Artificial subsurface lithoautotrophic microbial ecosystems and gas storage in deep subsurface

Anthony Ranchou-Peyruse

FEMS Microbiology Ecology, 2024, **100**, fiae142
DOI: 10.1093/femsec/fiae142
Advance access publication date: 24 October 2024
Minireview

Artificial subsurface lithoautotrophic microbial ecosystems and gas storage in deep subsurface

Anthony Ranchou-Peyruse*

Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau 64000, France
*Corresponding author. Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau 64000, France. E-mail: anthony.ranchou-peyruse@univ-pau.fr
Editor: [Marcus Horn]

Abstract

Over the next few years, it is planned to convert all or part of the underground gas storage (UGS) facilities used for natural gas (salt caverns, depleted hydrocarbon reservoirs, and deep aquifers) into underground dihydrogen (H_2) storage reservoirs. These deep environments host microbial communities, some of which are hydrogenotrophic (sulfate reducers, acetogens, and methanogens). The current state of microbiological knowledge is thus presented for the three types of UGS facilities. In the mid-1990s, the concept of anaerobic subsurface lithoautotrophic microbial ecosystems, or SLiMEs, emerged. It is expected that the large-scale injection of H_2 into subsurface environments will generate new microbial ecosystems called artificial SLiMEs, which could persist over time. These artificial SLiMEs could lead to H_2 loss, an intense methanogenic activity, a degradation of gas quality and a risk to installations through sulfide production. However, recent studies on salt caverns and deep aquifers suggest that hydrogenotrophic microbial activity also leads to alkalinization (up to pH 10), which can constrain hydrogenotrophy. Therefore, studying and understanding these artificial SLiMEs is both a necessity for the development of the H_2 industry and presents an opportunity for ecologists to monitor the evolution of deep environments in real time.

Keywords: artificial SLiME; deep microbial ecosystem; hydrogen; methanogenesis; UGS; UHS; UMR

Introduction

Dihydrogen (H_2), commonly called hydrogen, as an energy resource, is expected to play a central role in the future energy mix. Hydrogen combustion results in the production of only water. Excluding natural hydrogen produced through geochemical reactions (Zgonnik 2020, Boschee 2023, Truche et al. 2024), this molecule ought to be regarded not as an energy source, but rather as an energy vector, akin to electricity. By 2022, most of the 95 Mt of hydrogen produced was destined for use in the petrochemical, fertilizer, and steel industries (IEA 2023). The hydrogen was mainly produced by steam reforming of methane (CH_4) and coal gasification, generating ~830 million tonnes of carbon dioxide (CO_2) per year (IEA 2019). Recent technological breakthroughs, such as those involving electrolyzers or plasmas, hold promise for the production of decarbonized hydrogen derived from renewables, nuclear or even fossil fuels in the future (Tsai and Chen 2009, Sharshir et al. 2023, Alabbadi et al. 2024). Developmental targets for the hydrogen sector are extremely ambitious and are still closely linked to the future of renewable energy sources, which currently account for approximately one-seventh of the world's primary energy sources (Ritchie et al. 2020). Converting surplus electricity to hydrogen could be a viable solution and a cornerstone of climate transition objectives (Edlmann et al. 2023, Ember 2023). In all cases, storage is essential to the operation of an energy network; it ensures the preservation of surpluses for use in response to consumption peaks and changes in financial strategies and supply (i.e. market resilience in the face of crises). In Europe, gas manufacturers are working to convert or complement their existing natural gas transmission network for hydrogen, with the aim of eventually creating what is now referred to as the European Hydrogen Backbone (Guidehouse 2021, Neumann et al. 2023). This network is planned along a north–south axis, linking wind farms in the North Sea and solar farms in southern Europe and even northern Africa to 21 European countries by 2040. In 2019, Europe presented a target: a quarter of its total energy demand would be supplied by hydrogen by 2050 (Fuel Cells and Hydrogen Joint Undertaking 2019, Seck et al. 2022).

Advantages of underground hydrogen storage

The advantages of hydrogen include the fact that it is a gas and that the gas industry has been developing underground geological storage (UGS) facilities since the beginning of the 20th century (Molíková et al. 2022). Underground environments offer large storage capacities with limited surface footprints and excellent safety. In 2021, there were 667 natural gas storage sites worldwide, including 446 in North America and 139 in Europe (Cedigaz 2023). Together, these sites represented a storage capacity of ~424 billion m^3. There are three main types of UGSs: salt caverns, depleted hydrocarbon reservoirs, and deep saline aquifers (Fig. 1). This raises the question of whether UGSs used to store natural gas (essentially methane) can be converted to underground hydrogen storage (UHS) reservoirs. The main difference between methane and hydrogen is that the latter is a smaller, highly mobile and reactive molecule. Hydrogen can react with materials, minerals, certain ions, organic matter and microorganisms. Currently, pure hydrogen storage in salt caverns appears to be the most advanced and

