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Paper**



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Microbial risks associated with hydrogen underground storage in Europe

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Abstract

Salt caverns have been used for natural gas and oil storage in the past decades and are now under consideration to store hydrogen, which is needed in large quantities for the Green Shift of industry and society. However, salt caverns are not sterile environments and many specially adapted extremophilic microorganisms (halophiles) can live or even require high-salt conditions for their survival. These organisms will be in direct contact with the injected hydrogen, which is a ubiquitous energy source for many different microorganisms including sulfate reducers, methanogens (methane producers) or acetogens (acetate producers). While these microorganisms consume the hydrogen, they can induce microbial-triggered risks including loss of the stored hydrogen, risks to operational safety and deterioration in quality by H₂S production, biocorrosion and changes of the cavern properties due to biochemical reactions. The microbial communities living within salt caverns have been scarcely studied, but up until now all investigated caverns harbored a diverse set of microorganisms. Whether each cavern community is capable of consuming hydrogen is not clear and also if always similar communities are present. Salt caverns are leached in different geological salt structures, and the mineralogical composition differs significantly even within one salt dome. Such variations will affect the hydrochemical composition of the brine and maybe also the types of microorganisms.

We will present the overall topic of microbial induced risks within salt caverns and the latest findings of our CETP co-funded project HyLife where we investigate different potential storage locations in Europe. This will enable us to gain a better understanding of the overall role of microorganisms and their impact on hydrogen underground storage.

Key words: microbiology, hydrogen storage, H₂S, safety

1. Microbes are abundant in high-salt environments

Saline environments such as salt lakes, soda lakes, saltmarshes, deep sea brine basins, halite-rich sediments or evaporated sea water ponds are extremely common on Earth. Microbial life can be found over the whole range of salt concentrations from freshwater, sea water to high salt environments with salt concentrations up to saturation. High-salinity sites can have surprisingly high diversity, biomass and high cell numbers as high as $10E08$ cells/mL brine and can be inhabited by hundreds of different specialized microbial species, so-called halophiles [1-3]. As long as other factors like pH and temperature are ambient (for pH around 6 to 7 and temperature between 20 and 60 °C), high salinity is in itself not "extreme" for microbes and these environments can be as diverse as low saline ones [1]. Overall, it can be stated that the most important parameters for microbial life in general are temperature (known limits are -20°C to $+122^{\circ}\text{C}$), pH (most diversity at pH 7), salinity and available nutrients like carbon, nitrogen, phosphorous and different trace elements like iron and magnesium.

Living in high saline environments requires unique adaptation strategies, which can be found in both microbial domains Bacteria and Archaea. Normally we classify microbes into slightly-, moderately-, and extremely halophilic [4] (figure 1). Some microbes can only live at high salt environments (halophiles)[4] and in addition, there are halotolerant strains, meaning they do not need high salt, but they can tolerate it. In contrast to temperature where we have a clear threshold of microbial life (current known activity limit is $+122^{\circ}\text{C}$), there is no such limit for salinity and salt environments are highly permissive for microbial life [5]. Even on deliquescent liquids on pure halite in the Atacama Desert, active microbes could be found with astonishing cell densities (up to $10E07$ cells/g rock) [6]. This already indicates that salt caverns will be not sterile environments but will be also prone to microbial colonization.

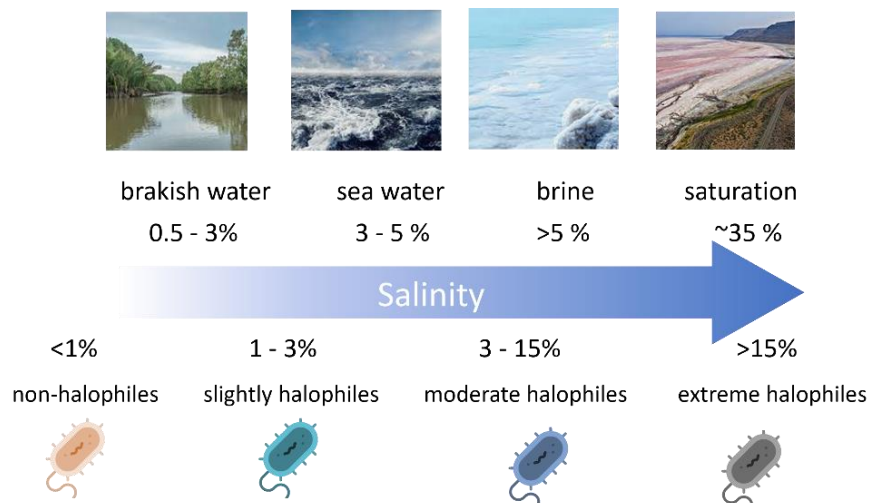


Figure 1: Different salinity environments on our planet ranging from fresh water up to saturated salt lakes. For all salinities there are specially adapted microbes. Image after [4]

