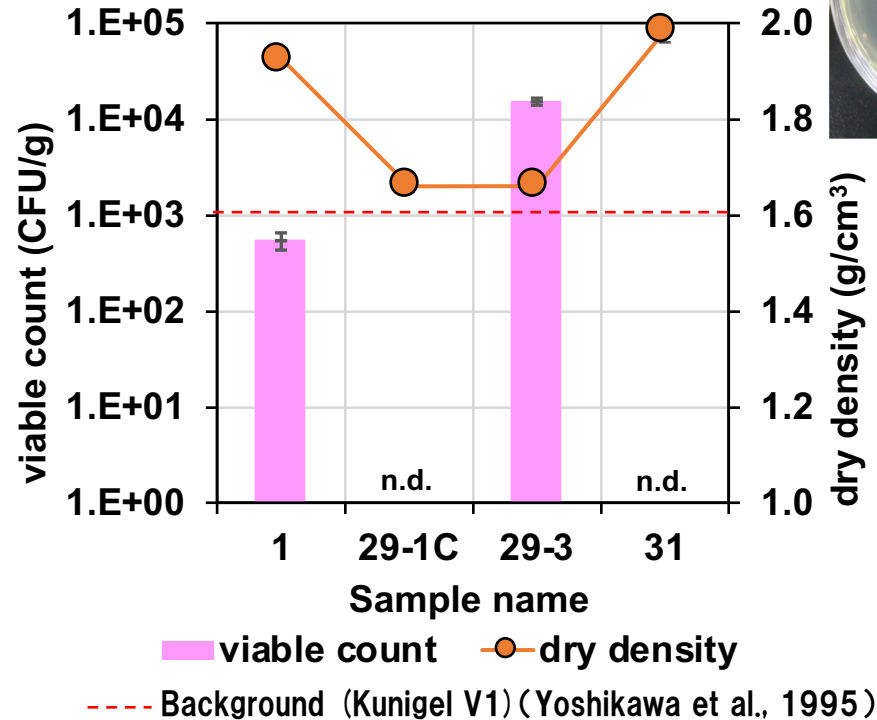
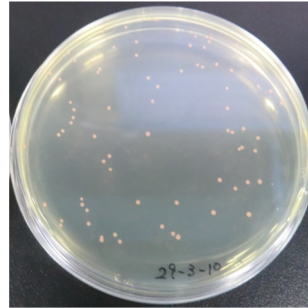


Sampling positions in the bentonite buffer material



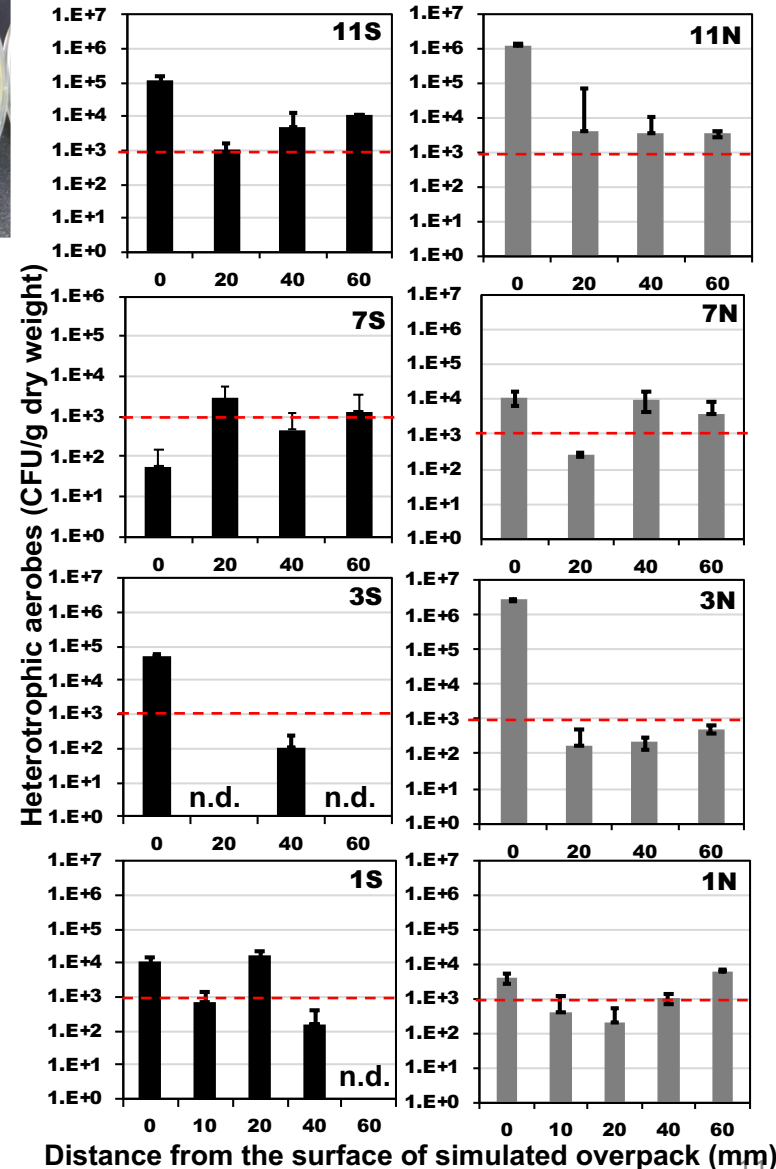
Culturable aerobic heterotrophic microorganisms in the bentonite samples