Received 25 March 2024; **revised** 9 July 2024; **accepted** 23 October 2024

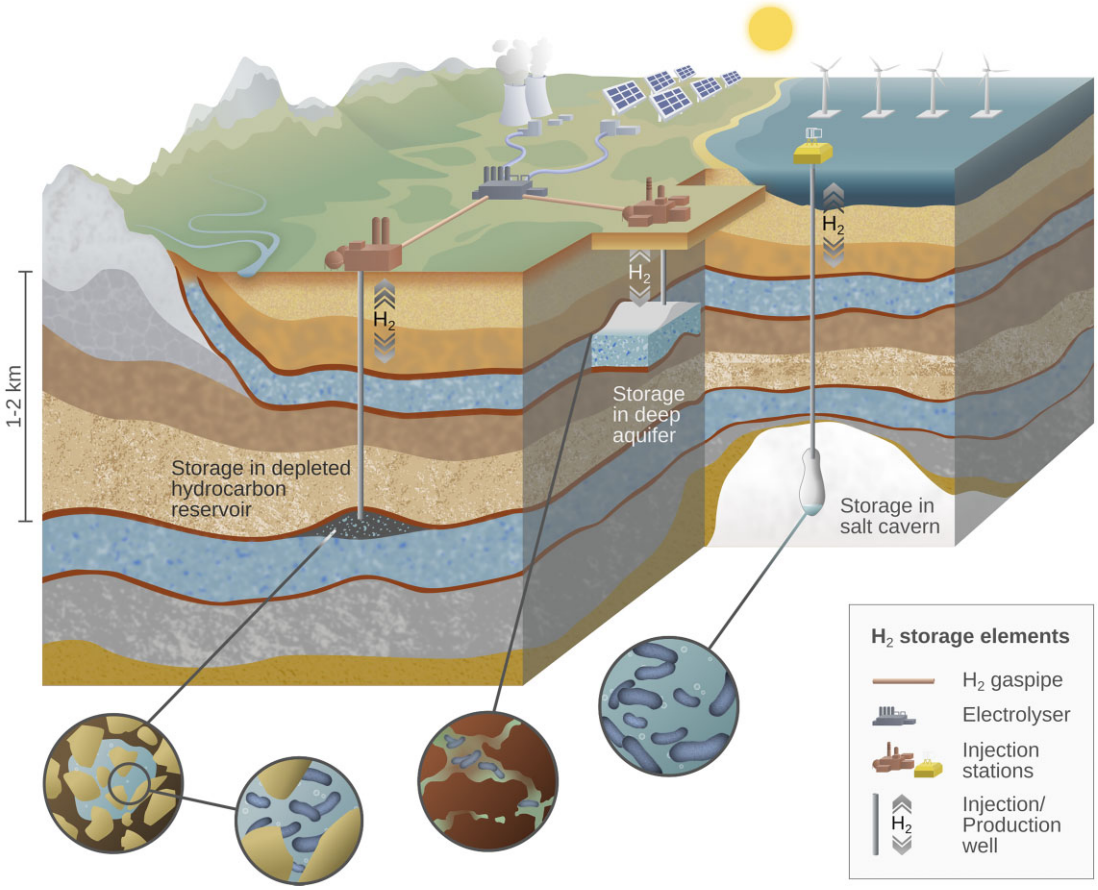

Figure 1. Examples of geological underground gas reservoirs (UGSs) harbouring indigenous or allochthonous microbes. (i) The bottom of salt caverns is bathed in brine in which hyperhalophilic prokaryotes are regularly detected. (ii) Depleted hydrocarbon reservoirs harbour rich microbial communities capable of thriving on ubiquitous organic molecules. (ii) Deep oligotrophic aquifers have been described to host SLiMEs. Illustration by Jagoba Malumbres-Olarte.

reliable technology, with decades of empirical knowledge from the Teesside (UK) and Texas (USA) sites, which have been in operation since 1972 and 1983, respectively. Porous reservoirs, on the other hand, have much larger storage volumes. The depleted Leman reservoir in the North Sea is estimated to have a storage capacity of ∼25 Mt hydrogen, which alone corresponds to >6000 salt caverns (Hassanpouryouzband et al. 2021, Londe 2021). Since 2015, >20 review articles have been published on this subject in various scientific journals, including seventeen in 2021 and 2022 alone (for the most recent, see Amirthan and Perera 2023, Muhammed et al. 2023, Sekar et al. 2023).

What geological storage of town gas taught us

Historically, the first publicly available information on hydrogen storage in porous reservoirs was related to the storage of manufactured gas, commonly known as town gas (Table 1), in a deep French aquifer (Beynes). From 1956 to 1972, the site stored a town gas composed of ∼50%–60% hydrogen (Foh et al. 1979, Taylor et al. 1986). As early as 1960, "*the possibility of bacterial sulphate reduction in aquifers was considered first (…), when it was learned that gas stored in France (….) had become contaminated with hydrogen sulphide. In coke oven gas and reformer gas stored at Beynes (near Versailles), 2–13 p.p.m. of hydrogen sulphide were found, although on one occasion, a concentration of 40 p.p.m. was recorded; the formation of hydrogen sulphide was attributed to the hydrolysis of organic sulphur compounds such as carbon oxysulphide*" (Pankhurst 1967). At the time, the hypothesis of hydrogen consumption by sulfate reducers was proposed because of increasing hydrogen sulfide (H_2S) concentrations, but the idea of hydrogen consumption quickly vanished in favour of geochemical explanations. No microbiological approach was pursued. The methanogenesis pathway, discovered ∼30 years earlier (Stephenson and Stickland 1933), was not considered. In the Czechia (Lobodice) in 1990, Šmigáň and colleagues demonstrated a loss of ∼10%–20% of the volume of gas stored in a reservoir similar to that in Beynes, and there was an increase in the proportion of methane in the gas mixture. Interactions between gases stored in UGS (CH_4 and CO_2) and autochthonous microorganisms have been known and studied for several years (Kleinitz and Böhling 2005, Ivanova et al. 2007, Basso et al. 2009, Gniese et al. 2014). Currently, with plans underway for the large-scale injection of industrial hydrogen into deep subsurface environments for storage purposes, questions about the interactions between hydrogen and the prokaryotic communities present must be addressed (Dopffel et al. 2023a). The aims of this minireview are to summarize the current understanding of the nature and functioning of the microbial communities that will interact with future underground hydrogen storage facilities (UHS), and to discuss them with a view to considering the development of these particular ecosystems in the context of industrial exploitation.

Table 1. UGS facilities worldwide used to store pure or mixed hydrogen.

Project	Operating UHS Site	Country	Commissioning	Depth (m)	Temperature	Objective	Presence of Artificial-SLiMEs	Reference
Underground Sun Storage	Depleted oil and gas	Austria	2016	1000	40°C	UHS (10% H_2)	Yes	Bauer 2017
Diadema	Depleted oil and gas	Argentina	2010	815	55°C	UMR (10% H_2)	Yes	Pérez et al. 2016
Ketzin	Depleted oil and gas	Germany	1960	250–400	34°C	Town gas (62% H_2)	Probable	Stolten and Emonts 2016
Beynes	Saline aquifer	France	1956–1974	430	27°C	Town gas (16%–50% H_2)	Contradictory but probable	Ebrahimiyekta 2017
Lobodice	Saline aquifer	Czech Republic	1965–1990	400–500	43°C	Town gas (50% H_2)	Yes	Šmigáň et al. 1990
Teesside	Salt cavern	UK	1972	380	N/A	UHS	Probable	Tarkowski 2019
Clemens dome	Salt cavern	USA	1983	850–1150	N/A	UHS	Probable	Tarkowski 2019
Moss bluff	Salt cavern	USA	2007	820–1400	N/A	UHS	Probable	Tarkowski 2019
Spindletop	Salt cavern	USA	2017	1500	N/A	UHS	Probable	Tarkowski 2019
Kiel	Salt cavern	Germany	1970s-1990s	1305–1400	N/A	Town gas (65% H_2)	Probable	Stolten and Emonts 2016

N/A, not available. (Adapted from Dopfell et al. 2021 and Sambo et al. 2022).