The microorganisms which can live at high salt conditions, need unique adaptations to overcome three main stress factors [7]: a) high osmotic pressure, b) low water activity, and c) high chaotropicity, which will be further explained below.

a) The high levels of salt can cause water loss from cells via osmotic pressure, leading to cell collapse (hypertonic) and ultimately death (figure 2). However, cells have two adaptive mechanisms that help them maintain internal cell/turgor pressure: the "salt-in" strategy and the "osmotic-solute/salt-out" strategy. The "salt-in" strategy involves maintaining a high salt concentration inside the cell to create iso-osmotic conditions. The main intracellular cation is potassium, which is actively accumulated. Enzymes and proteins have increased hydrophobicity and are enriched in specific amino acids to enable cell function. These microbial specialists are all moderate or extreme halophiles and require high saline conditions for correct protein folding and functionality, and they cannot survive in low-salt conditions. On the other hand is the "osmotic-solute/salt-out" strategy which involves the exclusion of salt ions from the cytoplasm. By simultaneously synthesizing or accumulating high concentrations of small organic compounds called osmotic solutes, such as glycine betaine, ectoine, glutamine, or different sugars, the cells prevent the unfolding and denaturation of proteins. These organic compatible solutes are osmotically active compounds that provide a stabilizing effect. These microbes can rapidly adjust the concentration of osmotic solutes inside the cell, depending on the outside salinity, thus tolerating changing salt conditions. Moderately halophiles and halotolerant microbes are using the osmotic-solute strategy.

However, the synthesis of these compounds is energetically costly and can limit microbial growth [8, 9].

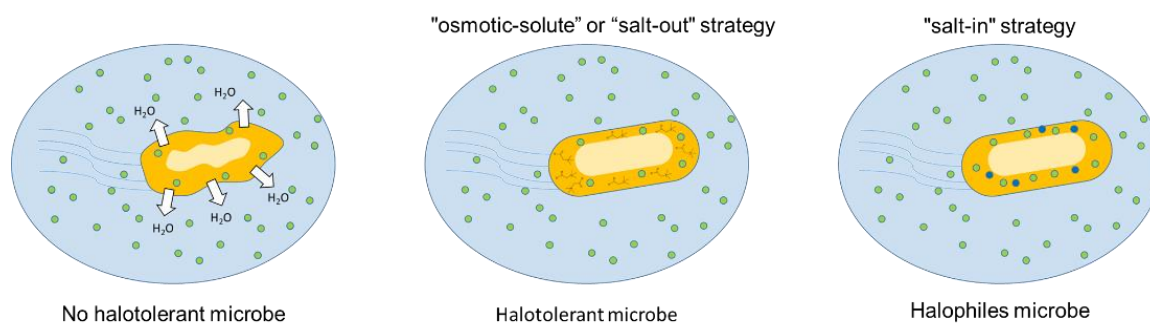


Figure 2: Graphic depiction of salt adapting strategies of microbes. Non halotolerant microbes will suffer water loss out of the cell/cell collapse under high ionic conditions (left bubble). Strategies to uphold cell integrity are "osmotic solutes" accumulating small organic molecules inside the cell (middle bubble) and "salt-in" which means the cells have the same salinity in the cell as the outside solution has (right bubble).

b) Low water activity is normal for high salinity of brines, which is the effective mole fraction of free available water. For instance, saturated NaCl brines have a water activity of 0.75 a_w at ambient conditions, with other ions like calcium or magnesium causing even lower water activity. Halophilic bacteria and archaea can grow in habitats with water activity as low as 0.748 – 0.635 a_w , while fungi can multiply actively at 0.611 a_w . However, certain compounds like glycerol can enable activity even below that value [10]. The water activity of salt caverns has not been reported until now.

c) Chaotropicity, which destabilizes cells, is an important factor limiting life in high salinity environments. Magnesium and calcium are chaotropic cations that cause a loss in cell function. This means that the presence of high concentrations of magnesium, calcium and other ions might be crucial and might be different for each single cavern based on the mineralogical properties [11].