Tsukinuno bentonite rocks

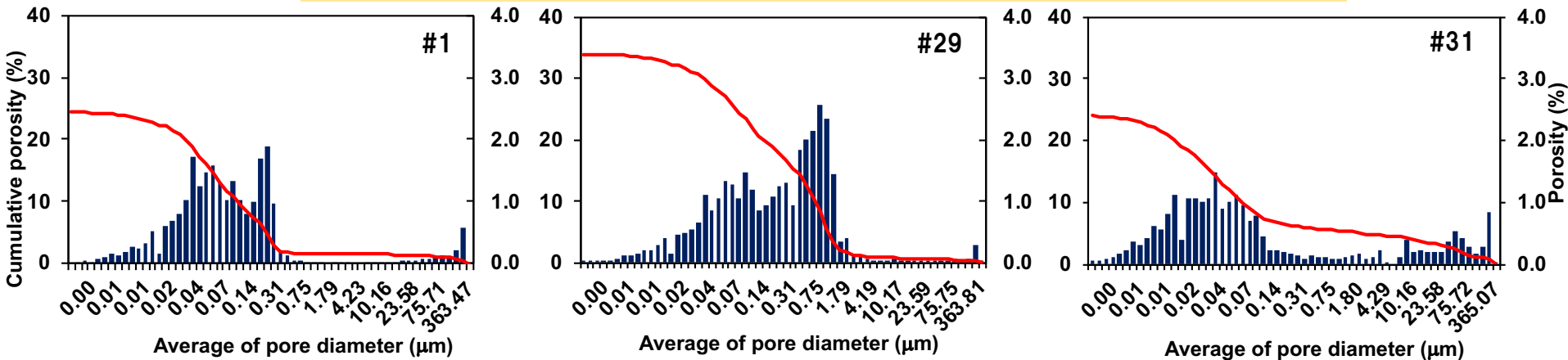


- Anaerobic microbes were not detected in all the samples.
- With the exception of a few samples, aerobic microbes were detected with low density, suggesting that their growth in the bentonite would have been suppressed.

In-situ experiment at Horonobe



Pore size distribution obtained by a mercury penetration method



Physical characterization of the Tsukinuno bentonite rocks

Sample name	Water content (%)	Dry density (g/cm ³)		Porosity (%)		Pore-size distribution			Viable count (CFU/g) 28days
		weight/volume	mercury penetration	weight/volume	mercury penetration	median pore diameter	Average pore diameter	maximum pore diameter	
1	7.9	1.91	1.92	24.2	23.7	0.064	0.041	0.204	540
29-1C	12.1	1.63	1.66	34.7	34.4	0.216	0.072	0.920	0
29-3	15.4	1.66	1.66	34.7	34.4	0.216	0.072	0.920	15206
31	18.3	1.70	1.98	35.0	24.3	0.047	0.029	0.037	0

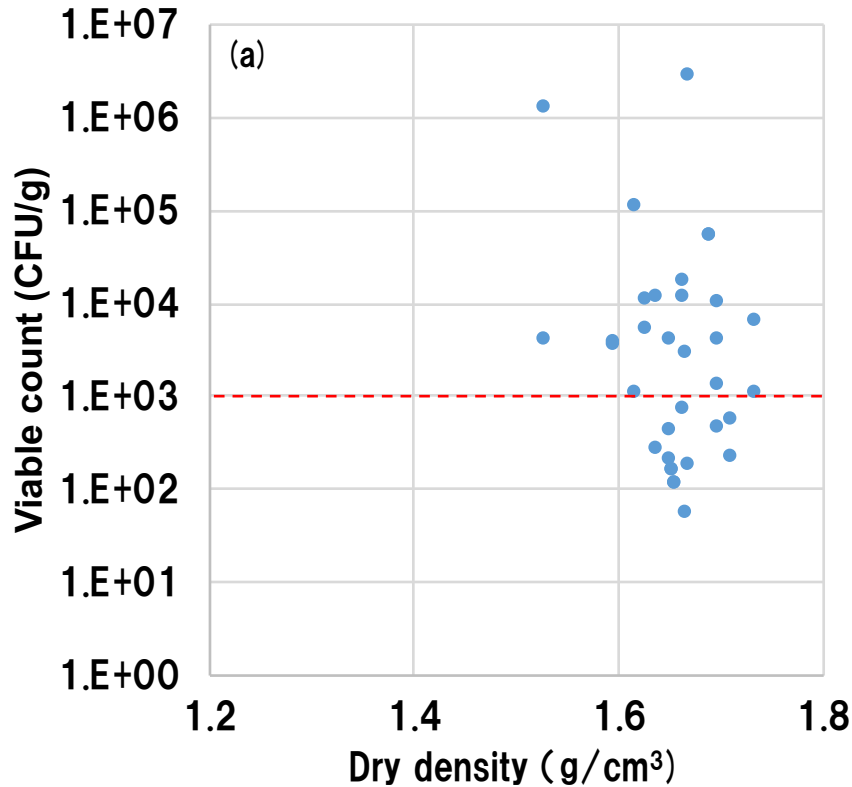
➤ It seems that there is a correlation between dry density, pore size distribution, and viable counts.