Hydrogen, a universal energy resource for prokaryotes

Across the three domains of life, Bacteria, Archaea and Eukaryotes, many organisms recover energy by oxidizing hydrogen as electron donor and even produce hydrogen as fermentation product (electron acceptor), e.g. via metalloenzymes called hydrogenases (Adhikari et al. 2016, Greening et al. 2016, Sukhanova et al. 2019). Despite their structural similarities, hydrogenases originated from convergent evolution but are not related (Boyd et al. 2014). Due to their diversity, they can function in a wide range of environments (temperature, pH, and salinity) and species. These enzymes catalyze the reversible $H_2 \leftrightarrow 2H^+ + 2e^-$ oxidation reaction. Some postulate that these capabilities appeared from the origins of life, facilitating the development of microorganisms possessing them when conditions on Earth were reducing prior to the great oxidative shift that took place ~2.4 billion years ago (Boyd et al. 2014). The low reduction potential of hydrogen makes it a highly energetic electron donor that can be used by many different microbial metabolic groups (Greening et al. 2016, Gregory et al. 2019). Microorganisms conserve energy by coupling hydrogen oxidation to the reduction of dioxygen, carbon dioxide, sulfate, fumarate, nitrate, and iron, enabling energy conservation by producing proton motive force, leading to adenosine 5'-triphosphate (ATP) synthesis and providing the energy required for cellular functions (Boyd et al. 2014). Nevertheless, the potentially negative effect of high hydrogen partial pressures on the growth of prokaryotes has been studied since the early 20th century (Larson et al. 1918). The issue has come to the fore again with the optimization of biomethanization processes and the inhibition of fermentation end-products, in particular, hydrogen generation itself (Hawkes et al. 2007, Cazier et al. 2015). In another context, increasing hydrogen partial pressure has already been reported as an inhibitor of homoacetogenesis (Braun and Gottschalk 1981, Morinaga and Kawada 1990, Kantzow and Weuster-Botz 2016). Dissolved hydrogen concentration has also been shown to influence planktonic or biofilm growth of methanogenic archaea, as well as their metabolic activity (Jensen et al. 2019).

Several microbial metabolic groups depend on the presence of hydrogen (hydrogenotrophic methanogens, acetogens, some iron and sulfate reducers, fumarate respiration coupled with hydrogen utilization, knallgas bacteria, etc.) or produce hydrogen (some phototrophs, nitrogen fixers, carbon monoxide oxidizers, and fermenters). These producer and consumer microorganisms can live in syntrophy (Pumphrey et al. 2011, Schroeder et al. 2011, Aüllo et al. 2013, Schwartz et al. 2013). For those interested in more general information on hydrogen-consuming prokaryotes, I recommend a review written by Thaysen et al. (2021). We can assume that many of these metabolic groups were confined to anoxic niches after the appearance of oxygenic photosynthesis on Earth. Notably, in the subsurface, hydrogen, even at low concentrations, can be the main energy source (i.e. electron donor) for autotrophic organisms possessing hydrogenases (Gregory et al. 2019). However, it has also been shown that microorganisms such as Actinobacteria and Acidobacteria can grow in soil solely by consuming hydrogen present in the lower atmosphere under microoxic conditions (Greening and Cook 2014). The physicochemical conditions of the environment, such as pH, as well as carbon sources and intermediates, can impact hydrogen production yields in the case of fermentative organisms (Khanal et al. 2004). These competitive or mutualistic interactions related to an often scarce resource, the concentration of which depends on its reactivity with minerals and organic matter (OM) and on physicochemical conditions, particularly redox conditions, will shape communities in terms of diversity and biomass (Watson et al. 2003, Al-Yaseri et al. 2023a). Thus, at temperatures compatible with microbial life, studies estimating hydrogen consumption efficiencies with carbonate rocks present contradictory results ranging from no reactivity (Hassanpouryouzband et al. 2022, Veshareh et al. 2022) to up to 6.6% loss in the first year of storage (Zeng et al. 2022). More recently, Al-Yaseri et al. (2023b) simulated pure hydrogen storage in carbonate rock for 75 days at a temperature of 75°C and a pressure of 48 bar. Although the study does not indicate the percentage of hydrogen lost, the reaction induced by its presence led to calcite precipitation, which in turn contributed to a significant reduction in porosity. Similarly, sulfide minerals containing Fe(II), such as pyrite, can be reduced by hydrogen. As from 90°C, the reaction of pyrite with hydrogen produces pyrrhotite and sulfide (Truche et al. 2010). The versatility of metabolism related to hydrogen and the complexity of microbial communities mean that their respective contributions to variations in hydrogen concentrations are not known (Adhikari et al. 2016).

Subsurface lithoautotrophic microbial ecosystems (SLiMEs)

At the surface, photosynthetic organisms are responsible for the primary production that forms the basis of various ecosystems. On a geological timescale, some organic matter can be transported to deep depths via the infiltration of meteoric waters and sedimentation processes. During the burial, organic matter will (i) be reduced by microbial consumption, (ii) increase its proportion of more recalcitrant molecules (particularly polyaromatics), (iii) evolve under the effect of increased pressure and temperature, and (iv) become trapped in pores that are difficult for microorganisms to access. Deep biosphere volumes are estimated to be between $7{\cdot}10^8$ km^3 (isotherm of 85°C) and $10.4{\cdot}10^8$ km^3 (isotherm of 122°C) according to recent reports by Magnabosco et al. (2018). Therefore, the deep subsurface could be perceived as an essentially oligotrophic environment, with microbial activity limited by the depletion of resources derived from photosynthesis. However, the idea of the existence of deep microbial ecosystems based essentially on hydrogen has gradually emerged (Gold 1992, Boston et al. 1998).

