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"Microbial life in salt caverns and their influence on H_2 storage – Current knowledge and open questions."

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ABSTRACT

Hydrogen will be one of the key components for renewable energy storage in the future energy systems as it can be stored in significant volumes to overcome daily to seasonal energy fluctuations. Subsurface storage in salt caverns will be a first step. These caverns are created by solution mining in underground salt formations. Despite the high salinity in this environment, salt caverns harbor microbial life. These microorganisms can not only survive in these caverns by using unique adaptation mechanisms, but they actually cause several risks to hydrogen storage. Different metabolisms can use hydrogen as electron donor, leading to hydrogen loss and in the worst case also to H_2S formation. The knowledge on salt cavern microbiology and subsequent possible effects of hydrogen is still in its infancy and only a limited number of salt caverns have been investigated so far. This review summarizes the current knowledge and key questions about halophilic (salt-loving) microbes, their adaptation strategies, their origin and potential consequences of their metabolisms. It also discusses the major factors influencing microbial activities and potential risks. This review emphasizes that more research and field trials with extensive microbial monitoring are needed before hydrogen storage in a biologically active system can be safely achieved at a global scale.

1. Why is H_2 storage important? - Introduction

To build a resilient and secure energy network, sufficient amount of energy storage is needed [1]. Very often energy production and energy demand are not equivalent, therefore a buffer is needed to ensure continuous supply. This holds true for all types of energy and energy carriers like electricity, stored in batteries, or natural gas, stored in tanks, ships, reservoirs and salt caverns. As the new renewable energy market evolves, hydrogen (H_2) will be one of the key pillars to decarbonize society and industry [2]. H_2 can be produced from renewable electricity like wind and solar via electrolysis. In contrast to electricity, H_2 gas can be stored in large volumes. Especially for the "green" H_2 produced from renewables, sufficient storage volume is needed because renewable electricity is heavily fluctuating due to changing weather conditions resulting in a constantly changing production rate, while at the same time the H_2 consumption may fluctuate at a daily to seasonal scale. In the early H_2 economy, container storage will be the first step. With a broader use of H_2, larger storage volumes are needed which simply cannot be realized with pressurized containers due to costs, space limitations and safety aspects. Storage in geological underground formations will be a favorable solution for mid-to large storage volumes due to the large rock volumes available in Europe [3–5]. Suitable underground options include hard rock caverns, porous rocks like gas depleted reservoirs or aquifers and solution mined salt caverns. Especially solution mined salt caverns are currently in discussion for H_2 storage with typical volumes up to 500.000 m^3 (corresponding to 133 GW h) storing the gas for days to several weeks or even months [6]. Salt caverns are very common in central Europe especially in the Netherlands, Germany, France and Great Britain, and can be created wherever suitable geological salt structures can be found [3,7]. Current salt caverns are used to store natural gas, oil, brine or chemicals, and the operating technologies are well developed. The existing and potentially new caverns might also be suitable for H_2 storage. Considering the available storage volumes, it has been estimated that up to 170 TW h can be stored in the existing salt caverns [8]. If all suitable salt structures were efficiently used for leaching new caverns, the overall onshore

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storage potential is estimated to be 23.2 PW h [9], which would be sufficient for all estimated H_2 scenarios envisioned by the European Commission [10]. To reach this potential, there are several hurdles which need to be overcome. As H_2 is very different from natural gas in terms of density, chemical reactivity and diffusivity, for example, all components like materials, safety valves, sensors, and compressors need to be qualified for H_2 suitability. But H_2 is also very different when it comes to biological systems. H_2 is a ubiquitous electron donor for many anaerobic microorganisms, which use H_2 to fuel their metabolism to be active and/or grow [11]. These microorganisms (both Bacteria and Archaea) can be found everywhere in nature especially in the subsurface and salt caverns are no exception [12]. When these microorganisms come in contact with H_2 they can trigger many consecutive reactions, changing properties of minerals and brines or producing toxic compounds like H_2S gas [13]. Due to the extreme conditions that develop in salt caverns only very specialized microorganisms, so-called halophiles, can survive but many aspects of these organisms are still unknown, including their origin, their activity level and also how to limit their metabolism.

This review aims to summarize the current knowledge of salt cavern microbiology and to answer the most common questions about how salt cavern conditions effect microbes and how microbes affect salt cavern operations especially regarding H_2 underground storage.