The smallest size of microorganisms: 0.1 ~ 0.2 μm

(Large enough to contain a sufficient amount of biomolecules necessary for the maintenance of biological activities such as cell membranes, proteins, ribosomes, and genomic DNA)

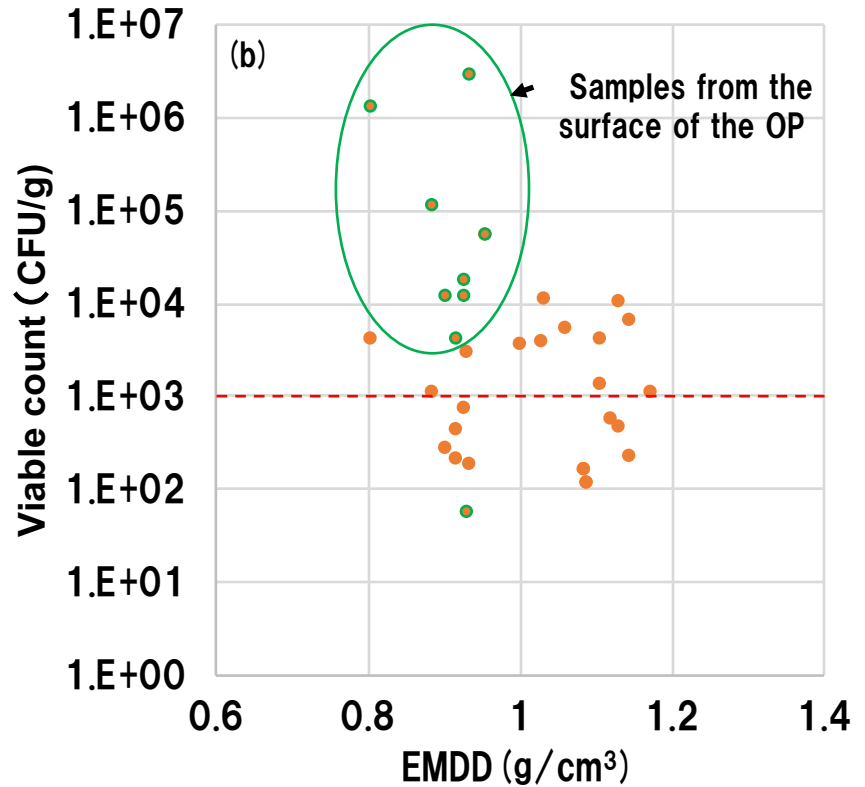
The relationship between dry density and viable count

Measured dry density



--- Background level (Yoshikawa et al., 1995)

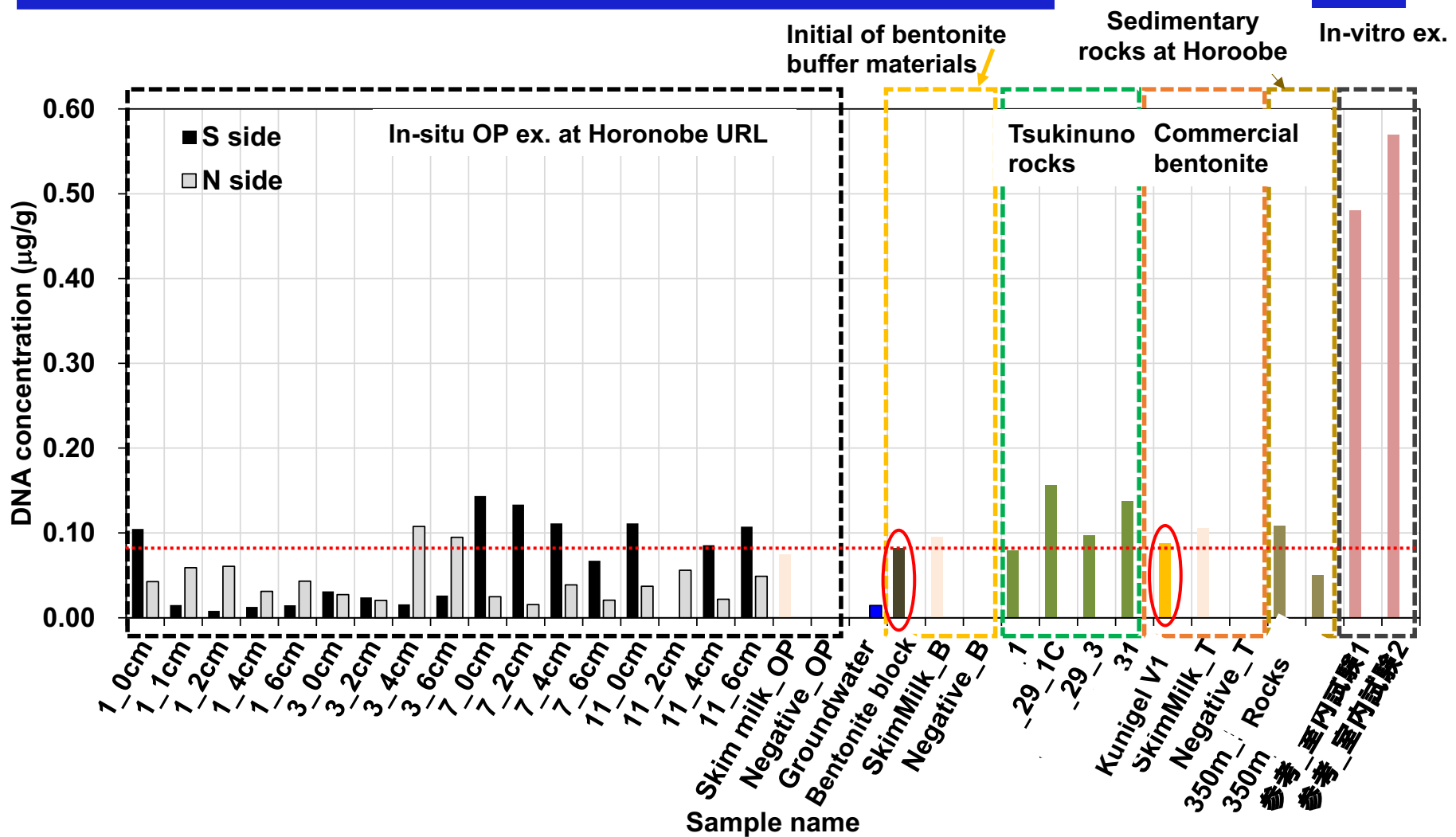
Effective Montmorillonite Dry density (EMDD) calculated by the ratio of silica sand



