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Halophilic Hydrogenotrophic Prokaryotes in Brine: The Implications of Microorganisms on Hydrogen Storage in Salt Caverns

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Summary

Utilizing hydrogen as an energy carrier is required to reach decarbonization goals (Nnabuife et al., 2023). Subsurface hydrogen storage in salt caverns addresses energy demand fluctuations and provides high storage volumes, while the crystalline structure of evaporite minerals maintains an impermeable container to store H_2 (Muhammed et al., 2022). Introducing hydrogen to the salt cavern poses a risk in the presence of hydrogenotrophic (hydrogen-metabolizing) prokaryotes such as sulphate reducing bacteria (SRB) and methanogenic archaea. Hydrogen acts as an electron donor for SRB to reduce SO_4^{2-} into H_2S, a corrosive and toxic gas, and for methanogens to reduce CO_2 into CH_4, which is emissive in nature. These microbial redox reactions compromise H_2 purity by the production of H_2S and CH_4 and reduce the volume of injected H_2 which results in resource loss, therefore posing a risk to a H_2 storage operation. This study, 1) examines the microbial community in salt cavern brine taken from the Lotsberg Salt Formation (LSF) in Alberta, and 2) monitors hydrogen purity while exposing enriched anaerobic salt cavern brine cultures from the LSF to a hydrogen headspace. DNA sequencing results of the salt cavern brine from the LSF indicates methanogens represented less than 0.1% relative abundance, and SRB were of extremely low abundances (*Desulfohalobium* at 0.1% and *Desulfovermiculus* and 0.2%). Aerobic halophiles were at the highest relative abundances (2%-24%). Their presence in the anoxic salt cavern environment is likely a result of brine cycling between the subsurface cavern to the surface brine pond. Following inoculation into anoxic growth media with an 80% H_2 / 20% CO_2 headspace, CH_4 concentrations were measured on days 45, 108, and 120 of incubation, with less than 0.1% CH_4 being present on day 120. Following 120 days of incubation, sulfide is present in the brine at concentrations of approximately 20ppm in cultures lacking acetate and formate, and 60ppm in cultures with acetate and formate. These findings indicate that SRB are likely out-competing methanogens for hydrogen. DNA extraction and 16S rRNA sequencing of the microbial community after incubation is ongoing.

Theory / Method / Workflow

The Lotsberg Salt Formation (LSF) in central Alberta belongs to the Elk Point group and is comprised primarily of large halite crystals, with lesser amounts of anhydrite, gypsum, and carbonate minerals (Yuan et al., 2024; Tobola and Kukialka 2020). Salt cavern conditions are characterized by high salinity, elevated temperatures, high pressures, limited nutrient availability, and lack of oxygen causing reducing conditions (Thaysen et al., 2021). When hydrogen is diffused into salt cavern brine it can act as an electron donor to hydrogenotrophic bacteria and archaea that are anaerobes or facultative anaerobes, and halotolerant or halophilic (Thaysen et al., 2021). This study uses brine collected from an active hydrocarbon storage salt cavern in the LSF to determine the microbial community before exposure to hydrogen gas and to examine the effect of H_2 gas exposure to microbial community composition, methane production, and sulfide production. Incubation conditions mimic the cavern operating temperature of 30°C.

Four liters of salt cavern brine was collected at the wellhead and anaerobic conditions were preserved by overfilling the sample collection bottles. This brine was filtered and DNA was extracted from the filter surface which was then prepared for PCR followed by 16S rRNA sequencing to determine the microbial community to the genus level. Anaerobic cultures were made following the defined media for *Methanocalculus halotolerans* detailed in the DSMZ (Dopffel et al., 2023). The growth medium was prepared at an increased concentration (10 times more concentrated) to compensate for adding only 5mL of the medium into 45mL of salt cavern brine. The addition of the methanogen medium to the salt cavern brine was done to stimulate growth of methanogens if present in small relative abundances. Six cultures were prepared and three included acetate and formate to stimulate growth. The anaerobic cultures were incubated at 30°C under 80% H_2 / 20% CO_2 headspace to facilitate methanogenesis and to replicate temperature conditions of the salt cavern. GC analysis for CH_4 was conducted on day 45, 108, and 120 of incubation. A sulfide test was conducted on day 120 of incubation. Monitoring of CH_4 and H_2S is ongoing.

