MOBILITY MISSION REPORT

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KLIKNETE NEBO KLEPNETE SEM A ZADEJTE TEXT.

MISSION TITLE

Impact of microbial activity on concrete durability

DESCRIPTION

Concerned organisations

SCK CEN
Technical University of Liberec

Concerned infrastructures or facilities

- Environmental monitoring facilities
- Specific microbiology facilities: glove box, flow cytometer, ATM measurement instrument

Concerned phases

Phase 5: Post-closure

Themes and topics

Theme 3: Engineered barrier system (EBS) properties, function and long-term performance

- Cementitious-based backfills, plugs and seals

Keywords

Microbial activity evaluation, low-pH concrete, aqueous environment, monitoring

EXECUTIVE SUMMARY

A significant discovery within the WP MAGIC framework involves the identification of a microbial community sourced from an underground water source. This community presents a mixed presence of sulfate-utilizing and heterotrophic bacteria. To gain insights into the potential effects caused by this microbial consortium on low-pH concretes (LPCs) over the long term, a monitoring experiment in laboratory conditions has been initiated.

The experiment aims to illustrate the performance of biofilm formation on LPCs and its potential effects under both anaerobic and aerobic conditions. Additionally, the experiment will explore further conditions involving the presence of a positive ureolytic strain, discovered during the WP MAGIC framework by the Czech team.

Various techniques, including ATP assay, flow cytometry, and colony-forming unit (CFU) assessments, will be employed to validate and determine the growth of microorganisms. Scanning electron microscopy (SEM) will further confirm the formation and attachment of biofilm on the LPCs. The experiment has been set up and is scheduled to continue for three months until February 2024.

1. MISSION BACKGROUND

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1.1. R&D background

In the context of the WP MAGIC framework within EURAD, the Czech artificial ageing experiment exposes low-pH concretes to various conditions, including ageing in an underground water source, thereby revealing the microbial community in this specific environment. During the WP MAGIC timeframe, the discovery of sulfate-utilizing bacteria, such as sulfate-reducing bacteria and sulfate-oxidizing bacteria, along with other heterotrophic strains, has been made. This discovery indicates potential effects caused by these bacteria on low-pH concretes (LPCs) under aqueous conditions.

One intriguing question arises: can this aqueous microbial consortium exert either beneficial or detrimental effects on the LPCs in the long term? To address this, a monitoring experiment in laboratory conditions is underway, providing valuable insights into this question.

Furthermore, a finding of a urease-expressing strain during the WP MAGIC shows potential beneficial effects (e.g., calcium carbonation) made by this strain on the LPCs. Therefore, a monitoring experiment also involving the incubation of this strain with LPCs can provide insights into this question.

1.2. Mission objectives

The primary objective of this internship is to confirm the urease-expressing activity identified in the findings. Subsequently, investigations into the consequential effects of this strain on low-pH concretes (LPCs) under both anaerobic and aerobic conditions will be conducted.

Additionally, the second goal involves examining the impact of microbiology from the underground water source on LPCs by monitoring experiments. This encompasses the validation of the presence of sulfate-utilizing bacteria and the exploration of their effects.

Moreover, a key goal is to impart a practical understanding of counting techniques such as flow cytometry and ATP measurements to the beneficiary. This knowledge transfer aims to equip the beneficiary with the skills and confidence to independently perform and analyze data for his future career

1.3. Mission request

The mission is to confirm the presence of metabolically active microorganisms in the used underground water using flow cytometry and ATP assay. Additionally, the subcultivation of potentially hazardous and/or beneficial bacterial strains will be pursued either concurrently or sequentially. Following validation, percolation experiments involving the LPCs and selected bacterial clones will be conducted.

1.4. Mission composition

Host organisation

SCK CEN – Nuclear Belgian Research Centre, Mol

Host facility

SCK CEN – Microbiology Unit

Mission dates

01 October 2023 – 30 November 2023

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

ATP assay

Description

Adenosine triphosphate (ATP) serves as the primary energy source for living cells and is integral to numerous metabolic pathways. When cells cease to synthesize and use ATP due to death, a rapid degradation of ATP is observed. This phenomenon underscores the utility of ATP as a marker for viable cells, as a high concentration of ATP indicates a substantial number of living cells.

The ATP assay is a procedure designed to measure cell viability based on ATP detection. Bioluminescent ATP assays are commonly employed for this purpose, owing to their high sensitivity, simple protocol, and rapid results.

