

4.4.P03***In situ* investigation under high pressure and temperature of the limits of life**

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Our current view that life requires low pressures, moderate temperatures, light as an energy source, or even oxygen as a final electron acceptor is slowly fading as we get a better view of the diversity of Earth's extreme biotopes. Many of these later do not rely on light and oxygen, a scarce resource on the young earth, but on alternate energy sources such as metals. Interestingly, they share strong similarities with potential extraterrestrial biotopes such as those found on Europe or the early Mars, as well as striking similarities with early Earth's postulated conditions. High-pressure biotopes are the most widely spread on today's Earth. Unfortunately, these biotopes are hardly accessible by standard techniques.

Experimental setup

We have developed an experimental incubator based on a modified Diamond Anvil Cell, with a wider observation angle (60°), and a thin (250-400 μm) observation diamond window to allow for lower detection limits using Xray or visible fluorescence. This incubator can be used between 0.1 MPa and 2 GPa, with typical gasket of 100μm thick Inconel alloy. Our experimental model consists of either microbial compounds components (nucleic acids, proteins, etc.) in aqueous solutions, or bacterial strains submitted to different regimes of P and T.

Development of novel low pressure indicators

The shift of ruby fluorescence, commonly used for pressure calibration, proved not to be sensitive enough for the preferred pressure-range needed to study organic materials *in situ*. Therefore, we have evaluated the use of different fluorescent chemicals to serve as pressure sensors inside the cell.

Behavior of cells and cellular components

Two sets of strains from low pressure environments were analyzed in our setup, namely *Escherichia coli* and *Rhizobium* sp. No growth could be obtained for *E. coli* at any pressure, while some growth occurred for the second strain up to 0.6 GPa. Pressure did not affect cellular shape, but severely limited bacterial mobility, probably due to increase in the viscosity of the medium. Increasing pressures significantly affected the activity of the fluorescent proteins analyzed, showing a first reduction in fluorescence ca. 0.3 GPa and a second decrease ca. 0.7 GPa that lead to the complete loss of activity.

Our results validate the use of the DAC for *in situ* analyses of biological components.

4.4.P04**Fe(II) and Mn(II) oxidizing bacteria associated with weathered oceanic basalts**

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Historically, the ocean crust has been considered to be highly oligotrophic and relatively unsuitable as a host for microbial organisms due to the lack of organic carbon or high concentrations of soluble energy sources. However, the juxtaposition of reduced basalts and oxygenated seawater offers a tremendous amount of potential chemical energy that could form a basis for life in the deep ocean. In particular, the geochemical and petrographic characterization of altered basalt surfaces suggest that Fe and Mn are the major elements that are actively redox cycled and are therefore the most likely candidates as potential electron donors for microbial growth.

Although novel neutrophilic, lithotrophic Fe(II)-oxidizing bacteria have recently been isolated from hydrothermal microbial mats (e.g. Loihi Seamount) and sulfide surfaces (e.g. Juan de Fuca Ridge), it is currently unknown whether or not these same organisms colonize adjacent basalt surfaces and/or could affect the rates of chemical alteration of ocean basalts.

Using basaltic glasses recovered from the cold, outer regions of Loihi Seamount (at -1000 to -1700m), we have isolated a diversity of Fe(II) and Mn(II) oxidizing alpha- and gamma-Proteobacteria. The Fe(II) oxidizing bacteria have been cultured with Fe(II) as a sole electron donor, whereas the Mn(II) oxidizing bacteria are oligotrophic heterotrophs. None of these isolates (nor their nearest relatives) were previously known to be metal-oxidizing bacteria, which has enormous implications for the interpreting the functional role of organisms identified in culture-independent (DNA-based) studies of basalt-associated biofilms.

In order to quantitatively assess how the metabolic activity of each of these Fe and Mn-oxidizing isolates may accelerate (or suppress) the rates of basaltic glass alteration, we are currently conducting a series of biofilm experiments to measure changes in basalt surface chemistry relative to abiotic controls.