What is a SLiME?

In 1995, Stevens and McKinley were the first to introduce the term SLiME for an "anaerobic subsurface lithoautotrophic microbial ecosystem" in describing a microbial community driven by geochemical hydrogen in a basaltic system. According to their conclusions, Stevens and McKinley assumed that these SLiMEs, which are dependent on geochemical fluxes, can be maintained indefinitely and that these ecosystems function independently of current or past photosynthesis. Although the existence of SLiMEs was questioned by Anderson et al. (1998), numerous examples of SLiMEs in different geological environments have been published (Fry et al. 1997, Kotelnikova and Pedersen 1997, Chapelle et al. 2002, Haveman and Pedersen 2002, Takai et al. 2003, Lin et al. 2005, Basso et al. 2009, Crespo-Medina et al. 2014) and have sometimes given rise to nuanced names such as HyperSLiME or UltraH3 for Ultramafics-Hydrothermalism-Hydrogenesis-HyperSLiME characterizing hydrothermal sites (Takai et al. 2006). It has been shown that a limitation to the development of these microbial ecosystems may come from the lack of electron acceptors; their production is countered by the strongly reducing conditions prevailing in the deep subsurface. The "infinite" persistence of SLiMEs in the absence of resources derived from photosynthesis has been called into question (Nealson et al. 2005), leading to the "true SLiMEs" criteria: (i) the electron source and (ii) the electron acceptors must be of geochemical origin and independent of photosynthesis; (iii) the organisms containing the appropriate metabolic pathways must be present and identifiable via molecular techniques; and (iv) the reaction products and their kinetics must confirm the suspected presence of SLiMEs. In addition to these "true SLiMEs", there are also deep microbial ecosystems that depend fully or in part on organic matter trapped in the rock that may gradually become accessible to microorganisms as the system evolves (fracturing, geological forces, water flow, etc.). This accessible organic matter can then be made available to fermentative microorganisms, which can produce carbon dioxide and hydrogen as metabolic wastes. This abiotic or biotic hydrogen will subsequently be consumed by acetogenic and methanogenic microorganisms under conditions that are unfavourable to sulfate and iron reducers (limitation in sulfate and absence of iron(III)), which are more often competitive or even inhibitory rather than noncompetitive as they use hydrogen (Cabrol et al. 2017).

SLiME in granitic rocks

SLiMEs have been revealed in a variety of geological configurations, including granitic, basaltic and sedimentary rocks. Granite forms the bulk of the continental crust, underpinning all continents. This type of rock has intrinsically low permeability, and its porosity decreases with depth and pressure. Over time, granite undergoes alteration processes that weaken it. Under the action of geological stresses, the rock breaks up, forming fractures of varying sizes ranging from less than a millimeter to several meters long (Pedersen 1998), increasing the porosity and permeability of the rock. Fractures can communicate with each other via sinuosity, allowing water to circulate freely and facilitating the transport of microorganisms (Motamedi and Pedersen 1998). Water conveys dissolved metals, carbon dioxide and sulfate, which dissolve at water–rock contacts, producing geochemical gradients that impact microbial diversity at granitic depths. Most granitic rocks have low but significant radioactivity, which can lead to the appearance of hydrogen via radiolysis of the water or hydrothermal alteration (Lollar et al. 2014, Truche et al. 2021). In 1998, Pedersen demonstrated the presence of hydrogenotrophic communities, such as homoacetogenic bacteria and autotrophic methanogenic archaea associated with acetotrophic methanogens, in granitic faults. Their presence and interactions are consistent with an active autotrophic biosphere based on hydrogen.

SLiME in basaltic rocks

Basaltic rocks are extremely heterogeneous in terms of porosity and permeability from the moment they are formed, and this heterogeneity increases with their alteration and the appearance of fractures (Helm-Clark et al. 2004). In oceanic settings, mantle peridotite rises to the surface during tectonic activity. In these serpentinization contexts, hydrogen in contact with carbon dioxide and carbon monoxide can sometimes lead to the abiotic formation of organic matter via Fischer-Tropsch-type synthesis reactions (Ménez et al. 2012, Wang et al. 2014, Barbier et al. 2020). The organic matter present in these environments can therefore be of abiotic or biotic origin when the environment has a temperature below 121°C and can be colonized by microorganisms, the latter also being able to consume the hydrogen transported by hydrothermal fluids. SLiMEs are also found in black smokers, which are hydrothermal ecosystems that combine specific macrofauna and thermophilic microorganisms (Nealson et al. 2005). Hydrogenotrophic microorganisms, in particular, methanogenic archaea (Wankel et al. 2011), find thermodynamically favourable conditions for the oxidation of hydrogen produced by the oxidation of rocks in contact with seawater, which explains their systematic identification in hydrothermal environments. However, Bacillota (Firmicutes), Chloroflexi and Pseudomonadota (Proteobacteria) seem to compose a stable community in serpentinized hydrothermal systems (Postec et al. 2015). The existence of HyperSLiMEs comprising hyperthermophilic microorganisms has also been shown in active hydrothermal regions of the Central Indian Ridge (Takai et al. 2004). Predictive biogeochemical models in deep environments tend to show that low hydrogen concentrations can quickly become limiting to the development of hyperthermophiles, explaining why syntrophy is important to allow otherwise non-favourable hydrogen production in these energy-poor environments (Ver Eecke et al. 2012).