● Bentonite samples collected from 0 cm area

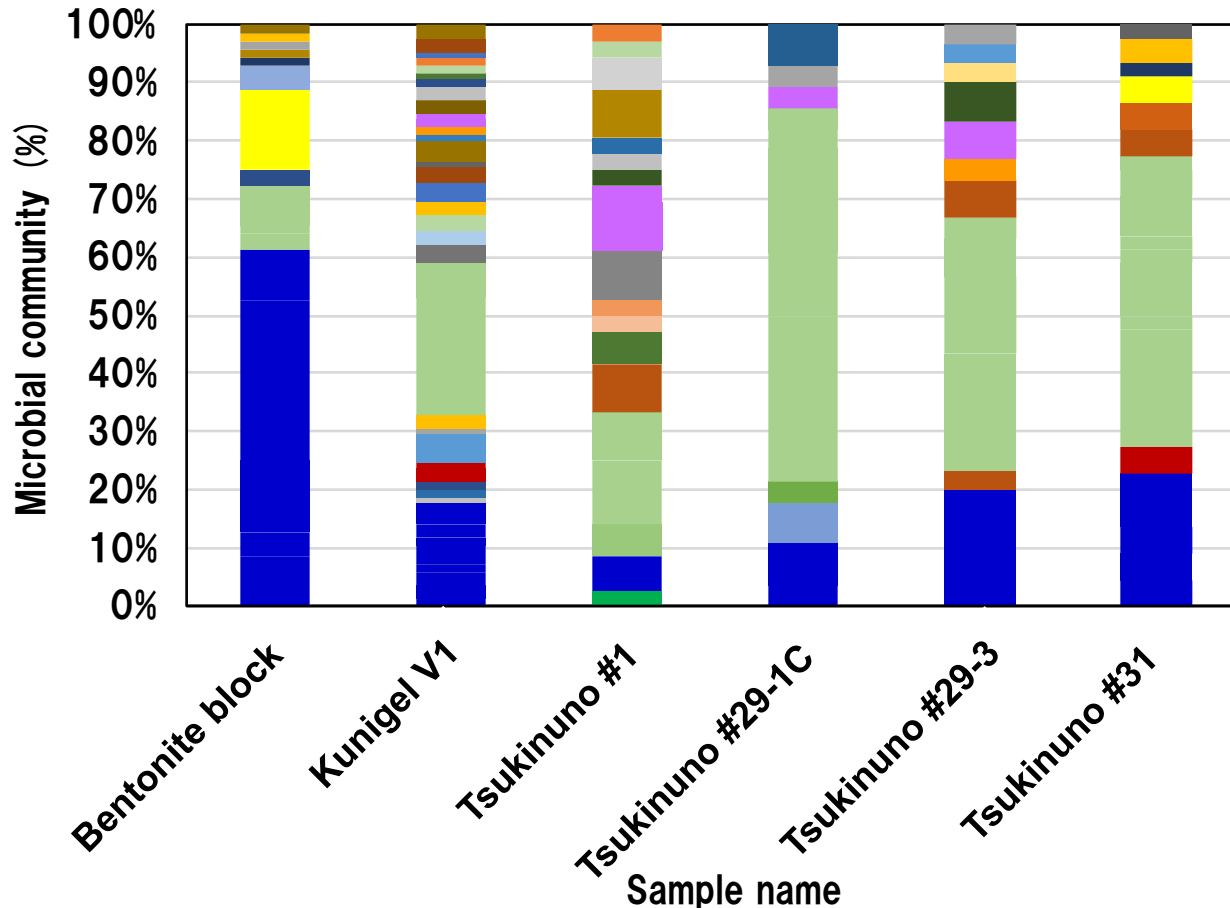
- In case of the bentonite buffer mixed with silica sand, macro-pores of about 10-50 μm diameter increase even in homogeneous compacted bentonite (Wang et al., 2012).
- The results indicate that microbial activity could have been suppressed under high dry density conditions, although microbes could survive in the buffer material when the condition of the dry density in buffer material would be heterogeneous.

