MOBILITY MISSION REPORT

This work has been partially supported by the EURAD project that has received funding from H2020-EURATOM 1.2 under grant agreement ID 847593.

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MISSION TITLE

Exploring the impact of gamma radiation on microbial diversity in bentonite: molecular quantification of target genes and 16S rRNA gene diversity analysis.

DESCRIPTION

Concerned organisations

- Technical University of Liberec
- Institute for Nanomaterials, Advance Technologies and Innovation (Department of Applied Biology)
- Universidad de Granada (Department of Microbiology, Faculty of Sciences)

Concerned infrastructures or facilities

- Genetic Laboratory

Concerned phases

- Phase 1: Perform quantitative PCR (qPCR) using the different target primers of the study (16S, apsA, dsrA).

- Phase 2: Preparation of libraries of the samples to sequence 16S rRNA gene to study microbial diversity.
- Phase 3: Analysis of the results
- Phase 4: Mission report writing

Themes and topics

- Theme 3: Engineered barrier system (EBS) properties, function and long-term performance
    - Clay-based backfills, plugs and seals

Keywords

Bentonite; qPCR; Microbial diversity; Sulfate-reducing bacteria; Radiation

EXECUTIVE SUMMARY

The objective of this project was to explore how gamma radiation impacts microbial communities, particularly sulfate-reducing bacteria (SRB), in FEBEX compacted bentonite.

For this purpose, prior to this stay, FEBEX bentonite was compacted in blocks, inoculated with a SRB consortium, amended with acetate and saturated at 100% with bentonite pore water. One set of samples were irradiated with gamma radiation (14 kGy) at the beginning of the experiment. All the samples analysed during this period of time were incubated for six months at 28 ºC in anaerobic conditions. Time 0 was also included as non-incubated natural powdered FEBEX bentonite and non-incubated irradiates powdered FEBEX bentonite (14 kGy).

This proposal is a continuation of a research stay (3 months – from 20.09.2023 to 19.12.2023) previously carried out in collaboration with the Technical University of Liberec within the ConCorD project Task 4 under the supervision of Mgr. Kateřina Černá (host supervisor) and Dr. Mohamed L. Merroun (home supervisor). During that research stay, a batch of irradiated and compacted bentonite samples was analysed using molecular techniques that cannot be used in the home institution (University of Granada) such as qPCR of different markers and 16S rRNA sequencing. Considering the outstanding results achieved and analyzed during that stay, the new stay aimed to extend the analysis to additional samples from the same experiment, representing different study times (Time 0 and 6 months).

Therefore, the main objective of this short research stay have involved utilizing qPCR to quantify 16S rRNA gene to assess the total bacterial biomass in each treatment. In addition, the quantification of the genes involved in the reduction of sulfate such as dsrA and apsA have also been performed. The qPCR results for the 16S rRNA gene generally showed that the presence of bacteria in these samples was very low. The results also indicated that the total bacterial biomass in the non-irradiated samples, incubated for 6 months, was slightly higher compared to time 0 (powdered bentonite). This suggests the possibility that certain microorganisms were capable of growth under conditions of compaction and anaerobiosis during the incubation period. Regarding the irradiated samples, both treatments (with and without SRB concortium) showed a weak positive

signal for the presence of the 16S gene. Gamma radiation at a total dose of 14 kGy at the beginning of the incubation period would have negatively affected the indigenous bacteria of the bentonite, and they were unable to recover during the incubation time. The two treatments with SRB consortium, both non-irradiated and irradiated (14 kGy), tested positive for the two genes associated with sulfate reduction (apsA and dsrA). Additionally, the non-irradiated sample without the consortium at 6 months also tested positive for this group of bacteria. This latter result suggests the presence of SRB indigenous to bentonite in the samples, and that the 6-month incubation period likely promoted their growth, as these genes were not detected at time 0.

On the other hand, libraries of the DNA samples from the different bentonite treatments were prepared for Next Generation Sequencing. The diversity data is still pending to be analysed. Once analyzed, we will be able to understand the changes that the bacterial communities have undergone according to the treatment and study conditions.

1. MISSION BACKGROUND

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1.1. R&D background

The aim of this project is to investigate the **effect of gamma radiation on the microbiology of FEBEX bentonite populations**, mainly sulfate-reducing bacteria (SRBs).

For this purpose, FEBEX bentonite was compacted in blocks at a density of 1.6 $g \cdot cm^{-3}$. A customized consortium of sulfate-reducing bacteria (*Desulfovibrio, Desulfotomaculum, Desulfuromonas, Desulfosporosinus*) and *Geobacter* were used to inoculate the bentonite samples. In addition, controls without spiked bacteria were also prepared for comparison. Small pure-copper coupons simulating metal canisters used for confining nuclear waste in deep geological repositories were included in the core of each block to analyze their corrosion. Acetate (1.5 mM) was added as energy and carbon source for the enhancement of SRB growth. All the samples were 100% saturated with bentonite pore water. One set of samples were irradiated at the beginning of the experiment (14 KGy). All the samples were incubated for six months at 28 ºC in anaerobic conditions. Time 0 was also included in the experiment as natural powdered bentonite and irradiated powdered bentonite (14 kGy).

The experiment was prepared by UGR and CIEMAT research teams for WP ConCorD-Task 4.