SLiME in sedimentary rocks

Finally, sediments are reservoirs of solid and dissolved organic matter that are trapped during sedimentation and subjected to microbial transformation, often into carbon dioxide and methane (Bagnoud et al. 2016; Pedersen 2000). Depending on their origin, sedimentary rocks can be highly porous and permeable (more or less consolidated sands, sandstones, etc.) or they can be highly impermeable (clays), which restricts the mobility of microorganisms, limiting nutrient transfer and reducing microbial activity (Lovley and Chapelle 1995). The development of biofilms in pores and fractures also affects fluid diffusion (Pedersen 2005). In these environments, it is theoretically possible that upwelling hydrogen produced at greater depths by faulting may reach the sedimentary strata. Although often oligotrophic, organic matter has been identified as the main energy source in these sedimentary environments (Krumholz et al. 1997). Fermentative organisms participate in the decomposition of this complex and sometimes recalcitrant organic matter into smaller compounds, such as acetate, coproducing carbon dioxide and hydrogen. These compounds can then be consumed by microbial communities composed of sulfate reducers, acetogens and methanogens (Basso et al. 2009, Adhikari et al. 2016). Although hydrogen may be involved in the functioning of communities in these environments, they do not strictly constitute SLiMEs since hydrogen is derived from the fermentation of organic matter originating from the surface and sequestered over geological time.

Hydrogen storage in salt caverns: hypersaline artificial SLiMEs

Salt caverns are artificially created by leaching underground rock salt deposits via freshwater injection and brine pumping (Bordenave et al. 2013, Dopffel et al. 2024). These cavities are inert, impermeable and nonporous at depths of ~500–2000 m but are very sporadically distributed worldwide. Due to their impermeable properties, they can be used not only for long-term gas storage but also for day-to-day storage (Donadei and Schneider 2016). During operation, a significant amount of brine systematically stagnates at the bottom of the structure, and the gas remains moist. The amount of residual brine can be estimated at ~5%–10% of the volume of the cavity in operation (INERIS 2021). From a microbiological point of view, brine represents an extreme environment, allowing only hyperhalophilic microorganisms to survive, while nonadapted microorganisms disappear and suffer osmotic stress (Fig. 1). Salinity places very strict limits on the diversity of microbial communities present in these cavities and seems to favour archaea, although bacteria can also be found in high-salinity environments. Methanogens (*Methanohalophilus* spp. and *Methanolobus* spp.), sulfate reducers (*Desulfovermiculus* spp. and *Desulfovibrio* spp.), and acetogens (*Acetohalobium* spp.) have been identified, as well as prokaryotes affiliated with the taxonomic groups Halobacteria, Halanaerobiales, and Balneolales (Bordenave et al. 2013, Schwab et al. 2022). Some of these microorganisms may have been introduced into the caverns during cavern construction or operation, but there is also evidence that some of these microorganisms were trapped during salt formation and the precipitation of minerals such as halite (Vreeland et al. 1998; Park et al. 2009, Jaakkola et al. 2016, Dopffel et al. 2024) during water evaporation. During halite leaching, microorganisms trapped in fluid inclusions and able to survive (sporulation, high concentration of glycerol in cytoplasm) are released (Lowenstein et al. 2011).

Under the hypersaline conditions prevailing in working cavities (solubility limit; ~270 $g{\cdot}l^{-1}$ NaCl), microbial activities are shown to slow (Oren 2011, Bordenave et al. 2013, Schwabb et al. 2022). Moreover, the cavity surfaces that can support biofilms, or even those containing sessile organisms, are extremely limited compared to those of a porous reservoir. The majority of microorganisms growing in salt caverns are found in the residual brine at the bottom of this confined environment. The nutrients required for microbial growth can only come from three sources: dissolution of minerals, injection of water from the surface when the cavern was created, and injection of gases during storage operation (with co-injection of hydrocarbons, metals, NOx, SOx, CO_2, etc.). Recycling of necromass elements is also possible. In the context of pure hydrogen storage, it is foreseeable that low concentrations of certain elements, in particular, NOx and iron, slow down the development of the microbial community (Schwab et al. 2022, Dopffel et al. 2023b). We assume that microbial cells can also be found on the damp surface of cavity walls in contact with stored gas (Dopffel et al. 2024) et, or in the gas itself in microaerosols, such as those found in clouds. In the latter two cases, a high hydrogen concentration is likely to have a toxic effect on bacterial and archaeal cells, as has been demonstrated before (Bonchosmolovskaya and Miroshnichenko 1994, Kantzow and Weuster-Botz 2016). In the case of hydrogenotrophic microorganisms present in brine, the low solubility of hydrogen, due to the salting-out phenomenon, limits its accessibility and therefore its consumption theoretically (Hagemann et al. 2015, Sainz-Garcia et al. 2017). In these deep anoxic and highly reduced environments, the expected hydrogenotrophic functional groups are (1) sulfate reducers, (2) methanogens, and (3) acetogens, with reactions such as the following (Veshareh et al. 2022):

$$0.028\,C_2H_3O_2^- + 0.024\,HCO_3^- + 0.016\,NH_4^+ + H_{2(aq)} + 0.236\,SO_4^{2-} + 0.272\,H^+ \rightarrow 0.08\,CH_{1.8}O_{0.5}N_{0.2} + 0.236\,HS^- + 1.03\,H_2O, \quad (1)$$

$$0.264\,HCO_3^- + H_{2(aq)} + 0.006\,NH_4^+ + 0.258\,H^+ \rightarrow 0.03\,CH_{1.8}O_{0.5}N_{0.2} + 0.234\,CH_{4(aq)} + 0.778\,H_2O, \textit{and} \quad (2)$$

$$0.499\,HCO_3^- + H_{2(aq)} + 0.004\,NH_4^+ + 0.255\,H^+ \rightarrow 0.02\,CH_{1.8}O_{0.5}N_{0.2} + 0.239\,C_2H_3O_2^- + 1.01\,H_2O. \quad (3)$$

In 2023, Dopffel and colleagues carried out enrichments using sampled brine (salinity of 27%; pH 7.4). In the presence of a hydrogen-only gas phase, while the potential for hydrogenotrophy was present, it was limited by the low nutrient concentration present in the brine. Microbial activity led to an increase in pH (up to pH 9; i.e. consumption of carbon dioxide and/or bicarbonate, and proton). Subsequently, high pH values can drastically reduce microbial activity. In addition to all the essential nutrients that are difficult to access in this closed ecosystem, these microorganisms require the presence of carbon dioxide in addition to hydrogen for the production of their biomass and sometimes as a terminal electron acceptor. In the case of pure hydrogen storage, it is unlikely that there will be sufficient carbon dioxide dissolved in the brine to enable sufficient microbial activity to significantly interfere with storage activity. Because of equilibrium between the gaseous and liquid phases, successive injections and withdrawals of low-carbon dioxide gas are expected to greatly reduce the

quantities of CO_2 available for microbial life; phenomenon amplified by carbonate precipitation induced by pH increase. Nevertheless, in the case of salt caverns formerly used for natural gas storage, it is likely that hydrocarbon storage over several decades resulted in the introduction of hydrocarbons (Schwab et al. 2022) that can serve as carbon sources for heterotrophic organisms and lead to low carbon dioxide emissions during oxidation. Thus, we can conclude that salt caverns used for industrial storage of pure hydrogen will contain hypersaline artificial SLiMEs with low microbial diversity and activity that is sufficiently constrained by the conditions of the environment, probably increased pH, eliminating major risks for the perennial storage of hydrogen. From a fundamental point of view, these ecosystems are unique examples, and their study will enable microbiologists and exobiologists to refine their knowledge and concepts of the limitations of life in the subsurface.