The assay begins with the activation of luciferin by ATP, generating luciferyl-adenylate and pyrophosphate. Subsequently, luciferyl-adenylate reacts with oxygen to produce oxyluciferin in an excited state and CO2. As the excited state returns to the ground state, it emits green light, transitioning into yellow luminescent light (550 – 570 nm). A luminometer is then used to detect the intensity of the luminescent signal.

When ATP is the limiting component in the luciferase reaction, the luminescence is directly proportional to the ATP concentration. A higher luminescent signal indicates elevated ATP level

Usage

The use of ATP assays during the mission is to measured the potential viability cells presence in different monitoring condition. The Intracellular ATP Kit HS from BioThema was used.

Briefly, 50ul of samples were incubated with 50ul of Eliminating Reagent ín 10 minutes at room temperature. Then a 50ul of Extractant B/S was added to lyses the cells with mixing. 400ml of ATP Reagent HS was added and the mixtures were measured light emission Ismp. Finally, adding 10ul of 100nmol/L ATP standard and measuring the second light emission I(smp+std)

The amount of ATP is calculated bsed on the equations:

Luminometer: ATP smp = Ismp / (I(smp + std) - Ismp)

Benefits

This method is a good indicator of viability cells during the mission

Limitations

The drawback of the method is unable to provide an precise estimation of number cells based on the generated ATP.

Furthermore, presence of some chemical components in the media culture can also affect the efficiency of the assay. To overcome this, an internal standard is used.

Applicability

This assay in extreme environment can be used to have an overview of microbial activity in the aqueous samples.

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

Flow cytometry (FC).

Description

FC is a technique ultilizing the use of a laser beam projected through a liquid stream containing cells or other particles. When these particles are struck by the focused light, they emit signals that are detected by sensors. These signals are then converted for computer storage and subsequent data analysis, offering valuable insights into various cellular properties.

In this mission, the samples were labelled with fluorecesent marker (SYRB Green) which intercalate into the DNA of bacteria. Then the samples were injected into the machine and the functioning process was managed by the fluidics system.

Usage

The purpose of employing Flow Cytometry (FC) in this mission is to enumerate microbial cells during monitoring experiments. At the sampling time, 250 µL of each sample was utilized and stained with 2.5 µL of SYBR Green. Following a 30-minute incubation at 37 degrees Celsius, the mixture was injected into the Flow Cytometer

The operational conditions throughout the mission were as follows:

- Forward Scatter (FSC), Side Scatter (SSC), Fluorescein Isothiocyanate (FITC), and Propidium Iodide (PI) were utilized in both area and height for gating purposes.
- A stop condition of 100 µL was determined with a fast flow rate.
- Threshold settings were FITC at 5000 and SSC at 1000.

Subsequently, the acquired data was analyzed to determine the number of cells at different sampling points..

Benefits

One notable advantage of employing this technique is its ability to conduct high-frequency analyses of individual particles. Flow Cytometry allows for rapid measurements within a short timeframe. As it enables the counting of individual cells, it proves particularly valuable for the simultaneous characterization of mixed populations. This

capability is particularly relevant in experimental conditions encountered during this mission.

Limitations

One notable drawback of employing Flow Cytometry (FC) in this mission is its susceptibility to interference from the background of the solution. Specifically, during monitoring experiments, leachate from low-pH concretes (LPCs) can impact the accuracy of the results. Consequently, efficient analysis demands a high level of experience to mitigate these challenges effectively.

Applicability

Applying the same working conditions, with minor modifications, in our home context is advantageous. As we are studying materials with similar characteristics and examining the effects caused by microbiology, referencing results from this mission can provide valuable insights. This comparative approach enhances our understanding and contributes to the depth of our studies.

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

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Description

Klikněte nebo klepněte sem a zadejte text.

Usage

Replace this entire field with a description of your operation or study of this practice, technique, method, tool or system during the mission.

Benefits

Replace this entire field with a description of the benefits for implementing this practice, technique, method, tool or system.

Limitations

Replace this entire field with a description the limitations of this practice, technique, method, tool or system.

Applicability

Replace this entire field with a description of how this practice, technique, method, tool or system could be implemented in or adjusted to your home context.

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

Klikněte nebo klepněte sem a zadejte text.

Description

Replace this entire field with a description of the implementation of this practice, technique, method, tool or system at the host organisation.

