

# Detection and Characterization of Active Microbial Cells in Salt Cavern Brine

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## Abstract

Salt caverns have been used for decades as natural gas storage facilities and are now target of large-scale underground H<sub>2</sub> storage to secure national energy transition goals. Contrary to CH<sub>4</sub>, H<sub>2</sub> is a universal electron donor for microbial anaerobic respiration. Suitable electron acceptors are sulfate and carbonate, which dissolve from gypsum, anhydrite and lime that can make up 10 % of subsurface salt formations. Whilst sulfate reduction is inherently linked to the formation of H<sub>2</sub>S, microbial CO<sub>2</sub> reduction can generate acetate, which can be used as carbon source by diverse microorganisms. Thus, supporting other microbial side effects, such as H<sub>2</sub>S formation, clogging and H<sub>2</sub> consumption. However, microbial diversity and activity in salt caverns are selectively controlled by salt concentrations close to saturation and limited availability of organic carbon. If these conditions allow for microbial activity was investigated in our study.

To circumvent long enrichment times associated with high salinity and limited nutrient availability, we used a stable isotope labelling approach combined with nano-scale secondary ion mass spectrometry analysis (SIP-nanoSIMS) to investigate H<sub>2</sub>-dependant microbial activity in two brine samples and compared them with that of an extremely halophilic enrichment culture (MP-32). Heavy carbonate and water (<sup>13</sup>CO<sub>2</sub> and <sup>2</sup>H<sub>2</sub>O) served as tracers for microbial activity. Microbial H<sub>2</sub> consumption was additionally investigated in microcosm experiments with brine and rock salt over a period of 200 days. Setups with MP-32 served as a positive control. Subsequently, MP-32 was selected for metagenome sequencing to explore potential metabolic pathways and strategies for osmoadaptation.

Analysis of the microbial community composition in brine revealed that members of the Desulfohalobiaceae, Halobacteria and Halanaerobiales were present in all caverns and their relative abundance increased during incubation with H<sub>2</sub> as electron donor although sulfate reduction was not observed. But incubation with H<sub>2</sub> resulted in an increased uptake of <sup>13</sup>C from <sup>13</sup>CO<sub>2</sub> in 1.6 to 3.6 % of the cells compared to incubations without H<sub>2</sub>. Uptake of <sup>2</sup>H from <sup>2</sup>H<sub>2</sub>O was detected in 20 to 30 % of the cells and was higher when H<sub>2</sub> was not offered as an electron donor. Similar results were obtained from the enrichment culture

MP-32, which was grown in medium with reduced salinity compared to the salt cavern brine. Uptake of  $^{13}\text{C}$  was 10-fold higher when incubated with  $\text{H}_2$  and nearly all cells incorporated  $^2\text{H}$  with and without  $\text{H}_2$ . A total of eight metagenome-assembled genomes (MAGs) with a completion of more than 90 % could be recovered from MP-32. Two of them belonged to Desulfohalobiaceae and can be characterized as autotrophic sulfate reducers by means of the Acetyl-Coenzyme A pathway that compensate osmotic stress by synthesizing small organic molecules. Collectively, our findings provide a new approach to study microbial activity that is strongly impacted by high salinity and an improved understanding of their genomic potential.

## **Keywords**

salt cavern, halophile, SRB, underground gas storage, nanoSIMS, anaerobe

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