Depleted hydrocarbon reservoirs: artificial SLiMEs saturated with organic compounds

The microbiological study of hydrocarbon reservoirs began with the first discoveries of Edson S. Bastin in 1926, when he identified the first sulfate reducers in these environments (Bastin 1926, Bastin et al. 1926). In the 1950s, evidence of methanogenesis in these reservoirs represented the first work to demonstrate the ubiquity of these metabolic pathways in these ecosystems (Kuznetsov 1950, Ekzertsev and Kuznetsov 1954). The biogenic nature of methane in many reservoirs was soon demonstrated in Canada, Germany, Italy, Japan, Russia, and the USA (Rice and Claypool 1981). Although methanogenesis is not exclusively hydrogenotrophic, hydrogen seems to play an important role. The case of the Olla Oil Field (Louisiana, USA) is very interesting. Between 1983 and 1986, $2.2{\cdot}10^8$ m^3 of carbon dioxide was injected as part of an enhanced oil recovery (CO_2-EOR) procedure, and approximately one-third of this volume remained underground at the end of operations (Tyne et al. 2021). Thirty-five years later, it was found that 13%–19% of the residual carbon dioxide had been consumed by methanogens and had been transformed into methane. The origins of the hydrogen formed were not rigorously demonstrated, but its consumption and hence presence are not in doubt. Some hydrocarbons can be degraded via syntrophy between fermentative bacteria and methanogenic archaea (Schink 1997, Meckenstock 1999, Vogt et al. 2011). The very nature of hydrocarbon reservoirs, which are capable of holding oil and gas for millions of years, made them an early choice as UGSs for natural gas. In 2022, storage in depleted reservoirs accounted for ∼81% of the world's natural gas in storage (Cedigaz 2023). The theoretical possibility of sequestering up to 900 Gigatons of CO_2 (Bourg et al. 2015) has led to an abundance of literature on the subject, with numerous reviews on the sequestration of this molecule (Ajayi et al. 2019, Davoodi et al. 2023). More recently, the growing interest in the production and mass storage of hydrogen has led to an assessment of the effect of mass injection of this molecule into depleted gas reservoirs. Unlike salt caverns, which are home to simplified, less active microbial communities, porous reservoirs (depleted reservoirs and aquifers) are home to numerous microorganisms capable of consuming hydrogen (Fig. 1). Based on this observation, two questions have rapidly emerged: (i) Is it possible to maintain perennial hydrogen storage and thus create a vast UHS? (ii) Can we imagine coinjecting hydrogen with carbon dioxide to upgrade the latter into methane and create underground biomethanation reactors (UMRs)?

The Schneeren hydrocarbon-depleted reservoir has been described as hosting a microbial community dominated by hydrogenotrophic methanogens (Ehinger et al. 2009). In 2023, Dohrmann and Krüger evaluated the microbial consumption of hydrogen in high-pressure reactors (100 bar) when using such a site as a UHS (Dohrmann and Kruüger 2023). Hydrogen consumption was shown to be rapid (i.e. around twenty days). Initially, the community was dominated by fermenters belonging to the families of *Spirochaetaceae* and *Clostridiaceae*. In formation water rich in dissolved organic material (i.e. hydrocarbons) and possible necromass (depressurization during sample ascent [2700–3500 m depth], sample storage, community adaptation, etc.), it is logical to observe this dominance at the start of the experiment. The share of these fermentative organisms subsequently dropped during the first hydrogen injection, to the benefit of sulfate reducers belonging to the genera of *Desulfotignum* and *Desulfovibrio*. The microbial community composition shifted to higher relative abundance of sulfate reducers and acetogens such as representatives of the genus *Acetobacterium*, and hydrogen consumption was strongly dependent on the presence of sulfate. No hydrogenotrophic methanogenesis was demonstrated during this study. Conversely, in the study of the Underground Sun Storage project, reactor tests clearly showed a significant increase in the relative abundance of methanogenic archaea in hydrogen-supplemented enrichments (Bauer 2017). Similarly, an increase in pH was also noted; an increase that can most likely linked to lithoautotrophic growth (*i.e.* consumption of carbon dioxide and H^+). Recent work by Khajooie and colleagues (2024) et al. (2024) has questioned calculations of methanogenesis yields only based on laboratory enrichments of bulk solution, as the surface area offered by rock porosity for biofilm development, particularly for hydrogenotrophic methanogens, would have a stimulating effect on their activity.