Different microbial adaptations to these different salt stresses make halophilic microbes not only interesting from an academic point of view (for example as proxy for possible exoplanet life and life signatures and the aspect of long-term survival in evaporites [12]) but they are highly relevant for many biotechnological uses such as a source for salt-stable industrial enzymes, source of novel antimicrobial compounds or as biotreatment options [13]. On the other hand, these halophilic microbes can actually cause issues for the energy transition. Here specifically hydrogen underground storage might be impacted by unwanted microbial activity.

2. Hydrogen - a source of energy for microbes

Hydrogen is one of the oldest and one of the most attractive energy sources (electron donor) for many different microorganisms in oxygen-free subsurface environments such as salt caverns. Hydrogen-oxidizing enzymes, called hydrogenases, split the hydrogen into protons and electrons, which provide the energy for many cellular reactions. In principle, microbial hydrogen consumption can be coupled to the reduction of nitrate, ferric iron, sulphate, elemental sulfur or carbon dioxide (CO_2) to produce nitrogen, ferrous iron, hydrogen sulfide (H_2S), acetate or methane (CH_4) [14]. All of these metabolisms consist of diverse redox chemical reactions where one chemical compound is oxidized (an electron donor) with simultaneous reduction of another compound (an electron acceptor) along with the free energy released from the donor. As a general rule, higher energy-yielding reactions (i.e., nitrate-reduction) out-compete the lower ones (i.e., sulfate-reduction), given that the necessary electron acceptors are present, and the amount of hydrogen is limited. However, when the electron donor is available at extremely high abundance, the impact on the microbial community structure and the rate of reactions that might occur simultaneously remain unclear and higher hydrogen consumption might be possible. For salt caverns, sulphate reduction, methanogenesis and acetogenesis (table 1, figure 3) are the most important metabolisms since nitrate, oxidized iron and manganese will be very low. Activity of microorganisms capable of these metabolisms is a concern in three particular areas of hydrogen storage operations: economic effects due to hydrogen loss, health and safety effects due to H_2S formation, and effects related to purification measures upon withdrawing the hydrogen [15].

Table 1: Main hydrogen consuming processes and the expected effects in a cavern. Free Energy ΔG^0 describes the energy microbes gain from a reaction.[14]

Hydrogen-consuming process	Reaction	Free Energy ΔG^0	Will result in the following
Sulphate reduction	$\frac{1}{4} \text{SO}_4^{2-} + \text{H}_2 + \frac{1}{2} \text{H}^+ \rightarrow \frac{1}{4} \text{HS}^- + \text{H}_2\text{O}$	-38.0	<ul style="list-style-type: none"> • H₂S formation • hydrogen loss • pH increase of brine • microbiologically influenced corrosion
Methanogenesis	$\frac{1}{4} \text{HCO}_3^- + \text{H}_2 + \frac{1}{4} \text{H}^+ \rightarrow \frac{1}{4} \text{CH}_4 + \frac{3}{4} \text{H}_2\text{O}$	-33.9	<ul style="list-style-type: none"> • hydrogen loss • CH₄ production • pH increase of brine • microbiologically influenced corrosion
Acetogenesis	$\frac{1}{2} \text{HCO}_3^- + \text{H}_2 + \frac{1}{4} \text{H}^+ \rightarrow \frac{1}{4} \text{CH}_3\text{COO}^- + 2 \text{H}_2\text{O}$	-26.1	<ul style="list-style-type: none"> • hydrogen loss • Acetate production which could trigger growth of other microbes

The process of highest concern is sulfate reduction and the subsequent formation of sulfide (HS⁻/H₂S). Sulfide can be released to the gas phase as H₂S or remain bound in the liquid as metal sulfide, depending on the pH of the brine and the metal ions [16]. H₂S is a very toxic and corrosive gas. As salt caverns often contain high concentrations of dissolved sulphate, the overall potential of H₂S production is high. Sulphate reduction by hydrogen-oxidizing microbes might also lead to an increase of pH of the brine depending on activity and buffering components [17]. Changes in pH can subsequently trigger geochemical reactions within the cavern.