2. What is a solution mined salt cavern? - Construction and operating technologies of salt caverns

The solution mining technique is a well-established method for constructing caverns in salt deposits suitable for storage [14]. One important aspect is the availability of large volumes of water (either fresh water or seawater) and a disposal system for discharging the produced brine. The cavern shapes depend on the salt structures and the leaching method and can range from pancake-shape to cigar-shape. Immediately after leaching, brine is left in the bottom part of the salt cavern commonly referred to as the cavern's sump, which cannot be removed. This sump contains insoluble minerals and brine and is over-saturated in salt (Fig. 1). The chemistry of this brine sump will strongly depend on the mineralogy of the salt structure and the other minerals like anhydrite, which will release sulphate. It can be assumed that the majority of the microbial cells will be present in the cavern sump but due to evaporation and "condensation" in the cavern during pressure changes [15] there could also be cells within the brine film covering the cavern walls. Here it must be mentioned that in some caverns, the volume of the residual brine that engulfs the insolubles in the sump, will over time reduce or even become insignificant due to the gradual withdrawal of the wet gas from the cavern. The most important cavern parameters are discussed in Table 1.

There are currently six salt caverns in operation for H_2 storage, and many new cavern storage facilities are being converted from natural gas to H_2 storage. They are located in Teesside (UK) since 1972, US Texas (Clemens Dome, Spindletop, Moss Bluff) since 1983 (Clemens Dome) and 2007 (Moss Bluff) [16]. The H_2 is used for nearby chemical industry plants and no problems or incidents have been reported. However, operational details and specific cavern conditions are not available for these caverns, so it is not clear whether microbiological reactions required treatment and how much H_2 was lost via diffusion during operations.

3. How can microbes survive in salt brine? – Halophilic life

On Earth there are many different salt-saturated environments which occur either naturally or are of anthropogenic origin. Examples for natural NaCl-rich habitats are in-land salt lakes, soda lakes, saltmarshes, deep sea brine basins, halite-rich sediments or evaporated sea water ponds. Human made examples are salterns with salt crystallizer ponds, salt mines or salt caverns. All these places vary in their solute

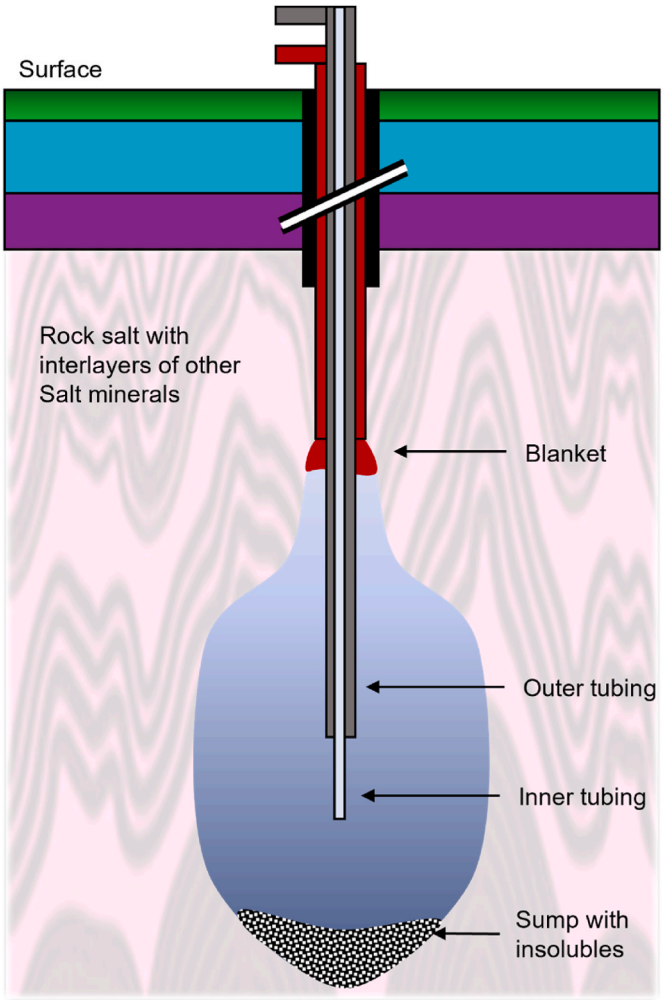

Fig. 1. Depiction of a typical salt cavern.

composition, but most are saturated or oversaturated with respect to salt. Here it is not only sodium and chloride, but many other elements can play a role like Ca, Mg, K, S, Fe, Br or Si, which are normally trace elements but can accumulate to significant concentrations in the high-salt habitats. Still, all of these mentioned high-salinity sites harbor microbes [20]. In contrast to other extreme environments (like low pH or very high temperature) saline environments are very rich and diverse in microbial life. They can have very high biomass and cell numbers (reported up to 10E08 cells/ml) and hundreds of different types of microbes. In fact, some research suggests that "only" high salt is not extreme, neither thermodynamically nor biochemically [21]. Still, high saline brines cause stress for microbial cells due to several factors, such as a) high osmotic pressure; b) low water activity; c) high chaotropicity [22].