Thaysen and colleagues carried out a major survey of 75 depleted hydrocarbon reservoirs (Thaysen et al. 2023). Using criteria based mainly on reservoir temperature and salinity, they determined four risk categories based on chemical and biochemical reactions: no risk (above 122°C), low risk (above 90°C), medium risk (above 55°C and salinity above 1.7 M NaCl), and high risk (below 55°C). These risks are also associated with sulfate concentrations and hydrogenotrophic sulfate reducers, which consume hydrogen and produce sulfides that can lead to porosity clogging by iron sulfide precipitation, corrosion, lower stored gas quality and additional gas treatment costs. The authors then plotted different hydrogenotrophic community compositions as a function of different environmental factors (Fig. 2). As expected, in the absence of sulfate, the two main functional groups are methanogens and acetogens, and the dominance of one over the other is essentially linked to temperature. Temperatures above 30°C would be more favourable to methanogens than to acetogens based on cultivated strains information (Thaysen et al. 2021). In the presence of sulfate, sulfate reducers would be favoured by a pH > 7 and by environments with high sulfate concentrations, whereas low-pH environments would tend to place them at a disadvantage compared to the other two functional groups. The results of this work showed that nine of the UGSs studied could be considered to have no microbiological risk (i.e. sterile because > 122°C), 35 had low risk, 22 had medium risk and nine had high risk (Thaysen et al. 2023). Beyond the risks of such UHS reservoirs, this also means that in the context of such storage, ∼90% of these sites studied represented new artificial SLiMEs. Risk was strongly correlated with the production of sulfide by sulfate reducers and essentially with the impact of this sulfide on infrastructure and storage

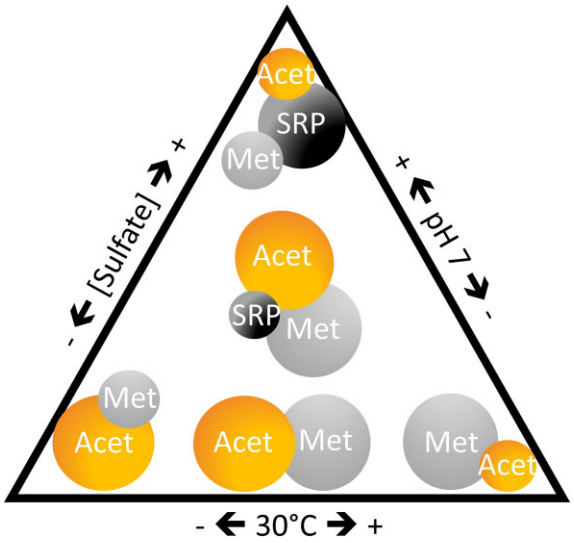

Figure 2. Schematic representation of the structuring of hydrogenotrophic communities based on the three functional groups assumed to predominate in deep environments: sulfate-reducing prokaryotes (SRPs), methanogenic archaea (Met), and acetogenic bacteria (Acet) (adapted from Thaysen et al. 2023).

activity (*i.e.* corrosion, pyrite precipitation, and souring). However, in the context of UHS, it is essential to maintain a certain quality in the stored gas. Thus, methanogenesis also represents a problem to be accounted for, as it modifies the quality of the stored gas, transforming hydrogen and acetate (from acetogenesis) into methane and thereby lowering gas pressure during storage (Shojaee et al. 2024). Thus, simply storing carbon dioxide (without hydrogen) in such reservoirs would make it possible for a signifiant proportion of the sequestered carbon dioxide to be converted into methane, albeit with rather slow kinetics (Tyne et al. 2021, Wang et al. 2024). Nonetheless, the large-scale introduction of hydrogen in such ecosystems would undoubtedly accelerate the microbial processes at work. This is why in the main studies on hydrogen storage in depleted hydrocarbon reservoirs, coinjection or the presence of carbon dioxide is proposed to produce underground methane reactors or UMR (Pérez et al. 2016, Dupraz et al. 2018, Strobel et al. 2020, Veshareh et al. 2022) and produce important artificial SLiMEs.

More recently, a study combining an on-site gas injection and production test (Lehen depleted gas reservoir; Austria) and mesocosm tests demonstrated methanogenic activity, with a particularly high representation of members of the families *Methanothermobacteiaceae* and *Methanobacteriaceae* (61% of the active community) (Hellerschmied et al. 2024). The injected gas contained 9.89% (v/v) H_2 and 0.19% (v/v) CO_2. The same taxonomic diversity analysis indicated a low representation of acetoclastic methanogens (genera of *Methanosaeta* and *Methanosarcina*). Sulfate reducers were poorly represented (<2%) and tended to decrease in the field trial. Putative acetate oxidizers, fermenters, and acetogens were identified. Experiments in mesocosms showed higher methanogenesis production (0.26 mmol·l^{-1}·h^{-1}) than on site. Hellerschmied and her collaborators explained these differences by the heterogeneity of the reservoir (porosity, permeability, water saturation, etc.), as well as differences in the H_2/CO_2 ratio on site and in mesocosms with 52/1 and 4/1 (optimal ratio), respectively. Understanding the limitations of methanogenesis in this reservoir (40 000 m^3) and overcoming them would theoretically enable the production of 114 648 m^3 of methane per year through four storage cycles.

Surprisingly, a study conducted in two Italian hydrocarbon reservoirs showed no significant hydrogenotrophic activity (Vasile et al. 2024). However, these two reservoirs presented a high risk according to the Thaysen classification criteria cited above. They had temperatures between 45 and 48°C, a salinity of ∼50 g·l^{-1} NaCl, and a pH slightly lower than 6. The sulfate concentration was 126 mg·l^{-1} in the former and <50 mg·l^{-1} in the latter. No evidence was revealed of major hydrogen consumption after four months of incubation with a mixture of either 10% H_2 and 90% CH_4 or 99% H_2 and 1% CO_2. The authors also found no evidence of significant sulfate-reduction activity (*i.e.* no sulfide detection) or methanogenesis (i.e. no methane production). The low consumption of hydrogen by microbes was attributed to the acidic pH and a lack of carbon dioxide under the conditions of the first incubation (H_2 10%; CH_4 90%). Apart from this example, it seems likely that the large-scale storage of hydrogen in numerous depleted reservoirs with temperatures compatible with microbial life will lead to the creation of artificial SLiMEs in which a large proportion of hydrogen is converted into methane. Depleted hydrocarbon reservoirs represent a total volume of 89 billion m^3 in Europe and 339 billion m^3 worldwide (Pappenreiter et al. 2019, Hellerschmied et al. 2024). These reservoirs would then become UMRs of sufficiently large volumes to decrease or dispense with the extraction of fossil natural gas (Bellini et al. 2022, Molíková et al. 2022, Vítězová et al. 2023) if the reaction kinetics are compatible with human activity. They would also pave the way for a competitive carbon dioxide capture and injection industry to fuel the process.