DNA concentration of the bentonite samples



- The DNA concentrations extracted from the samples of in-situ corrosion experiment were detected at very low level, and the values were below the initial one before installation.
- It is suggested that the microbial activity in the bentonite buffer material would be suppressed under high temperature and high dry density conditions.

Microbial composition based on 16S rRNA gene analysis for Tsukinuno bentonite rocks



■ Deltaproteobacteria, *Desulfovibrio*. etc. Sulfate redction

■ Betaproteobacteria, *Thiobacillus*; Fe/S oxidation

■ Betaproteobacteria, *Rhodocyclaceae*, Fe/S oxidation

■ Gammaproteobacteria. *Sulfurivermis fontis*; S oxidation

■ Firmicutes; Fermentation, spore-forming bacteria

■ Actinobacteria; Microbes surviving under extreme condition such as low-organics, -water content

■ Methanobacteria, *Methanomicrobia*; Methane production

Microbial composition based on 16S rRNA gene analysis for the in-situ corrosion Ex. at Horonobe URL (near OP surface area)

- Sulfate reducers and Fe/S oxidation bacteria, potentially related to metal corrosion, were detected in the bentonite buffer near the OP surface.

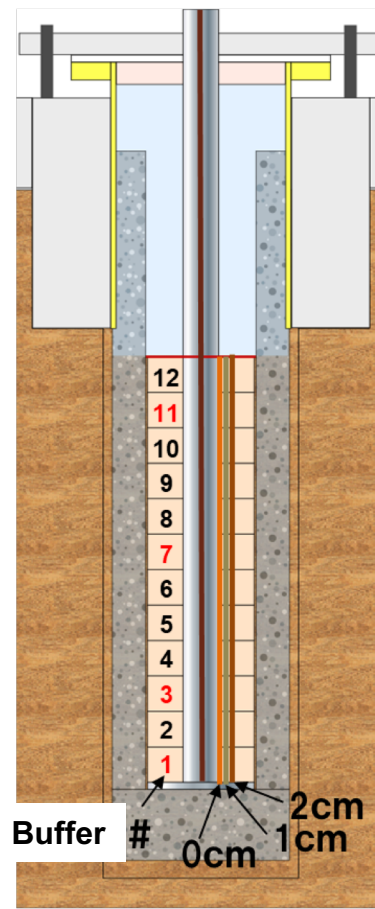
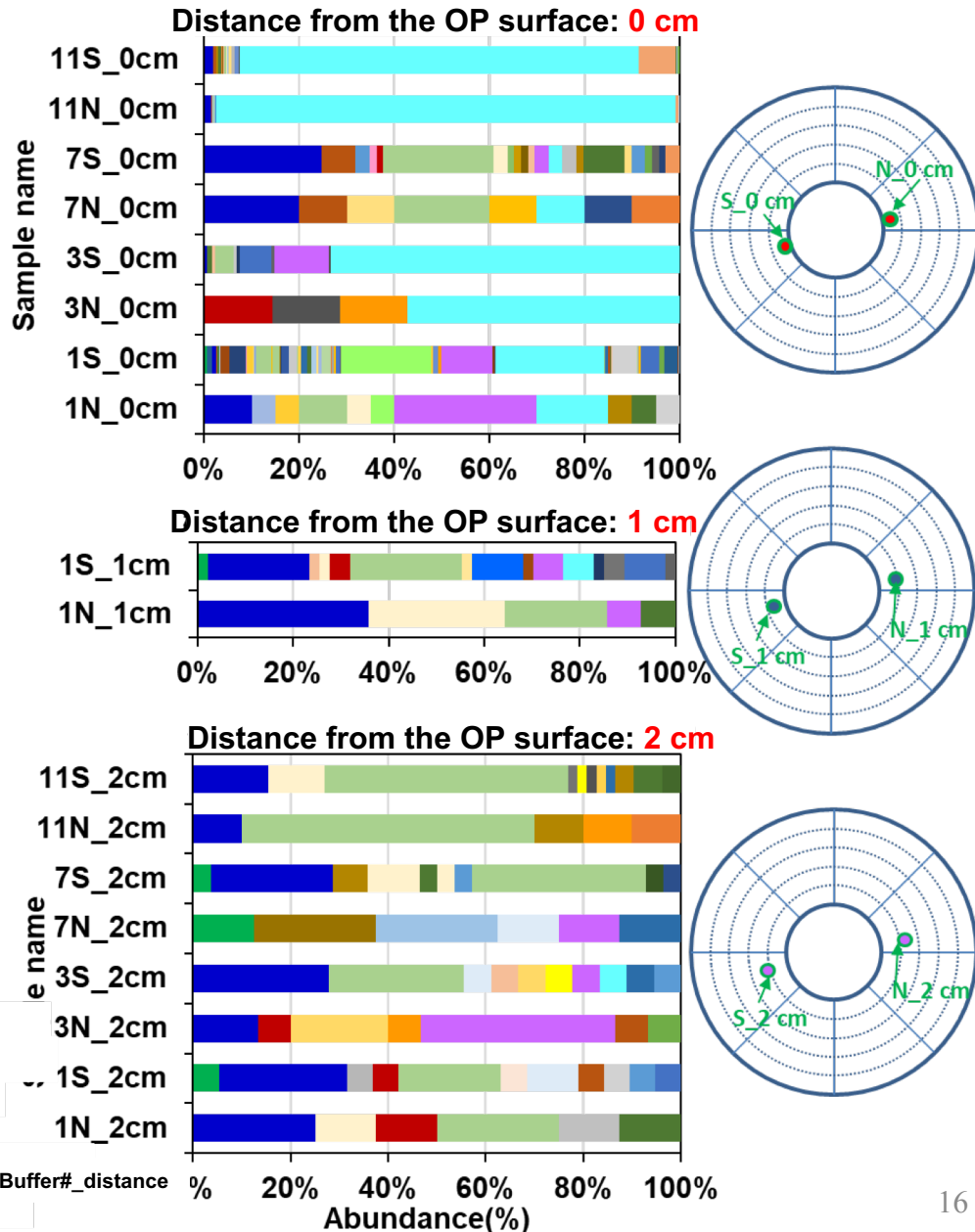