Osmotic pressure will lead to the loss of water out of the cell, as biological membranes are permeable to water, causing cell collapse and cell death. Two main adaptive mechanisms are known for cells to keep up the internal cell/turgor pressure, the so-called "salt-in" strategy or the "osmotic-solute" ("salt-out") strategy [23]. Both are fundamentally different, but both help the cells to cope with the hyper-saline environments. The "salt-in" strategy means that the cells have a high salinity inside the cell resulting in isosmotic conditions. Especially K^+ is the main intracellular cation, which is actively accumulated. To still enable cell function, the enzymes and proteins have an increased hydrophobicity and are enriched in specific amino acids (aspartic acid and glutamic acid). These amino acids will increase the negative charge on the protein surface and therefore increase H_2 bonds between the

Table 1
Typical geomechanics and geochemical properties of a salt cavern and their expected effects on microbes.

	Range	Effects on microbes
Depth [m]	500–1500 [17]	–
Temperature [°C]	20–80 [12]	Current known temperature range for microbes: −20 °C to +122 °C. At T > 90 °C low cell numbers can be expected.
Salinity [%]	saturated	High salinity can only be tolerated by halotolerant or halophilic microbes. There is no general salinity limit known for microbes.
Overall pressure range [bar]	45–202 [18]	Pressure will lead to certain changes in the cells and might affect activity. No general pressure limits are known for microbes.
Sump volume [m^3]	Depends on cavern shape salt lithology and leaching process	Most microbes will be in the brine phase either in the sump or brine films on the wall. Cell numbers are expected to scale with brine volume
Typical sulphate content [g/l]	2–5 [12]	With more sulphate available, increased activity of sulphate-reducing microbes can be expected. Overall H_2S potential is higher.
Pressure changes/ max withdrawal rates	Up to 20 bar/day 1,36 Mm^3 - 5,91 Mm^3/ day	Very fast pressure drops can destroy microbial cells. The expected drop in a cavern is not fast enough to have a significant effect.
Typical organic carbon content [mg/l]	8–500 [12,19]	Organic carbon is an important growth factor for microbes. With higher carbon content, cells are able to multiply and be more active.

protein-surface and water molecules. This improves both the structure and function of enzymes at high salt concentrations. However, this also means that these microbial specialists need high saline conditions for correct protein folding and therefore functionality and they cannot survive in low-salt conditions. The most extreme halophiles are all "salt-in" strategists and many of them are expected to be present in salt caverns [24,25]. More moderate halophiles or halotolerant microbes have much less salt inside their cell (cytoplasm) compared to the outside. These "salt-out" strategists synthesize small organic compounds, so-called osmotic solutes including glycine betaine, ectoine, glutamine or different sugars. Organic compatible solutes are osmotically active compounds, often polar and highly soluble. They display a general stabilizing effect by preventing the unfolding and denaturation of proteins enabling cells to be metabolically active without any major modifications on their cell membranes or proteins. Depending on the outside salinity, these microbes can rapidly adjust the concentration of osmotic solutes inside the cell (either synthesis or excretion) thus tolerating changing salt conditions. This strategy provides the cells with more flexibility, but the synthesis of these compounds is energetically costly which can limit their growth [20,23].

High salinity brines not only cause high osmotic pressure but also low water activity (water activity = effective mole fraction of free available water). For saturated NaCl brines, the water activity is 0.75 aw at ambient conditions (pure water = 1 aw). There are brines which can have even lower water activity, which is caused by other ions like calcium or magnesium [26]. Pure strains of halophilic bacteria and archaea grow in habitats with water activity as low as 0.748–0.635 aw and fungi have been reported to actively multiply at 0.611 aw [22]. Certain compounds like glycerol can enable activity even below that value. Also microbial activity has been reported for extremely low water-activity brines, deep-sea hypersaline anoxic basins [27] and Kryos Basins [28,29]. The water activity of salt cavern brines has not been reported so far but is to be expected to be at least 0.75 aw or lower, depending on the brine composition.

Chaotropicity is an important factor limiting life in high salinity environments. Certain cations like magnesium and calcium are chaotropic, meaning destabilizing, causing loss in cell function. The complex aspects of chaotropicity on life is an area of ongoing research and reviews can be found elsewhere [30,31]. Overall, the influence of chaotropic ions on microbial activity in salt cavern brine remains unexplored.