1.2. Mission objectives

1) Quantification by qPCR of 16S rRNA gene amplicon V4 region of the highly compacted bentonite incubated for 6 months together with non-incubated bentonite powdered samples as time 0. In addition, the quantification of the genes aspA (adenosine-5'-phosphosulfate) and dsrA (dissimilatory sulfite reductase) involved in the reduction of sulfate.

2) Preparation of libreraries of 16S rRNA gene to perform the sequencing of the samples.

1.3. Mission request

To study the effect of gamma radiation on the total biomass and the diversity of microbial communities present in bentonite using molecular methods for the quantification of genes of interest.

1.4. Mission composition

Host organisation

Technical University of Liberec

Host facility

Institute for Nanomaterials, Advance Technologies and Innovation.

Mission dates

06 May 2024 – 17 May 2024

2. MAJOR PRACTICES, TECHNIQUES, METHODS, TOOLS OR SYSTEMS OPERATED OR STUDIED

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2.1. Practice, technique, method, tool or system operated or studied during the mission

Quantification by qPCR of 16S rRNA gene amplicon V4 region of the compacted bentonite incubated for 6 months and time 0 powdered samples. Quantification by qPCR of specific genes for SRB group such as apsA and dsrA.

Description

qPCR is a molecular biology technique used to quantitatively analyze DNA, RNA, and gene expression. It enables the measurement and amplification of genetic material helping to determine the quantity of a specific target sequence in the sample.

Usage

This technique has been used to quantify bacterial diversity and specific genes involved in sulfate reduction that are representative of the presence of the SRB group.

Benefits

The qPCR data complement the diversity data obtained by sequencing, so having both enhances the study and improves it.

Limitations

The bentonite samples under study have a very low diversity and the extracted DNA had impurities that inhibited the activity of the enzyme involved in qPCR. Therefore, being a very sensitive technique, it requires numerous attempts to obtain reliable data.

Applicability

Quantification of genes of interest and obtaining complementary data to the rest of the molecular techniques.

2.2. Practice, technique, method, tool or system operated or studied during the mission

Preparation of libraries for Next Generation Sequencing (NGS).

Description

The preparation of libraries was carried out for the sequencing of the DNA extracted from the different treatments. This preparation consists of a first and second consecutive PCR,

DNA purification with magnetic beads, measurement of the concentration of amplified DNA and preparation of dilutions.

Usage

The purpose of the preparation of the libraries is the sequencing of the 16S rRNA gene to obtain microbial diversity data.

Benefits

The preparation of libraries and therefore the sequencing of the samples allows to know the bacterial diversity present in each study sample down to the genera level. It is a simple technique that provides information of great relevance for the study.

Limitations

The bentonite samples under study have a very low diversity and the extracted DNA had impurities that inhibited the activity of the enzyme involved both PCRs. Therefore, sometimes, it requires several attempts to obtain data.

Applicability

NGS is applied to study the genetic material of entire microbial communities, providing insights into the diversity and function of microorganisms in the different treatments under study.

2.3. Practice, technique, method, tool or system operated or studied during the mission

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Description

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Usage

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Benefits

Limitations

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Applicability

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2.4. Practice, technique, method, tool or system operated or studied during the mission

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Description

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Usage

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Benefits

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Limitations

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Applicability

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3. MISSION FINDINGS AND CONCLUSIONS

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3.1. Lessons learned and conclusions

During this new research stay, I continued to deepen my knowledge of the qPCR technique and the analysis of the data. In addition, I continued to learn how to prepare libraries for next generation sequencing of the 16S rRNA gene. As a Ph.D student, learning these molecular techniques will help me in the future to implement them in other samples and to continue growing as a scientist.

In addition, this short period of time has allowed me to continue to strengthen relationships with external collaborators and to maintain active collaborations with other research centers.

3.2. Relevant findings and conclusions for home organisation

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3.3. Relevant findings and conclusions for host organisation

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3.4. Relevant findings and conclusions for other organisations

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4. POTENTIALS FOR IMPROVEMENT OR DEVELOPMENT

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4.1. Generic potentials

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4.2. Potentials for home organisation

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4.3. Potentials for host organisation

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APPENDICES

Mission journal

06th-10th May: During this week, qPCRs were carried out with the study samples for the 16S rRNA gene and for the specific sulfate-reducing genes (apsA, dsrA).

13th-17th May: Library preparation of the samples for Next Generation Sequencing. Writing the mission report and analysis of the main results.

Mission bibliography

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MISSION BENEFICIARY

Mar Morales Hidalgo
PhD Student
Department of Microbiology, Faculty of Sciences
University of Granda, Spain

PARTNER EXPERTS CONTRIBUTING TO THE MISSION

Host organisation experts

- Katerina Cerná. Ph.D. Department of Applied Biology. Institute for Nanomaterials, Advance Technologies and Innovation

Home organisation experts

- Mohamed L. Merroun. Full Professor. Department Microbiology, Faculty of Science, University of Granada.
- Fadwa Jroundi. Associate Professor. Department Microbiology, Faculty of Science, University of Granada.

Other organisations experts

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REPORT APPROVAL

Date	Beneficiary	Home mentor/supervisor	Host mentor/supervisor
17/05/2024	Mar Morales Hidalgo	Mohamed L. Merroun	Katerina Cerná
	Visa	Visa MERROUN MOHAMED LARBI - X1766260K Firmado digitalmente por MERROUN MOHAMED LARBI - X1766260K Fecha: 2024.05.16 14:34:39 +02'00'	Visa