Acetogenesis is performed by specific bacteria, which can convert CO₂/carbonate and hydrogen to acetate [18]. Acetate production will result in a slight decrease in the pH of the brine (depending on the amounts of produced acid). Acetogens live on the energetic edge, and many basic questions on how they can uphold activity and even growth under high salt conditions are still unanswered. The fact is that they are widespread in the underground and also under extreme salt conditions, either active or present in dormant forms (spores), and can consume significant amounts of hydrogen over time [19]. The produced acetate can be further used as a carbon source (or also as an electron donor) for other microbes, including sulfate-reducers, triggering intricate microbial synergies.

The third potential risk group, hydrogenotrophic methanogenesis using hydrogen and CO₂/carbonate, has never been reported for pure cultures at salinities relevant to salt caverns. However, there have been reports of hydrogenotrophic methanogens that tolerate high salt concentrations [8]. The underlying pathways related to hydrogen utilization and methanogenesis would require further investigation under hypersaline environments.

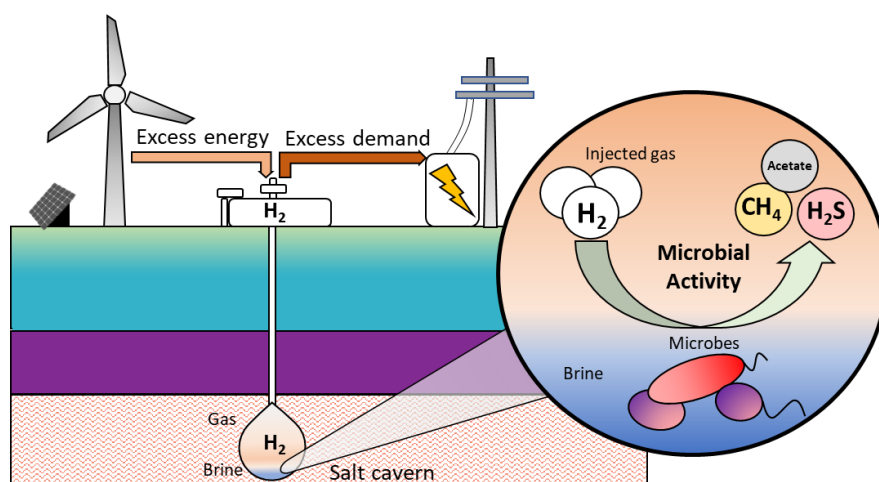


Figure 3: Schematic overview of a hydrogen underground storage concept in salt caverns using excess energy for producing hydrogen and withdrawal when needed. Right bubble: Microbes present in the brine and sump can use the injected hydrogen gas as an energy source and produce different products including H₂S, CH₄ or acetate.

As human activities in the underground expand into hydrogen storage, a significant challenge arises in understanding how the vast number of unknown species beneath the Earth's crust will respond. The impact of subsurface operations and their long-term effects on microorganisms, both on the individual level and as a whole community, is not well understood. Solution mining and operations of salt caverns

will lead to the introduction of microorganisms or activation of native species in the salt [20, 21], similar to reported observations in salt mines [22], fracking operations [23] and oil and gas reservoirs [24].

The microbial communities living within salt caverns have been scarcely studied, but up until now, all investigated caverns harbored a diverse set of microorganisms [25, 26]. Schwab *et al.* 2022 investigated five different salt caverns located in Germany and observed that the community (based on 16S DNA sequencing) was unique in all caverns, although four showed similar families present but in very different relative abundances. Whether all communities are capable of consuming hydrogen is not clear. An important knowledge gap is which halophilic species are present in caverns and do they affect hydrogen storage and, if yes, to which extend. Are there always similar microorganisms present due to the extreme environment, or does every cavern harbor a different community which will react distinctively to hydrogen injection? Salt caverns are leached in different geological salt structures, and the mineralogical composition differs significantly even within one salt dome [27]. Such variations will affect the geochemical composition of the brine, which will subsequently affect the types of microorganisms. Also, the depth of the salt cavern will affect the temperature within, which will directly influence the microbes.

3. HyLife-CETP Project goals

The use of renewable energy systems is becoming increasingly popular, and many of these systems rely on Power-to-Gas technologies to produce hydrogen, which is a universal energy carrier. Hydrogen can be used, transported, or stored for later use in various industrial sectors such as chemical industry, heavy transport, steel production and power generation. To ensure a reliable supply of hydrogen throughout the year, a flexible and large-scale storage facility is essential to balance expected fluctuations of energy production and demand [28]. Underground or subsurface storage has been proposed as a viable solution for mid to long-term storage due to the large available volumes (that could hold up to 920 TWh) and the low OPEX cost (compared to other storage solutions) (figure 4). This would be sufficient for a mid-range scenario of 2,500 TWh of annual hydrogen demand in 2050 with at least 30% storage capacity[28, 29].