4. What can they do? – General microbial activity at high salt

Halophiles are known to be highly proficient at the utilization of various metabolites, very often in a semi-symbiotic lifestyle. Metabolites are either excreted by primary producers and further used by other microorganisms. Or compounds are released from dead cell mass (necromass) produced by microbial cell turnover [21]. This "working together" enables halophiles to establish a chemolithotrophic community, which means that they are completely self-sustainable without any additional external input. Wide ranges of metabolic capabilities have been reported for halophiles, including organic fermentation, photosynthesis, sulfur cycling, iron cycling, nitrogen fixation, ammonia utilization, elemental sulfur disproportionation, acetoclastic and H_2-otrophic methanogenesis, syntrophic methanogenesis. The microbe with the highest salinity tolerance isolated up to this date are *Halarsenatibacter silvermanii* strain SLAS-1T from the alkaline hypersaline Searles Lake (CA, United States) (salinity optimum at 35 % NaCl) [32]. It is an anaerobic strain that grows on sulfide as the electron donor and arsenate as the electron acceptor. Within the Archaea, the order of *Halobacteriales*, contain the most extreme halophiles par excellence. These are highly specialized microorganisms, most of which do not grow at salt concentrations below 2.5–3 M [25]. Microbial activity in the environment at even higher salt concentrations has been reported but cultivated single strains are not available yet. For example, genomic evidence from salt-saturated environments showed high archaeal diversity, with the closest association to *Halobacteriales* (76 %) [33]. Based on the metagenomic analyses, it was suspected that high cell-to-cell interaction and amino acid metabolism are essential for supporting life in hypersaline also indicating the adaptability of microorganisms found within extremely saline environments [33]. It is important to note that most of the DNA sequences obtained from the near-saturation hypersaline environment could not be linked to any known culture representatives, indicating the vast potential for novel species and underlying mechanisms that remain unexplored [33].

5. Where do they come from? - Origin of salt cavern microbes

The origin of the halophilic microbes in salt caverns remains speculative, but several contamination sources are plausible:

Leaching: For solution mining of salt caverns fresh- or sea water is used. This water contains, not only organic matter and nutrients, but also microbes which are introduced into the subsurface. The water is usually not pre-treated in any way resulting in a massive input of biomass. Halophilic spores (duration forms) or single cells could be present in the leaching water by chance and find optimal conditions in the salt cavern as salinity increases. However, most marine or freshwater organisms will die when exposed to extreme salinity. Some may be able to survive via spores but will stay inactive. How the microbial community develops over time when a new cavern is leached, needs to be studied in more detail. It is known from fracking operations in the US that fresh water injected into shale becomes highly saline over time, which gives rise to the activity of halophiles (especially *Halanaerobium*) [34,35].

Interventions: Regular interventions of the salt cavern, for example work-overs, sonar surveys, integrity checks or similar, of the salt cavern

will always carry the risk of introducing new microbes into the cavern. Most chemicals and equipment are not sterile and can harbor microbial cells. Whether these microbes can survive the extreme salinity conditions is not clear, but it can be assumed that the risks are low.

Presence in salt rock: Halophilic microbes can already be present in the salt rock itself or the basinal brines [36] or maybe enter via meteoric waters (derived from precipitation). Various studies discovered both dead but also living microbial communities in water/brine inclusions present within different salt rock formations. For example, Vreeland et al., 2000 [37] reported possibly ancient microbial DNA in water inclusions in pure halite rocks. It is now an accepted theory that extreme halophiles can become entombed within brine inclusions of halite during evaporation processes [38,39]. It is reported that brine inclusions can make up to 5 % of the rock salt [40]. When the halite dissolves over time, the halophilic microbes are released back into the brine where they can resume normal growth. An experimental pure-culture study showed that all of the 14 haloarchaeal strains entombed in halite for 6 months were able to grow again when released into the brine [41]. Similarly, Huby et al., 2021 observed no changes in an environmental salt-lake haloarchael community when entombed for 21 weeks in halite crystals [42]. Brine inclusions within halite crystals can be highly abundant, up to 10E10 cm-3 [43] (see also Fig. 2). Within these brine inclusions, microorganisms are either metabolically dormant or can actively recycle organic compounds from dead biomass [44,45] or compounds leaked from other microbial cells, such as glycerol [46,47]. Additionally, some Haloarchaea are known to minimize their cell size to reduce metabolic requirements [48,49]. This means that survival within halite crystals may enable some halophiles to survive over geological time [44,50,51], potentially making halophilic archaea and bacteria the oldest living organisms on Earth. Schreder-Gomes et al., 2022 reported fluid inclusions and microscopically clearly visible cells in 830 million years old halite rock from 1500 m deep halite in Australia [52]. When a salt cavern is leached these entombed halophilic microbes are released into the salt brine, where they find optimal conditions to grow and be active. Thus, making it impossible to avoid microbial contamination of a salt cavern.