Image of the in-situ Ex.



Microbial composition based on 16S rRNA gene analysis for the in-situ Ex. at Horonobe URL (far from the OP surface area)

- Compared to the microbial composition between 0–2cm and 4–6cm, *Actinobacteria*, *Firmicutes* were more abundant in the buffer of 4–6 cm.
- Microbial population in the buffer of 4–6 cm was similar with the initial bentonite block and Tsukinuno rocks.

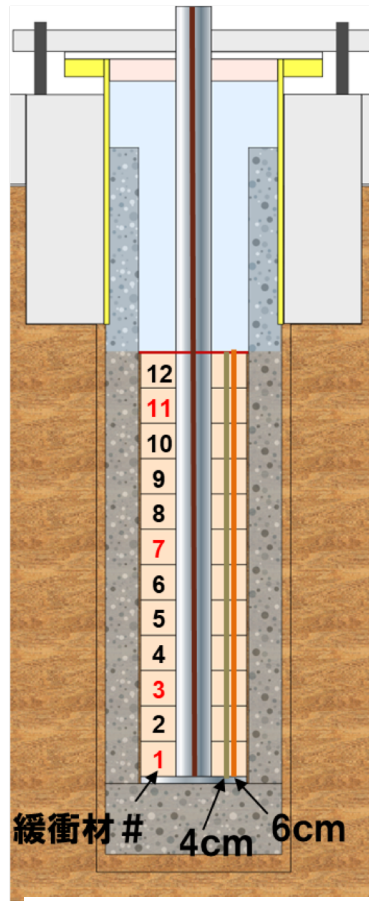
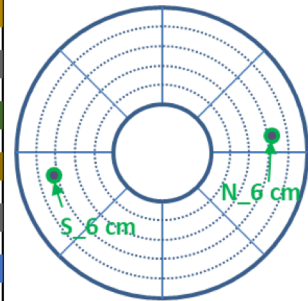
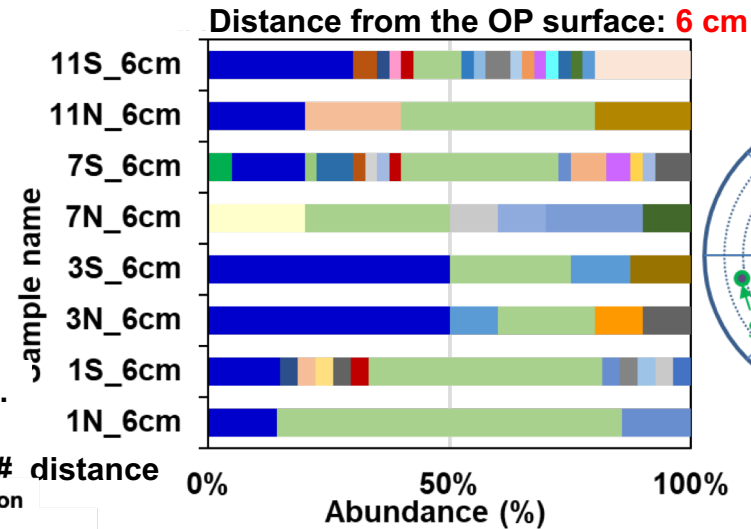
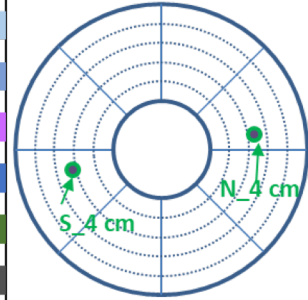
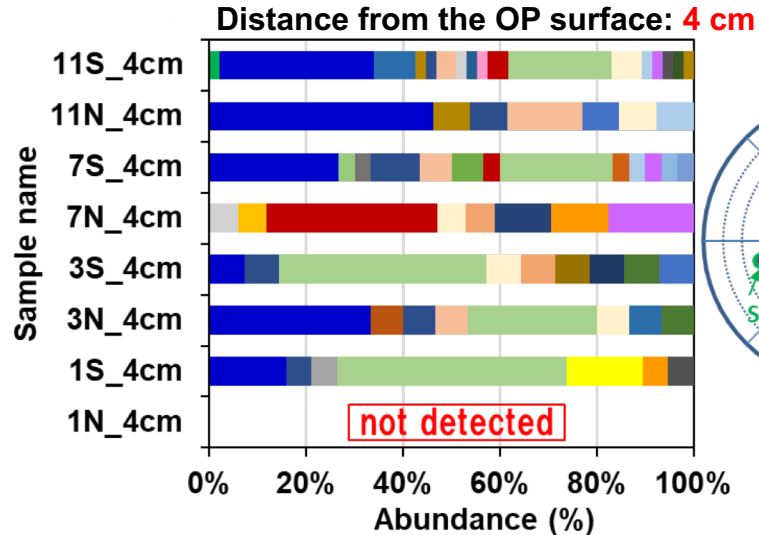