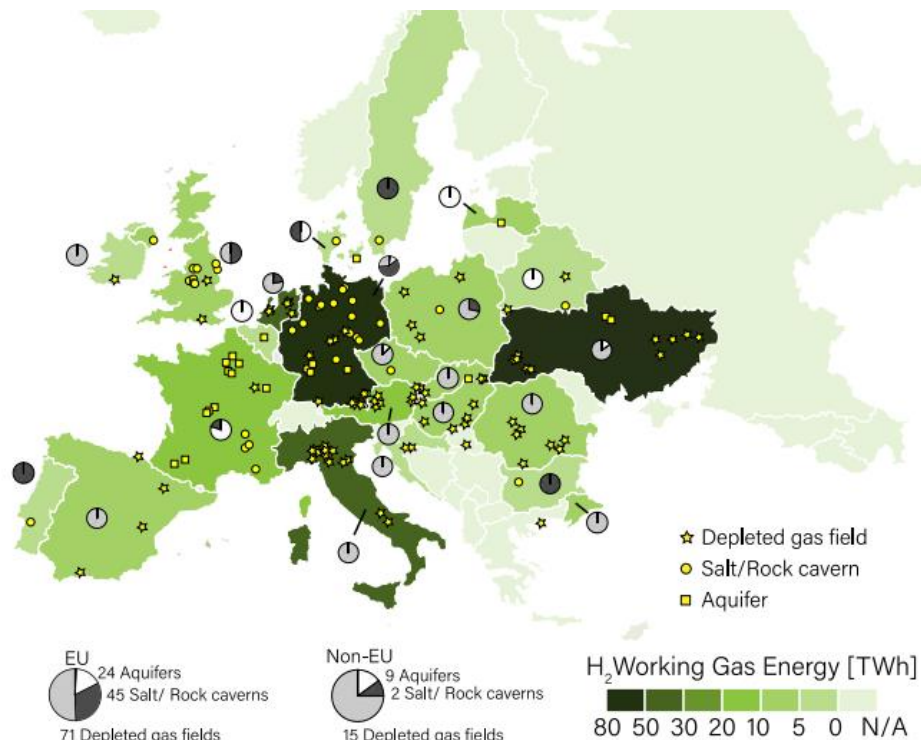


Figure 4: Hydrogen capacity of underground storage facilities in Europe. Pie circles for each country show the relative percentage of each field type. Picture taken from [30]

However, as described in the sections above, it is known that many of these potential underground storage sites harbor diverse microbial communities, and hydrogen is an excellent energy carrier not only for human industry but also for microbial metabolisms. While microorganisms consume the hydrogen, they can induce microbial-triggered risks especially deterioration in gas quality by H₂S production

[21]. As the demand for more hydrogen storage rises, it is, therefore, important to understand the extent of the microbial presence and activity within the different types of sites, especially salt caverns. Until now, most experience in subsurface gas storage is based on natural gas, which significantly differs from hydrogen regarding biological usability. Natural gas cannot be used as electron source by most microbes (although there are exceptions, see [31]). Overall, knowledge of microbial hydrogen-consuming activities in subsurface environments is scarce and seemingly site-dependent. To ensure a safe, efficient, and economically successful underground hydrogen storage, including in salt caverns, the understanding of the microbial impact is crucial. Salt caverns are confined spaces and are proposed to be ideal for short- to mid-term hydrogen storage, with opportunities for rapid injection and withdrawal of gases. Indeed most currently existing caverns are used for natural gas, oil, or chemical storage, all located onshore in different salt structures across Europe, especially in North-Eastern Germany, Netherlands, France, and Spain [32].

In our Clean Energy Transition Partnership (CETP) funded R&D project HyLife, we will provide valuable insights into the types of microbes present and how they will influence the stored hydrogen through extensive sampling across Europe. The project will produce aligned and tested methodologies to maximize comparability, microbiological data on community characteristics, growth, and kinetic data, which will all be accessible through an open database on obtained info within the broad range of storage site conditions. We will also assess related environmental risks, test potential mitigation options, and produce insightful techno-economic business analyses (figure 5). One major goal is to map microbial presence in the different European sites and pinpoint the most important factors for field screening to minimize risks when selecting sites. For the success of this project, we rely on field samples provided by operators, ranging through all relevant geological locations.