Travel through salt: Caporuscio et al., 2013 reported the migration of brine inclusions (explained in the section before) within single salt crystals as function of a thermal gradient [53]. They examined the behavior of individual brine inclusions and found that brine in both types of inclusions (liquid only and liquid/gas inclusions) was mobilized when the salt crystals were subjected to thermal gradients. That means that brine migrates toward the heat source through a network of channels. They reported that the salt dissolution/precipitation phenomena within the inclusion seemingly is controlled by a convection cell mechanism, leading to dissolution at the hot end of the inclusion. This allows the inclusion to migrate towards the heat source with varying velocity. This was dependent on 1) the thermal gradient, 2) the temperature of the hot side, and 3) the size of the inclusion and the chemical nature of the brine. Furthermore, dissolution precipitation creep of halite is a known deformation process which occurs relatively rapidly and can increase salt permeability [54]. This implies that brine inclusions can migrate along grain boundaries, and this could also lead to releasing more microbes into the cavern. If these inclusions contain dormant and living microbial organisms, this could also mean that microbes can travel through the halite into a cavern. Especially considering that salt caverns will have a different and changing temperature (due to gas pressure changes) compared to the surrounding halite rock, a small thermal gradient could result in the release of new, migrating fluid inclusions into the cavern. As the expected thermal gradient will be small, the rate of movement will be slow. Same is true if deformation processes occur during the cavern lifetime. Whether these processes really do lead to a continuous input of new halophilic microbes, should be studied in the future.

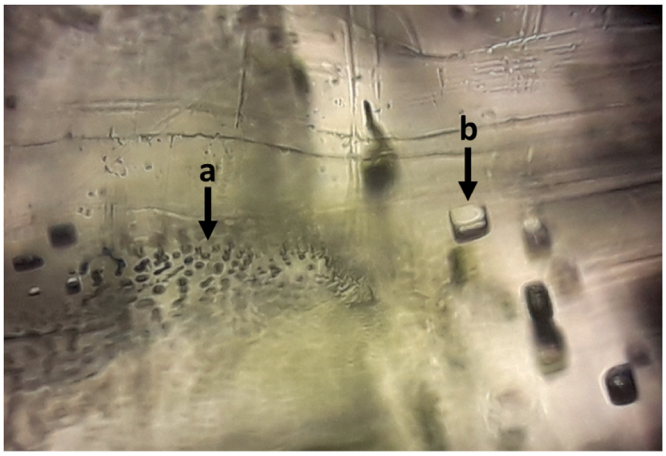

Fig. 2. Microscopy image of a Zechstein halite sample with inclusions visible. Assumed secondary inclusions are marked with (a) primary inclusion marked with (b). Image by Nicole Dopffel.

Overall, considering all the mentioned factors, it will be probably impossible to avoid microbial colonization of a salt cavern. Trying to avoid microbial cell input during leaching by sterilizing the leaching water will also not have the desired effect either. Nevertheless, more research is needed to understand the colonization of caverns in more detail.

6. What is the effect on H_2 storage? - Implications and possible risks

The activity of microbes will have an impact on three main areas of operation: i) economic effects due to H_2 loss and in case of sulphate reduction deterioration in gas quality and microbial-induced corrosion of tubings, ii) health and safety effects due to H_2S formation, and iii) purification measures upon withdrawing the H_2.

As mentioned, H_2 is a ubiquitous electron source for many different microbes [11]. They use H_2 as an electron donor to fuel their anoxic respiration. This H_2 oxidation is very widespread among microorganisms and large amounts of H_2 can be consumed. All known anaerobic (oxygen-free) metabolisms can use H_2 including nitrate reduction, manganese reduction, iron reduction, sulphate reduction, methanogenesis and acetogenesis (Fig. 3). For salt caverns, the most important metabolisms will be sulphate reduction, methanogenesis and acetogenesis since the amount of nitrate, reduced iron and manganese will be very low.

The most concerning process is sulphate (SO_4^{2-}) reduction and the subsequent formation of sulfide (S^{2-}) as a primary product. Sulfide can be released to the gas phase as H_2S or remain bound in the liquid as metal sulfide, depending on the pH of the brine and the metal ion content [55]. H_2S is a very toxic and corrosive gas, which is a common problem in sewers, water treatment plants, biogas plants or oil reservoirs [56–58]. Presence already at ppb levels can be smelled by humans, and ppm levels require specific safety and costly gas purification measures. As salt caverns contain very high concentrations of dissolved sulphate originating from sulfidic minerals like anhydrite, the overall H_2S potential is high. It has been noted that sulphate reduction on H_2 is a proton-consuming process and this will cause the pH of the brine to increase [19]. If the salt brines are low in buffering components and chemicals, this pH increase might lead to a reduced activity, but this effect has yet to be shown in a cavern. A positive side effect of an increasing pH will be that higher fractions of gaseous H_2S will dissolve in the liquid as aqueous S^{2-}.

