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D3.2 PUSH50 pressurized biological samples

WP 3 – Instruments and Tools: Development and Deployment

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History of the changes

Version	Date	Released by	Comments
1.0	17-08-18	Isabelle Daniel	First draft circulated internally at UCL
1.1	26-08-18	Alberto Striolo	Second draft circulated to the consortium for comments and approval
1.2	30-08-18	Natalie Nestorowicz	Final version after the reception of all the contributions' comments and feedback

Table of contents

Key word list.....	3
Definitions and acronyms	3
1. Introduction	4
1.1 General context	4
1.2 Deliverable objectives.....	4
2. Methodological approach	4
3. Summary of activities and research findings	5
4. Conclusions and future steps	5
5. Publications resulting from the work described (<i>if applicable</i>)	5
6. Bibliographical references (<i>if applicable</i>).....	5

Key word list

Definitions and acronyms

Acronyms

PUSH50

Definitions

Pressurized Under water Sample Handler 50 ml

1. Introduction

1.1 General context

D3.2 is the validation of PUSH50 to hold microbial samples under pressure prior to sampling at the CarbFix and the Cornwall sites (WP7). The PUSH50 facility at Lyon includes 50mL sterilizable PEEK variable volume, floating piston reactors housed in a high-pressure vessel rated for 100 MPa (1 kbar) and 160°C. They are certified for airline travel. Five vessels are ready to use at Lyon.

This equipment will allow to maintain/grow/store microorganisms under their original pressure, as we know that assessing the role of subsurface microorganisms by growing them under ambient pressure lead to underestimate their diversity and activity. The ideal situation includes recovery, storage and cultivation under pressure without 'breaking pressure', i.e., lowering the pressure for some time. If not possible, storing and cultivating under pressure is already a major step forward compared to previous investigations in continental settings such as those targeted in the S4CE project.

1.2 Deliverable objectives

The objective of D3.2 is to make sure that the PUSH50 vessels can be operated on the field sites of interest, where operations may modify the subsurface microbial communities, with potential consequences for the operations.

Initially PUSH50 was designed for sampling at sea during oceanic scientific cruises.

Before the S4CE consortium starts sampling continental subsurface microbial communities, the goal of D3.2 is to ensure that PUSH50 can operate under the conditions of the field sites selected in the project and hold pressurized microbial samples.

2. Methodological approach

We first checked the pressure and temperature conditions of operations and sampling at the CarbFix site to ensure they fit within the technical specification range of the PUSH50. Then we targeted a well characterized piezotolerant bacterial strain (which bears mild pressure) with a metabolism typical of those expected for instance at the CarbFix site, thanks to the earlier work done by the group at IPGP (Paris).

3. Summary of activities and research findings

Thanks to the visit at the CarbFix site by one of us – Dr Cardon, and extended discussions with Dr Bergur Sigfússon (Reykjavik Energy), we identified the pressure and temperature of interest at the Nesjavellir, CarbFix1 and CarbFix2 sites in Iceland. At CarbFix1 sampling should occur at a maximum depth of 400 m, which corresponds to the limit of the casing of the wells, and to a pressure of 4 MPa. Temperature in this location has been monitored sporadically between 2005 and 2007; the data show that temperature does not exceed 20°C at that depth. This is well within the P-T range of PUSH50 instruments. At CarbFix2, the temperature ranges between 15°C at the wellhead to max 80°C at 750 meters depth, although the casing doesn't go as deep.

The important positive observation is that these conditions are well within the biotic zone for both pressure and temperature.

The limitation is that pressure is too low to retrieve samples under pressure. Therefore, we will collect the samples and the water is pumped to the surface and we will pressurize again at the original pressure for transportation and further investigation.

In order to prepare as far as possible according to D3.2, Dr Aude Picard (S4CE – engineer in high-pressure microbiology May – July 2018) launched the culture of a synthetic piezotolerant bacterial strain and natural communities, whose metabolic activities resemble those expected at the CarbFix sites, targeting the microaerophilic metabolism of nitrate and sulfur. Dr. Picard also calibrated some internal standards for the measurement in situ of the metabolic activity of sulfur using Raman spectroscopy in the diamond anvil cell. The piezotolerant strain is now stored in one PUSH50 at 10 MPa.

4. Conclusions and future steps

As planned for deliverable D3.2, PUSH50 hold a pressurized microbial sample and the four other pieces are ready for sampling at the Nesjavellir, CarbFix1 and CarbFix2 sites in Iceland September 6th and 7th, prior to the S4CE all hands meeting.

5. Publications resulting from the work described (*if applicable*)

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6. Bibliographical references (*if applicable*)

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