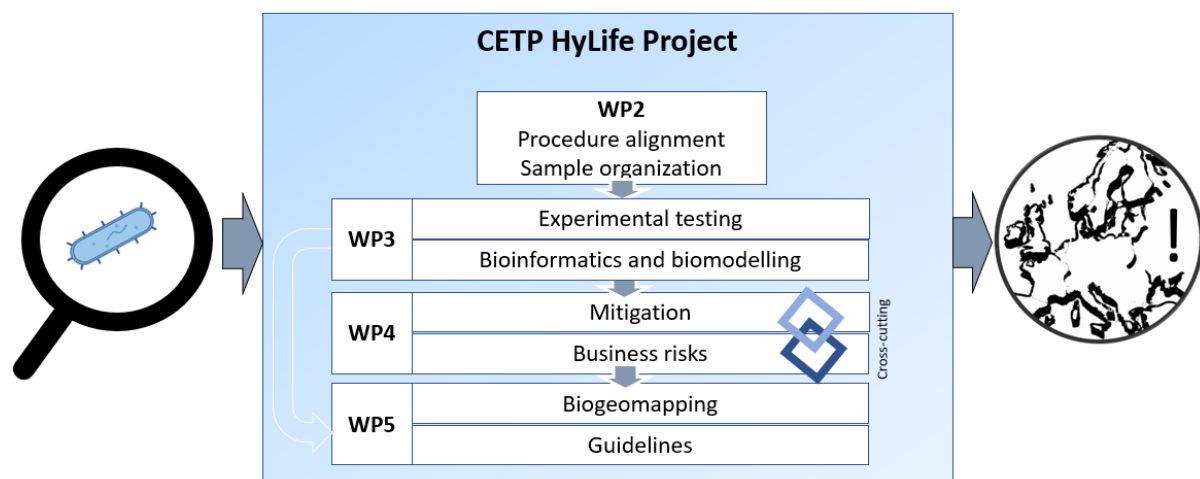


Figure 5: CETP HyLife project structure showing all relevant scientific work packages.

4. Method alignment as key success for establishing standards

The variation in geological and physical conditions at the potential hydrogen storage sites is high and it is therefore essential to investigate as many sites as possible to gain a holistic understanding of the overall impact of microorganisms on the hydrogen storage performance. This task cannot be achieved by one single lab due to the sheer number of samples and the necessary analytics. Our project HyLife is therefore based upon the building of a microbiological network across Europe on this topic. However, all the different approaches and methods used in individual labs located in Germany, France, Norway and Czech Republic had to be aligned at the beginning to assure maximum comparability between the analyzed samples.

A variety of different microbiological and chemical methods need to be applied to field samples provided by different operators in Europe. Anaerobic cultivation techniques enable us to obtain microbial growth rates, gas conversion rates and get an insight into the microbial metabolic potential. Gas- and liquid chemical analytics will reveal all major brine compounds and products of microbial metabolism. Further DNA-based methods need to be applied to obtain information on the microbial community structure both from the original brine and the culture samples to describe overall microbial diversity in brine samples and identify key microbial taxa responsible for the observed changes. Thorough discussions enabled us to align and develop best-practice protocols for sampling (figure 6) of brine for

microbiological and DNA analysis. All work is available online and supported by videos on YouTube (<https://youtu.be/e0utSDKVImk>; <https://www.youtube.com/IPUytd4UGIM>).



Figure 6: Sampling procedure proposed by our project to obtain anoxic samples for microbiological analysis at the wellhead: (A) Wear gloves and sterilize them before you start. (B) Flush the provided glass bottle with the nitrogen. (C) Flush the pipes properly before sampling. (D) Connect the tubing to the tap. (E) Sample the water while continuously flushing the bottle with nitrogen. (F) Fill the bottle to 2/3 volume and continue flushing with nitrogen. (G) Close the bottle and write the label. (H) Pack the bottle into a Styrofoam box.

Secondly, we focused on the setting-up the microbial enrichment cultures and their analytics. The enrichment cultures are crucial as they enable us to assess activity of the most concerning microbial groups which are sulphate reducing bacteria (SRB), methanogens or acetogens (see Table 1). The chosen enrichment design and set of analytical tools is going to be tested in round-robin test across the labs. Ongoing methodical alignment effort ensures that all results will be as comparable as possible and clear, feasible guidelines to assess microbiological risks will be developed for industry and operators for future practice.