Acetogenesis is performed by specific Bacteria, which can convert CO_2 and H_2 to acetate and thereby gaining minimal amounts of energy [59]. This still enables them to be metabolically active and grow under conditions that many other microorganisms cannot tolerate. Acetate production will result in a decrease in the pH of the brine, which could

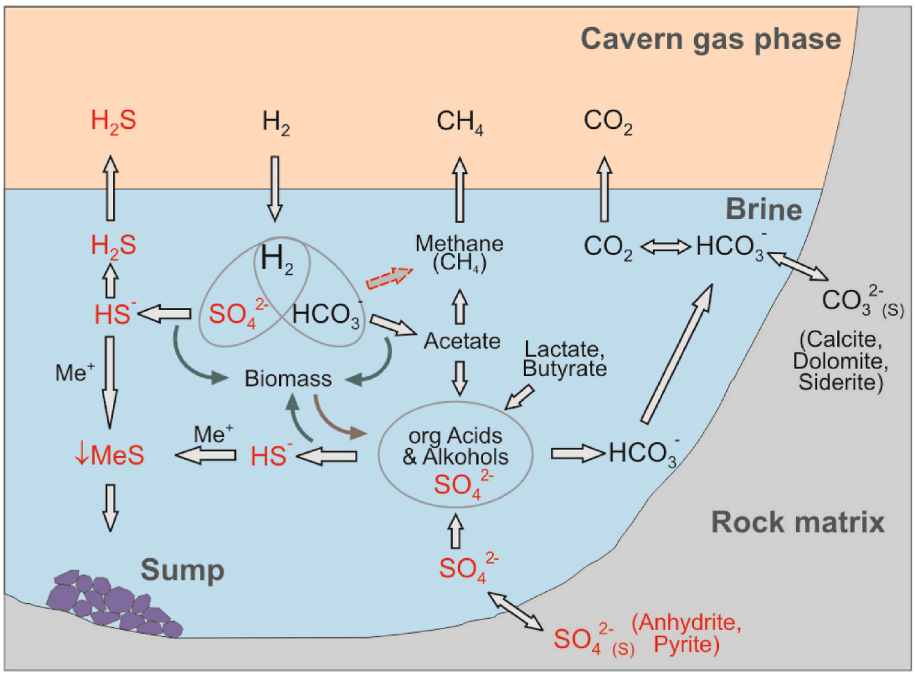

Fig. 3. Graphical illustration of potential metabolic activity in a salt cavern.

counteract the pH increase caused by sulphate reduction. Also, acetate can be used as a carbon and electron source by sulphate reducers, stimulating their activity. Few strains, i.e. *Natrionella acetigena* and *Acetohalobium* sp. can tolerate up to 4.4 M NaCl [60,61]. Acetogenesis at high salinity is not very well studied and many questions remain as to how they can uphold their metabolic energy level.

Hydrogenotrophic methanogenesis (Archaea using H_2 + CO_2) has never been reported for pure cultures at salinities relevant for salt caverns. Two halophilic hydrogenotrophic methanogens are reported, *Methanocalculus halotolerans* and *Methanocalculus natranophilus,* tolerating concentrations up to 2 M NaCl and 3.4 M NaCl, respectively [62,63]. Hydrogenotrophic methanogenesis has lower energetic yield compared to methylotrophic methanogenesis [64]. However, the calculation of energy yield and metabolic activity is based on our current knowledge at standard conditions [64,65]. The available energy (Gibbs Free Energy) at standard conditions per proton, which microbes can use at pH 7 is lower than that at pH 10 [65], so changes in pH might enable other metabolic groups to grow. Furthermore, there has been evidence of syntrophic hydrogenotrophic methanogens showing activity up to 3.75 M Na^+ [66] and detection of unidentified methanogenic sequences at near saturation conditions was possible [65]. The underlying pathways related to hydrogen utilization and methanogenesis, with or without syntrophic partners, would require further investigation under hypersaline environments.

However, it is not sufficient to consider only the primary, H_2-consuming processes when discussing microbial processes in salt caverns. Many other metabolisms can also play a role either by providing (i) carbon, for example CO_2 fixing microbes, providing (ii) other types of nutrients, for example amino acid or osmotic compounds, or (iii) sulfur-cycling microbes producing more sulphate from other sulfur species. This implies that the products of the various metabolisms by the different microbial groups, are themselves potentially utilizable substrates for other microorganisms, leading to intricate self-enforcing or self-limiting feedback processes. Recently, Haloarchaea capable of sulfur reduction have been found at salt-saturated conditions and their activities were stimulated by additional acetate [67]. Another aspect which can stimulate microbial activity are substances introduced during previous phases of cavern operation. These may include gas, oil, diesel as a blanket, or glycol or methanol as hydrate inhibitors or others and these substances can be used as carbon sources and trigger microbial growth/activity.

Overall, the loss of injected H_2 will strongly be dependent on the available electron acceptors, carbon sources as well as nutrients, the volume of the cavern sump and the cell numbers within as well as the types of microbes present. It can be assumed that this will differ between caverns, although the geological location and depth might be the same.