Usage

Replace this entire field with a description of your operation or study of this practice, technique, method, tool or system during the mission.

Benefits

Replace this entire field with a description of the benefits for implementing this practice, technique, method, tool or system.

Limitations

Replace this entire field with a description the limitations of this practice, technique, method, tool or system.

Applicability

Replace this entire field with a description of how this practice, technique, method, tool or system could be implemented in or adjusted to your home context.

3. MISSION FINDINGS AND CONCLUSIONS

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3.1. Lessons learned and conclusions

The first significant achievement is the positive confirmation of urease-expressing bacteria, specifically clone B62. Acquired from our home organization, this clone is now available for further applications in the mission.

Additionally, there is a positive assessment of sulfate-reducing bacteria (SRB) present in the underground water source. Using the modified P63 media from our host organization, the observation of black precipitation in the media confirms the presence of SRB.

The monitoring experiment, designed to run for three months until February 2024, has been initiated. Initial validation tests have already been conducted, confirming the presence of the microbial community under experimental conditions.

3.2. Relevant findings and conclusions for home organisation

This section is not mandatory but can be prepared with the mission supervisor or mentor from your home organisation. If applicable, replace this entire field with a description of about 200 words of findings and conclusions that are specifically relevant to your home organisation. If not applicable, remove the entire section.

3.3. Relevant findings and conclusions for host organisation

This section is not mandatory but can be prepared with the mission supervisor or mentor from the host organisation. If applicable, replace this entire field with a description of about 200 words of findings and conclusions that are specifically relevant to the host organisation. If not applicable, remove the entire section.

3.4. Relevant findings and conclusions for other organisations

Klikněte nebo klepněte sem a zadejte text.

4. POTENTIALS FOR IMPROVEMENT OR DEVELOPMENT

This entire section shall be maximum one page (remove this entire sentence).

4.1. Generic potentials

This section is not mandatory. If applicable, replace this entire field with a description of about 150 words of generic potential improvements or developments you can suggest for the practices, techniques, methods, tools or systems operated or studied during the mission. If not applicable, remove the entire section.

4.2. Potentials for home organisation

This section is not mandatory but can be prepared with the mission supervisor or mentor from your home organisation. If applicable, replace this entire field with a description of about 150 words of specific potential improvements and developments you can suggest for your home organisation. If not applicable, remove the entire section.

4.3. Potentials for host organisation

This section is not mandatory but can be prepared with the mission supervisor or mentor from the host organisation. If applicable, replace this entire field with a description of about 150 words of specific potential improvements and developments you can suggest for the host organisation. If not applicable, remove the entire section.

APPENDICES

Mission journal

This section is mandatory. Replace this entire field with the description of the daily activities and work carried out during the mission (this should be prepared during the course of the mission and should not exceed 1 page).

- Performing initial testing on the input samples (i.e underground water source S25, Biofilm from water box, the two clones used in the experiement). The tests were flow cytometry for counting the number of cells in the inoculum, ATP measurements to estimate the active cells in the inoculum, OD measurements to measure the
- Setting the monitoring experiment with different conditions.
- Performing sampling analysis of experiments at different time point (T0, T1, T2)
- Extracting bacterial community mocks with different extracting methods to findout the significant differences which contribute to construct the optimize extracting method toward Bentonite.
- Preparing and testing different culture medias to recultivate the sulfate-reducing bacteria and sulfate-oxidizing bacteria from the underground water source and biofilm respectively.
- Picking the potential and intesting colonies from the media for further experiments and characterization using sequencing.

Mission bibliography

MISSION BENEFICIARY

Trung Le Duc
Ph.D student
Applied Biology
Technical Univesity of Liberec, Czech Republic.

PARTNER EXPERTS CONTRIBUTING TO THE MISSION

Host organisation experts

- Mijnendonckx Kristel, Project leader, SCK CEN
- Smolders Carla, Lab technician, SCK CEN

Home organisation experts

- Veronika Hlaváčková, Senior researcher, Technical University of Liberec.

Other organisations experts

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REPORT APPROVAL

Date	Beneficiary	Home mentor/supervisor	Host mentor/supervisor
Date of last signee 18.12.2023	Trung Le Duc	Veronika Hlavackova	Mijnendonckx Kristel
	visa 18.12.2023	Visa 19.12.2023	15.12.2023