5. Characterization of the hydrogen consumption and definition of mitigation strategy

The hydrogen consumption rate is dependent on many environmental conditions such as the number of cells, the microbial diversity, the water (salinity, pH, etc.) and gas (total pressure, partial pressure) composition. These aspects can also change or evolve continuously through the hydrogen storage process conditions. Therefore it is planned in the CETP HyLife project to study the impact of the environmental conditions on the hydrogen consumption for the most relevant sites (considering their properties, the microbial diversity and activity). In order to constrain rates of consumption and to better understand which experimental conditions impact these consumption rates and what microbial community dynamics are involved, a series of experimental tests will be done by modifying experimental conditions like the composition of water, gas and rock, temperature and by adding specific compounds that should stimulate specific communities. As explained in the previous section, the key success of the project is the definition of common methodologies. The produced data will be directly used to define kinetic models for microbial growth and hydrogen consumption and give us the opportunity to test different mitigation options.

1) Mathematical modelling of community structures and growth dynamics

Understanding and predicting microbial growth kinetics in relation to the geological and physico-chemical properties of different hydrogen storage sites requires quantitative approaches, such as kinetic models of microbial community growth and hydrogen utilization. These models aim to relate the structure of the microbial communities in the original brines to measurements of microbial growth kinetics in

enrichment cultures (containing higher cell numbers but less diversity). To this end, the community functions (for example sulfate reduction or acetate production etc.) will be listed using the genetic information of the brine samples with bioinformatic approaches [32]. This allows microorganisms to be classified into functional groups that share the same sources of energy and nutrients ([33] Figure 7A,B). We will then develop chemical reaction models to describe the microbial growth of each functional group that accounts for the enrichment culture data (for example sources of nutrients and energy, pH, temperature, inhibitors; Figure 7C). Model predictions for different salt cavern sites will allow us to analyze the impact of different environmental conditions during hydrogen storage on microbial growth and hydrogen quality, and possibly identify important functions or interactions between groups in all or individual salt caverns. This will help to understand how salt cavern parameters influence the microbial activity during hydrogen storage.

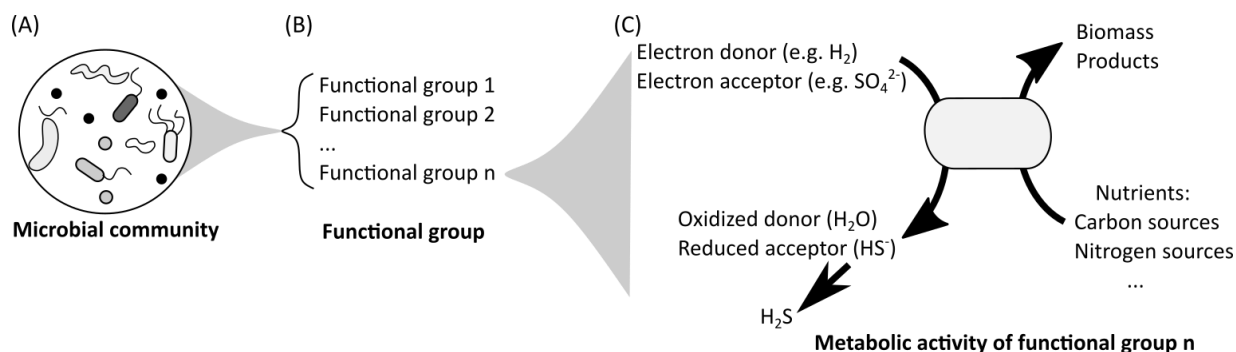


Figure 7: Kinetic modelling of microbial community growth. The microbial community (A) is described in terms of functional groups (B) whose metabolism is divided into three main reactions: catabolism, anabolism, and maintenance (C) (adapted from [33, 34]).

2) Modelling at the reservoir scale

One of the aims of the HyLife project is also to make progress in predicting the potential alteration in the quality of hydrogen gas due to microbial activity during its underground storage. This estimation of the evolution of the gas quality involves taking into account the impact of microbial activity at the storage scale, as well as other processes occurring in the salt cavern, such as gas mixing. Consequently, one of the kinetic models will be incorporated into a larger 3D model. Upscaling the kinetic model from laboratory experiments to a real storage site will enable us to analyze the impact of reactions on the gas, aqueous and mineral phases after several cycles of hydrogen injection and withdrawal in a salt cavern. Thanks to a larger set of hydrogen consumption kinetic parameters that will be acquired during the project, the 3D models will take into account microbial reactivity previously developed using GEM STARS [35], which will be extended and improved. These improvements will include a more accurate account of the effects of microbial activity on the conversion of hydrogen into methane and on the pollution of hydrogen by H_2S .

