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► **To cite this version:**

Anaïs Cario, Carole Lecoutre-Chabot, Olivier Nguyen, Samuel Marre, Yves Garrabos. High pressure microfluidics : new tools for investigating the deep biosphere. 17th European Meeting on Supercritical Fluids and 7th European Meeting High Pressure Technology, ITQUIMA, Apr 2019, Ciudad Real, Spain. pp.155-156. hal-02899996

**HAL Id: hal-02899996**

**<https://hal.science/hal-02899996>**

Submitted on 20 Jul 2020

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# High pressure microfluidics: new tools for investigating the deep biosphere

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## 1. Introduction

Over the last 10 years, a new field of investigation called "high pressure microfluidics" has gained increasing interest [1]. It is based on the idea of combining the advantages of microfluidics (reduction in size, rapid screening, *in situ* analyses, reproducibility, hydrodynamic control, improvement of heat and mass transfers, low consumption of reagents during optimization phases, etc.) with fluid systems used under high pressure (and high temperature) conditions. The development of this high pressure microfluidic technology has opened up many opportunities to study and characterize a large number of processes using high pressure fluids (up to a few hundred bar at present) such as hydro- and solvothermal processes, thermodynamics measurements [2], studies of geofluids flows in model porous media [3] or supercritical fluids [4]. More recently, it appears that such tools are particularly well adapted to the investigation of microbiology under extreme conditions such as microorganisms living in the deep.

## 2. Results and discussion

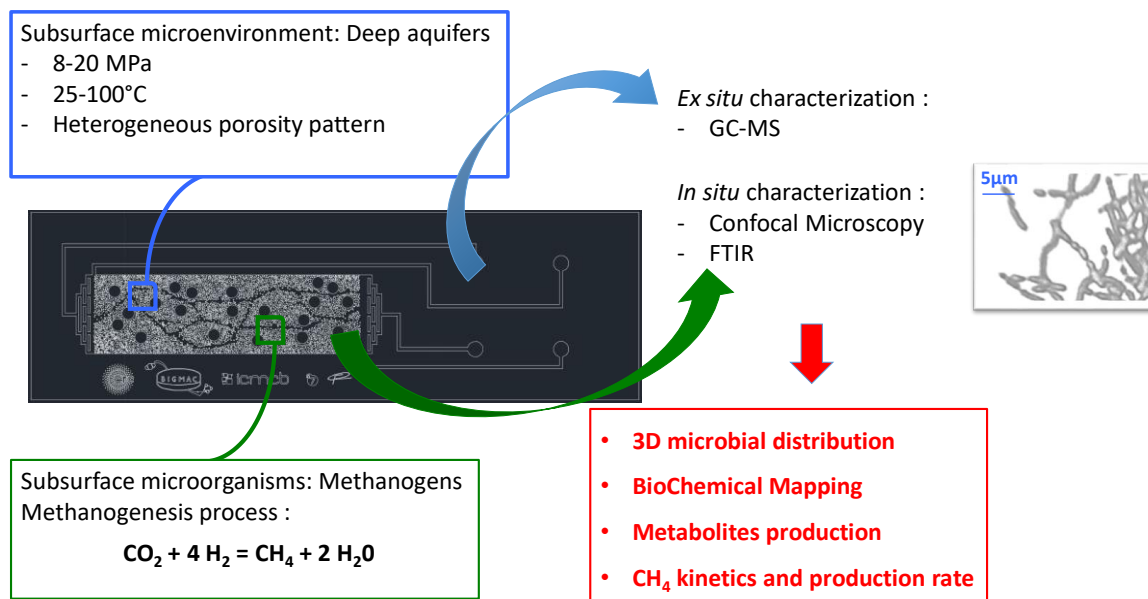
A majority of Earth's prokaryotes reside in the deep biosphere (deep sea or deep underground environments) where little is known about how inherent elevated pressures impact the underground geochemistry and the inhabiting microbial communities. The deep biosphere represents the unseen majority (~ 99 %) of the total biosphere on Earth and has gained increasing attention given its significance in a variety of critical processes and topics (carbon cycle, origin of life, biomining, etc.). However, the complex sampling procedures on site result in small amounts of biological materials, while the samples analysis (sorting of the various strains, cultivation, cytometry and metabolisms determination) mostly requires depressurization, which could induce some uncontrolled bias during the investigations. Although some HP experimental means exist (macroscopic batch reactors or diamond anvil cells), the microbiology under extremes conditions is still scarcely studied. Indeed, conventional cultivation and analysis techniques offer both limited optical access and *in situ* characterization, while generally requiring sufficient amount of sample, thus narrowing the ability to investigate deep subsurface microbial communities.

HP microfluidics tools are able to overcome these limitations being able to propose fast screening approaches and *in situ* monitoring in real conditions, both in batch and continuous mode using small quantities of biological materials.

In this presentation, we will first detail the interest of this technology and the different strategies developed to manufacture and use high-pressure microreactors. Then, we will present the use of on-chip biocompatible high pressure geological laboratories (BioGLOCs) for the culture and the study (Figure 1) of methanogenic microorganisms living in deep geological environments. As part of the ERC project "BIG MAC" [5], which aims to mimic deep geological environments, we study the bioconversion reactions of CO<sub>2</sub> into methane by methanogenic archaea. BioGLOCs are a significant tool to mimic the *in situ* biogeological reservoirs conditions to study CO<sub>2</sub> bioconversion within deep aquifers. These tools could provide new insights into bioremediation process to restore CO<sub>2</sub> as a valuable energy resource (*i.e.* CH<sub>4</sub> via methanogenesis process) and could also find wider applications in geological-related and deep-sea field studies.

## 3. Conclusions

In conclusion, high pressure microreactors turn out to be excellent experimental tools for investigating microbiology under extremes conditions at the lab scale. After summarizing the advantages of these new experimental approaches compared to traditional instrumentation, we will give some perspectives concerning other future applications of this technology applied to the study of extremophile microorganisms (thermophile, piezophile).



**Figure 1.** Example of a high pressure geological laboratory on a chip for investigating methanogens subsurface microorganisms for CO<sub>2</sub> bioconversion to methane in deep saline aquifers.

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