7. What can be found in a solution mined salt cavern? – Current knowledge on salt cavern microbiology

The current knowledge on the microbial diversity in salt cavern is very limited. Only a few studies have investigated salt caverns microbiologically and we need to be careful to draw conclusions from these few caverns and to extrapolate the lab derived results to salt caverns in general.

Bordenave et al., 2018 [68] studied two salt caverns in Alberta, Canada. One used for oil sand storage and one is still brine filled. They observed dominance of Archaea in the oil sand cavern and the brine cavern was dominated by Bacteria (Firmicutes, specifically *Acetohalobium*). Overall, they found DNA related to different metabolic groups including fermenting microbes (*Halanaerobium*), sulphate reducers (*Desulfovermiculus, Desulfovibrio, Desulfohalobium*), acetogenic bacteria (*Acetohalobium*) and methylotropic methanogens (*Methanohalophilus*, *Methanohalobium*). Enrichment of the brine samples with H_2 + CO_2 showed acetate formation at high salinity and acetate + methane production at low salinity.

Schwab et al., 2022 [12] investigated five caverns located in Mid-Germany all used for natural gas storage. Two of them were previously used for town gas storage. All caverns seem to be relatively similar regarding chemistry and physical parameters. Still, DNA analysis of the cavern sump revealed a high diversity in each cavern with most prevalent phyla Proteobacteria, Halobacterota, Bacteroidota, Firmicutes, Actinobacteriota, Halanaerobiaeota and Desulfobacterota. One cavern was dominated by the fermenter *Halanaerobium*, one by the fermenter Balneolaceaea, two caverns were dominated by the archaeal group Halobacteriaceae, one cavern showed the metabolic-diverse family Moraxellaceae but only in one of the duplicate samples. The caverns were notably very high in organic carbon content especially

methanol and ethanol, which were introduced during work over procedures.

In a follow up study, Schwab et al., 2023 [60] reported a halophilic enrichment culture mixed from samples from several salt caverns and saline aquifers. The enrichment culture contained six different members of Halanaerobiales including *Halanaerobium* in addition to *Acetohalobium*, *Methanohalophilus* and *Desulfonatronovibrionaceae*. The enrichment culture was able to consume H_2 under different salinities. Especially acetogenesis and sulphate reduction could be detected. Methane was produced from methanol. They showed that especially acetogenesis can cause a major H_2 loss. Addition of carbon sources (CO_2) did not change the community but the rate of sulphate reduction. Acetate production from acetogenesis stimulated sulphate reduction. Sulphate reducers used the acetate as carbon and maybe electron source leading to H_2S formation without direct H_2 consumption by the sulphate reducers. Sulphate reduction was also increased in presence of methanol. This shows that an already simple enrichment culture has a complex interplay between the members depending on available nutrients affecting each other. A natural salt cavern community could have even more complex interplays.

Bock et al., 1994 [69] enriched oil-degrading aerobic *Bacillus* and *Corynebacterium* from salt caverns used for oil storage in Germany. Other isolation studies from halite very often find *Bacillus* or *Bacillus*-types strains, which are known to produce very durable and long-living spores [70,71]. If they will play a role in a salt cavern is unknown. Because many isolation studies focus on aerobic strains, the presence of active anaerobes which will dominate anoxic salt caverns is not very well studied.

There are many different reports on microbial diversity in salt mines reporting diverse microbial communities very often with high abundance of Archaea (especially Halobacteria). There are indications for chemolithoautotrophic lifestyles and active sulfur cycling in salt mines indicated by the presence of *Thiohalorhabdus*, *Halorhodospira* and *Desulfohalobium* [72].

Overall, certain halophilic microbes seem to occur predominantly in the saline subsurface including *Acetohalobium*, *Halanaerobium* and *Halobacteria*. If they will also be key players when H_2 is injected into the salt caverns is unknown. Overall, the community development over time when in contact with H_2 needs to be further studied, which will also help to understand if certain metabolic groups or certain families will always be the main H_2 consumers.

8. What can we do? - Operational implications

Overall, the amount of theoretically possible H_2S will depend on the amount of sulphate present and the physico-chemical conditions of the brine and the total volume of the sump. It will be advisable that the cavern should contain as little liquid sump as possible, which will depend on several factors including cavern shape and amount of insolubles. With high sump volumes the cavern can contain increased microbial cells numbers and more sulphate. One of the most important factors will be the input of nutrients like carbon, phosphate or nitrogen, which should be carefully avoided during the lifetime of an H_2 cavern. For example, some workover fluids contain methanol [12], which is a very good carbon source for microbes and could trigger growth. Also, the history of a cavern might influence the activity. As mentioned above, if the cavern was previously used for oil or chemical storage (e.g., butanol) then left over carbon could be sufficient to fuel microbial growth.