3) Mitigation options in salt caverns and regulatory aspects:

Storage of hydrogen in salt caverns creates an unprecedented challenge in biofouling control as the addition of a hydrogen into an established halophilic microbial community may have large impacts on community dynamics, hydrogen biotransformation and infrastructure integrity. Mitigating these consequences will require the development of a novel microbial control program that will initially have to adapt available technologies for a unique application. Challenges that will need to be overcome include performance limitations of established microbial control technologies in halophilic environments along with biocide delivery and regulatory constraints of this novel application.

A key objective within the HyLife-CETP project is to evaluate potential mitigation strategies for microbial proliferation in salt caverns that will require the development of a novel microbial control assessment program leveraging cultures isolated from field sites around Europe. Microbial control strategies that are currently registered for application in Europe need to be screened for performance against halophilic, hydrogen-consuming microbial communities cultured from the brine fluids. Performance will be initially evaluated by the impact on growth kinetics and correlated hydrogen consumption rates followed by optimization of successful candidates under real-world conditions. Optimization would include manipulation of dosage rates, synergies between different compounds and application strategies designed to reflect the constraints of application in the field. Concurrently, non-registered microbial control approaches identified from the literature or other industries will be assessed using similar criteria. One

important aspect is that promising technologies will be evaluated for upscaling potential, taking into account manufacturing, toxicological, financial, intellectual property, and, very importantly, regulatory hurdles.

European biocide regulations will profoundly impact mitigation strategies. The Biocidal Products Regulation (BPR) is a scheme that constitutes a comprehensive framework established by the European Union (EU). It imposes strict requirements for authorization, market entry, and use of biocidal products within the region. Under the BPR, biocidal active substances and biocidal products undergo thorough scientific assessments before receiving approval for entry into the EU market. These assessments evaluate the efficacy and safety of active substances, as well as their potential risks to humans, animals, and the environment. A registration and authorization are conducted per specific product type (PT), each with its own criteria for intended use. Active substance or biocidal product dossiers submitted by the manufacturers are reviewed by all the member state representatives. Each member state can make their own decision on authorization of the product within their country. Complexity of the BPR scheme requires attention to the compliance, since there are cases when a biocide is approved for one PT but banned for another, or when authorization is granted by some but not all the member states, resulting in limitations on its use even within the EU. In addition to the BPR operating at the regional level, local regulatory schemes exist, also requiring compliance and registration with specific national standards.

Importers and manufacturers bear the costs and other resources associated with product registration and authorization. Furthermore, regulatory authorities may periodically withdraw approval for existing products or introduce new restrictions, thus impacting market dynamics. Given the complexity of these regulations within the EU and the significant resources required, primarily the biocides already approved for use in the EU would undergo efficacy testing for salt cavern application. Currently, there are no biocides registered for this specific application. Therefore, after selecting the most effective products for such use, it will be necessary to register these products under a certain PT, which is yet to be determined. This process will involve conducting efficacy testing, risk assessments, as well as ensuring all other requirements are met, as defined under the BPR.

6. Outlook

Our HyLife project aims to help the energy industry find the best places to store hydrogen safely and efficiently. We are working with four microbiological labs across Europe to study the effects of microbes on hydrogen storage in different types of storage facilities. As the demand for hydrogen storage increases, it's important to choose the right site to avoid risks to the sites and the business. We are gathering samples from different locations in Europe, and we are thus closely working with industry operators, which will allow us to map microbial presence and/or activity throughout different European storage sites. At the current state we are in contact with 21 storage operators in 8 countries, which will provide us samples over 2023 until 2025. We will continuously look for more operators to join the project to get a good coverage of different storages in different geological locations with different chemo-physical properties. This enables us to get a clear view on microbial presence in salt caverns.

Our research will provide guidelines and help stakeholders make informed decisions about where to store hydrogen. By understanding the biological processes involved, we can develop strategies to prevent and mitigate unexpected failures and ensure safe, long-term storage of hydrogen. This will help accelerate the transition to a more sustainable energy future in Europe.

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