A microbial ecosystem in Opalinus Clay rock fueled by hydrogen gas

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A significant part of microbial biomass is found in the deep subsurface and little is known about metabolisms in these environments (McMahon and Parnell 2014). This remote biosphere is decoupled from sunlight-derived organic carbon and rely exclusively on geogenic gases, such as methane or hydrogen (Lovley and Chapelle 1995).
Opalinus Clay is a low-porosity clay rock, which is supposed to host nuclear repositories in Switzerland. In this regard and thanks to the underground rock laboratory of Mont-Terri in St-Ursanne, it has been extensively characterized (Bossart and Thury 2008). It is well established that pristine rock, because of its small pore size distribution, is not suitable for hosting microbial activity. But as soon as space is provided, because of the presence of organic matter (i.e., acetate) and electron donor (i.e., sulfate) in Opalinus Clay rock, microbial activity can take place (Stroes-Gascoyne *et al.* 2007). Thus, this project aimed at characterizing the biological response to H_2, which will be produced by the anoxic corrosion of steel canisters, in repository-relevant conditions. An *in-situ* experiment was carried out where H_2 was amended to borehole water for more than 500 days.
H_2 was rapidly consumed concomitantly with a production of hydrogen sulphide, an increase in planktonic cell density, and a decrease in CO_2 concentration, suggesting that autotrophic sulfate reduction was the dominating process in this system. A metagenomic sequencing allowed the assembly of 15 high-quality genomes, together representing 75% of the microbial community. Metaproteomic analysis highlighted the presence of a complete carbon cycle. Two organisms oxidize hydrogen: a sulfate-reducing bacterium (Desulfobulbaceae) representing 42% of the community, and a Rhodospirillaceae reducing sulfite. Biomass produced by autotrophs is then oxidized to acetate by a *Hyphomonas* species. Fermentation products are finally oxidized to CO_2 by several species of heterotrophic sulphate-reducing bacteria (Figure 1).
This work provides the first insight into a complete carbon cycle in the deep subsurface and significantly expands our knowledge of microbial metabolisms in the deep biosphere. It also suggests that allowing microbial activity in nuclear waste repositories is beneficial, by buffering the pressure increase due to *in-situ* H_2 production.

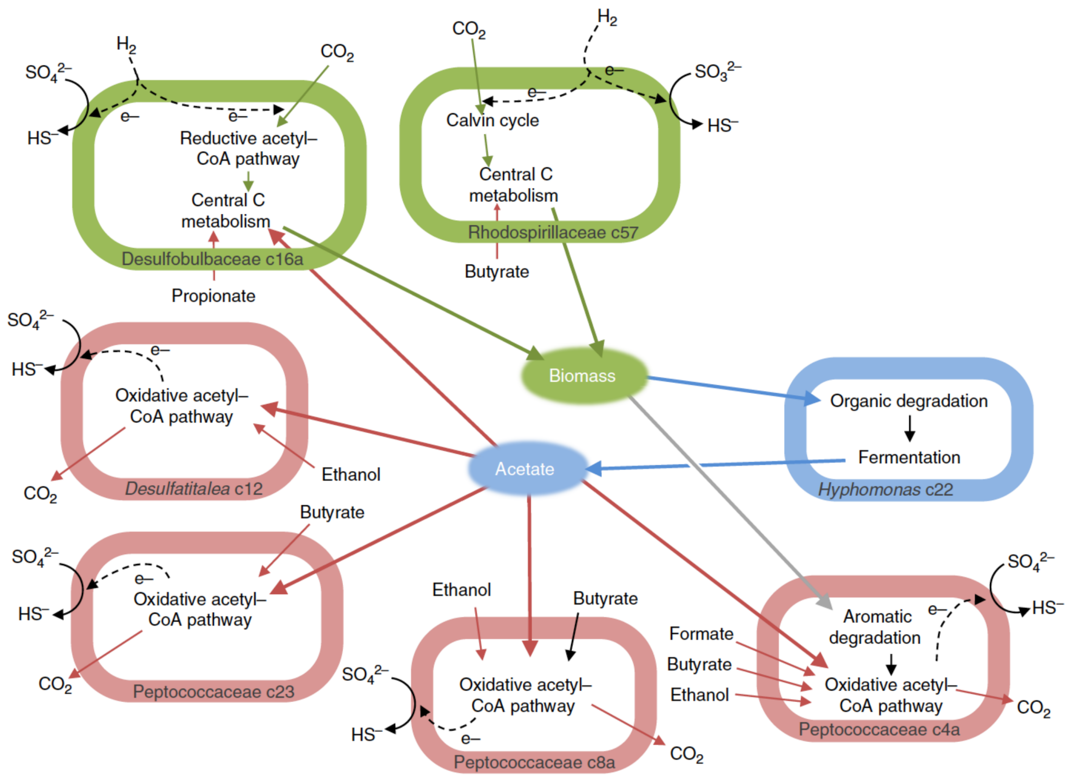

Figure 1. Microbial ecosystem fueled by hydrogen in Opalinus Clay rock.

REFERENCES
Bossart P, Thury M. Mont Terri Rock Laboratory. Project, Programme 1996 to 2007 and Results. Bern: Federal office of topography swisstopo, 2008.
Lovley DR, Chapelle FH. Deep subsurface microbial processes. Rev Geophys 1995;33:365–81.
McMahon S, Parnell J. Weighing the deep continental biosphere. FEMS Microbiol Ecol 2014;87:113–20.
Stroes-Gascoyne S, Schippers A, Schwyn B et al. Microbial community analysis of Opalinus Clay drill core samples from the Mont Terri underground research laboratory, Switzerland. Geomicrobiol J 2007;24:1–17.