Results, Observations, Conclusions

16S rRNA gene sequencing indicates no methanogens over relative abundances of 0.1% in the returned brine (Figure 1). SRB are present at low relative abundances, with the genera *Desulfohalobium* at 0.1% and *Desulfovermiculus* at 0.2% of sequences. Figure 1 shows the dominant genera comprising up to 24% of the microbial community, and all of them are known aerobic halophiles (Oren 2008).

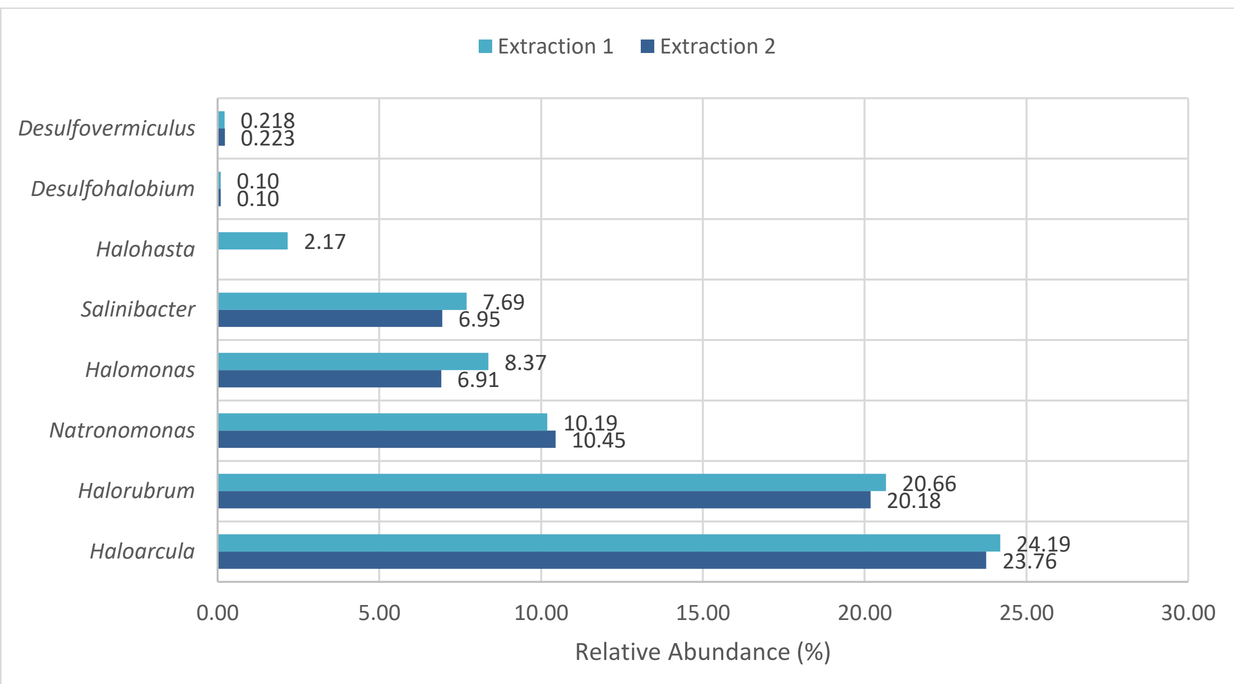

Figure 1. 16S rRNA sequencing results from two DNA extractions of salt cavern brine prior to hydrogen gas exposure displaying SRB and other genera with relative abundances above 2%.

The LSF salt cavern is connected to a surface brine pond, where excess brine is stored between injection and withdrawal cycles to maintain cavern pressure. This brine is cycled between the oxic brine pond and the anoxic salt cavern and carries the microbial community with it. Aerobic organisms are dominant in the brine sample which may be due to larger brine volumes occupying the surface pond for longer periods of time than in the salt cavern. CH_4 concentrations peaked at 0.43% in the anerobic cultures at 108 days, but decreased to less than 0.1% by 120 day. H_2S concentrations were approximately 20ppm in cultures lacking acetate and formate, and 60ppm in cultures with acetate and formate following 120 days of incubation. If methanogens and SRB are both present in the anaerobic enrichment cultures, the SRB will out-compete the methanogens due to having a higher substrate affinity for hydrogen (Purdy et al., 2003).

Novel/Additive Information

Production of H_2S rather than CH_4 implies that SRB pose a greater risk in a salt cavern environment for H_2 storage. Further testing will include microbial community sequencing of enriched anaerobic cultures, development of anaerobic cultures with SRB targeted medium, and bridging laboratory findings to salt cavern applications via simulation.

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