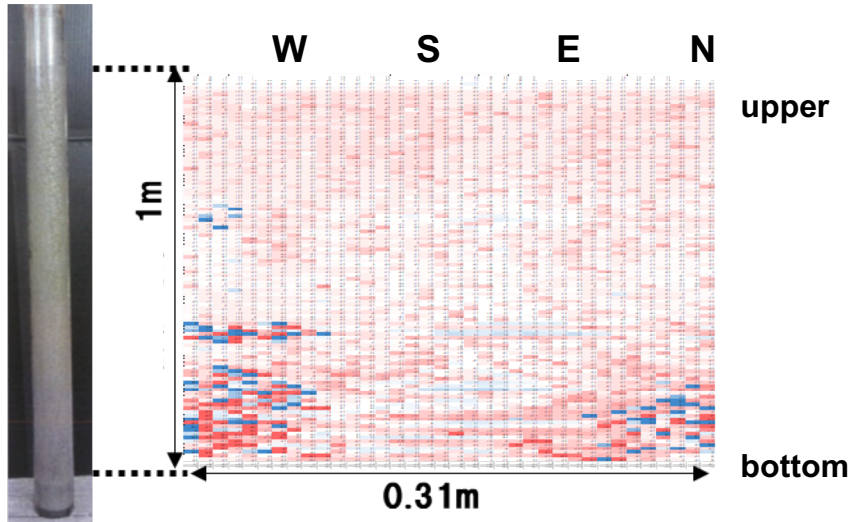
Image of the in-situ Ex.



- Buffer# distance
- *Deltaproteobacteria, Desulfovibrio*, etc.; Sulfate reduction
 - *Betaproteobacteria, Thiobacillus*; Fe/S oxidation
 - *Betaproteobacteria, Rhodocyclaceae*, Fe/S oxidation
 - *Gammaproteobacteria, Sulfurivermis fontis*; S oxidation
 - *Firmicutes*; Fermentation, spore-forming bacteria
 - *Actinobacteria*; Microbes surviving under extreme condition such as low-organics, -water content
 - *Methanobacteria, Methanomicrobia*; Methane production

Result for the corrosion of overpack

The result of corrosion depths measured after acid-treatment



Mineral composition of corrosion products on the surface of corrosion sensors

	45 °	135 °	225 °	315 °
#10	siderite (powdered XRD)	<u>akaganeite</u> <u>magnetite</u> (non-destructive XRD)	siderite (powdered XRD)	No data
#6	No data	siderite goethite magnetite (Micro-Raman Spectroscopy)	No data	No data
#2	siderite (powdered XRD)	<u>akaganeite</u> <u>magnetite</u> (non-destructive XRD)	siderite (powdered XRD)	No data

Siderite: Fe(II), magnetite: Fe(II)/Fe(III), Akaganeite: Fe(III) [β -FeOOH]

[pH and redox condition in the buffer material]

Initial: pH ~8, oxidizing condition

After tens of days: low-oxygen concentration

After hundreds of days: pH ~8.5-9, low-oxygen concentration

[monitoring result of the corrosion rate]