Monitoring: As mentioned earlier, the highest activity is expected to be at the brine (sump)-gas interphase. Caverns are regularly (usually every two years) inspected for integrity. These routine surveys provide a good opportunity for accompanying microbiological studies of the brine, which is sampled using deep samplers. The direct analysis of brine samples provides a valuable insight into the actual microbiological processes taking place and give indications of ongoing microbial processes. To ensure consistent gas quality, gas samples are constantly tested for impurities such as H_2S during normal operation. The occurrence of H_2S will be in most cases a direct indicator for microbial activity.

As H_2 can also chemically react with minerals, brine and also steel infrastructure it might be difficult to distinguish between chemical and microbial reactions. Stable isotope analysis of the gases H_2, CO_2 and methane as well as of dissolved sulphate were suggested as a suitable method. The elements carbon (C), hydrogen (H), oxygen (O) and sulfur (S), all exhibit different stable isotopes. As microorganisms are isotopically selective, the ratio of the heavy to the light isotopes ($^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) changes during biological processes. Stable isotope analysis of methane in town gas stored in the porous underground reservoir Lobodice revealed methanogenesis as the main process responsible for the decrease in H_2 and CO_2 concentration [73]. Comparable studies for underground hydrogen storage in salt caverns are lacking. Using stable isotope analysis to trace microbial H_2-stimulated processes during underground H_2 storage requires knowledge of the extent of isotope fractionation under in-situ conditions. Thus far, the influence of environmental parameters such as pH value, pressure, salinity on the extent of isotope fractionation has rarely been investigated. A recent study showed that temperature and pH had no influence on carbon isotope fractionation during methanogenesis [74]. In contrast, growth under high pressure significantly lowered carbon isotope fractionation during methanogenesis [75]. Systematic laboratory and field studies are needed to validate stable isotope analysis as a monitoring tool for tracing microbial hydrogen-consuming processes.

Treatment: As the caverns are confined spaces with limited new liquid input it should be possible to treat microbial H_2S production or contamination. Within the oil and gas industry or the water treatment industry the use of biocides is very common and might be applicable for salt caverns. Here, however, the compatibility between the chemistries and the extreme salt conditions must first be assessed. Not only could the high salinity affect the chemicals, but they could also be adsorbed by the insolubles in the sump rendering them inactive. Nitrate, which is often used as an inhibitor of sulphate reduction for oil and gas [76], might in the context of H_2 storage inhibit H_2S formation but could activate H_2-consuming nitrate-reducing organisms. Also, the major excess of H_2 could stimulate both nitrate reduction and sulphate reduction simultaneously. This, however, needs to be tested in more research trials. Changing the pH of the sump might be another option, as very low or very high pH will lead to reduced microbial activity, with most microbes thriving around pH 7. Here, it needs to be considered that there are microbes that can grow at very high (alkaliphile) or very low pH (acidophile) and might consume H_2. To name two known extreme H_2-consumers, *Acidithiobacillus ferridurans* has an optimum pH of 2.1 [77], and *Desulfonatronovibrio hydrogenovorans* an optimum of pH 9.7 [78]. Of course, whether these specialists will be present in the caverns need to be investigated for each site.

Also, drastic pH changes could be buffered by minerals in the sump or release bound H_2S, so the effect will strongly depend on the cavern mineralogy. Another treatment option would be to block of the sump/gas interface to avoid the direct contact between the gas and most of the brine phase. This could be achieved by the addition of, for example a liquid polymer to form a diffusion barrier between the gas and the brine. It has not been tested whether this would indeed stop H_2 diffusion into the brine and if the microbes on the cavern walls will still cause problems. Also, polymers are known to cause immense problems during decommission of salt caverns, therefore the usefulness needs to be carefully evaluated.

9. Conclusions

At very high salinities, microbial activity is often much slower, as microbial cells rely on energy-demanding strategies to uphold cell activity in the presence of high osmotic pressure, low water activity and

low nutrients. Still high salinity environments are known for a diverse microbial world with sometimes surprisingly high cell numbers and immense metabolic potential. As so far, each investigated and published salt cavern contained these halophilic microorganisms, which might cause H_2 loss and even H_2S formation. As the communities seem to be relatively diverse, it is not clear if H_2 will trigger different group members in each cavern or whether certain specialists will strive preferentially. Especially *Acetohalobium*, a H_2/CO_2 acetogen, seems to be present in several studied caverns, which could lead to acetate formation which subsequently also can trigger sulphate reduction. Many questions remain about the overall risk (both the extent of H_2 loss/H_2S formation and also the time scale) of microbes in salt caverns. Monitoring and mitigation options are of highest priority for future research projects. With many new salt cavern projects storing H_2 underway, we expect many new insights into microbial activity in the foreseeable future, which will help to enable safe and resilient H_2 storage in salt caverns.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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