Initial (a few days): tens of micrometer/y

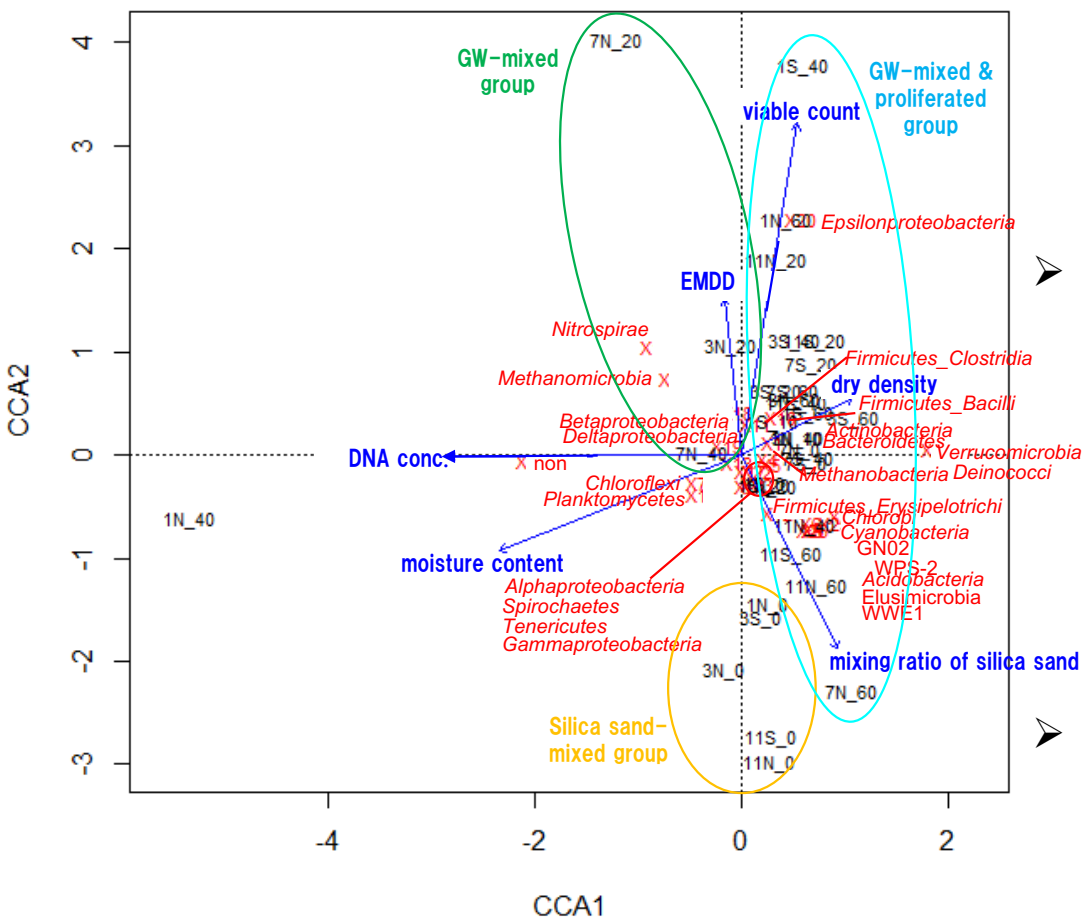
After hundreds of days: a few micrometer/y

- ✓ Non of sulfide minerals as corrosion products was detected on the OP surface, although sulfate reducers were detected in the buffer materials near the OP.
- ✓ It is suggested that microbial sulfate reduction would not have been occurred in the buffer.

Canonical correlation analysis (CCA) plots for the in-situ experiment at Horonobe

multivariate analysis using the data of microbial community and physical parameters

- The CCA analysis implied that aerobic and fermentative microorganisms might have grown in the buffer material when the condition of the buffer material would be heterogeneity or during the swelling process.
- Although microorganisms living in the groundwater also intruded into the buffer material along with groundwater intrusion, their significant growth was not observed, suggesting that microbial growth inside the buffer material was suppressed under high effective montmorillonite density.
- It is suggested that effective montmorillonite density, rather than dry density, may be a more realistic and reliable factor for microbial suppression in buffer materials under full-scale conditions such as in-situ environments.



- Microbial activity would be suppressed and the effect on corrosion of the metal would be limited under **homogeneous condition** with high temperature and high dry density conditions.
- The results of microbial investigation for the 10 Ma of the Tsukinuno bentonite rocks suggest that microbial activity could be suppressed for a long period of time under high dry density condition.
- Oxygen intruded in the buffer material was quickly consumed associated with the formation of iron oxide minerals, and microbial consumption.
- It is suggested that effective montmorillonite density, rather than dry density, may be a more realistic and reliable factor for microbial suppression in buffer materials under full-scale conditions such as in-situ environments.
- On the other hand, it cannot be denied that when the temperature in the buffer material decreases and/or the integrity of the EBS would be no longer maintained, microbes surviving as spores or under dormant state might be able to grow in the buffer materials.
- To suppress microbial activity in the buffer material for a long period of time, it is very important that installation of the engineered barrier should be homogeneity, which makes pore-size keep smaller than microbial size.

Thank you for your attention!

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Acknowledgement



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