Deep aquifers: oligotrophic artificial-SLiMEs

In the first 2 km underground, aquifers represent a cumulative water volume of ∼22.6 million km^3 (Gleeson et al. 2016). Under certain conditions (porosity, cap rock properties, geometry, etc.), deep aquifer anticlines can be converted into UGSs, with gas taking the place of formation water within the pores and permeable spaces of the rock (Fig. 1). Deep aquifers are oligotrophic porous systems. Little organic matter is available for the growth of the microbial communities that reside there, which have low abundances but high diversity. When organic matter is available, it is often limited in quantity, as it is trapped in rocks during rock formation and often consists of recalcitrant compounds. Several examples of SLiMEs have been described in deep aquifers (Stevens and McKinley 1995, Basso et al. 2009, Silver et al. 2010).

Between 1950 and 1990, large volumes of town gas were stored in European deep aquifers (France, Germany, and Czechia). At that time, the microbiology of deep environments was an emerging discipline. In the case of the Lobodice storage site (Czechia), two studies carried out in the early 1990s during the migration from town gas to natural gas storage showed strong methanogenic activity, during which approximately one-third of the hydrogen was converted into methane in just seven months (Šmigáň et al. 1990). In these studies, only methanogenic archaea were considered. In 2017, multiple samplings were carried out on French deep aquifers used to store natural gas (Ranchou-Peyruse et al. 2019). The aim of this study was to compare the phylogenetic diversity of sulfate reducers and methanogens present at 34 sites in seven different UGSs. In simplified terms, 15 sites were located in the Aquitaine sedimentary basin (southwestern France) with low salinity (conductivities between 296 and 560 µS·cm^{-1}) and sulfate concentrations (between 5 and 20.7 mg·l^{-1}, only one site had a concentration of 106 mg·l^{-1}). The other 19 sites were in the Paris sedimentary basin (around Paris), with relatively high salinity (266–5740 µS·cm^{-1}) and sulfate concentrations (34–1679 mg·l^{-1}),

with two sites showing lower concentrations of ~0.3 mg·l^{-1}. Phylogenetic studies focused on sulfate reducers and methanogens. The approaches were based on the targeting of markers for these two functional groups: the desulphite reductase gene, *dsrB*, which is characteristic of sulfate reducers, and the *mcrA* gene, which encodes the F420 cofactor of methanogens. At most sites, sulfate reducers dominate methanogenic archaea, often to a very large extent, even in waters with low sulfate levels, as in the Aquitaine basin. However, a recent study showed that even with a low abundance of methanogens, these sites had significant potential for methanogenesis (Ranchou-Peyruse et al. 2024). Surprisingly, there was only one site largely dominated by methanogens, which turned out to be the one that had hosted a town gas storage facility >50 years earlier (i.e. Beynes). Moreover, the sulfate concentration in the formation water at this site is abnormally low (0.4 mg·l^{-1}). These results are surprising. Deep porous systems are complex and these results seem to indicate that a signifiant proportion of hydrogen remained trapped in storage for >50 years after hydrogen storage was stopped and the reservoir was converted into natural gas storage, allowing the artificial SLiME to be maintained. Recently, microfluidic approaches simulating natural rock porosity (≈30%–60%) and permeability have revealed residual hydrogen trapping phenomena (Lysyy et al. 2022). Significant hydrogen trapping has been confirmed by core-flood experiments on a heterogeneous sandstone core (Boon and Hajibeygi 2022). It has also been hypothesized that this residual hydrogen could accelerate microbial activity and contribute to sulfate depletion during these slow recharges through the movement of the formation water. It also seems highly likely that the fact that these deep aquifers are confined ecosystems, because of the low formation water velocity (few m·year^{-1} (Labat 1998, Ranchou-Peyruse et al. 2019)), contributes to the stability of physicochemical conditions driving these microbial ecosystems (temperature, pH, redox, ion concentrations, etc.). We speculate that the town gas storage at Beynes enabled the emergence of one of the first artificial SLiMEs, initially dominated by sulfate reducers (a sulfide peak of 40 ppm noted in the late 1950s), and that when sulfate began to become limiting, methanogens began to dominate the microbial community. When these observations were reported, the pressure drop during storage was attributed to hydrogen leakage from the cover layer, but today, it could well be reinterpreted as the conversion of a signifiant volume of carbon dioxide and hydrogen into methane. However, although microbial activity seems evident, no mention of microbial activity was made during the operation of the town gas storage reservoir, jeopardizing gas activity.

In 2022, Haddad and colleagues published a research paper on a high-pressure reactor simulation of natural gas storage in which 10% H_2 was injected (Haddad et al. 2022). Thirty-two years after the end of town gas storage at Lobodice, this experimental study was the first examining hydrogen storage in deep aquifers by simulating *in situ* conditions as close as possible. The site studied was located in the Paris Basin at a depth of 989 m. The rock, formation water, and indigenous microorganisms sampled using a bottom sampler (Ranchou-Peyruse et al. 2023) were incubated under conditions simulating those *in situ* (47°C, 95 bar, anoxia). As soon as hydrogen was injected into the system, the microbial community evolved, favouring hydrogenotrophic microorganisms. In the first phase, sulfate was consumed, and formate production was observed. No sulfide was detected in the gas phase. This was explained by the precipitation of framboidal pyrite in the rock, by the presence of pyrite precipitates in the reactor and by the alkalinization of the medium, which prevented sulfide from being present in its H_2S form (Haddad et al. 2022, Mura et al. 2024). However, the disappearance of sulfate and the presence of bacteria phylogenetically affiliated with the families of *Ammonificaceae*, *Desulfotomaculaceae*, *Desulfovibrionaceae*, *Desulfurisporaceae*, and *Thermodesulfovibrionaceae* left no doubt of the sulfate reduction activity that led to the disappearance of sulfate after 65 days of incubation.

The presence of formate is very interesting. All microbiological reviews of UHSs cite acetogens as functional groups that are favoured by a massive influx of hydrogen. However, experimental studies simulating UHS in deep aquifers have not revealed a high acetate production associated with the presence of hydrogen and carbon dioxide. On the other hand, formate has been systematically observed. In 2017, Berta mentioned the appearance of formate in experiments carried out at low pressure (5 bar) with a gas phase composed of H_2/CO_2 (99/1) (Berta 2017). While an increase in total pressure does not a priori appear to influence formate production during acetogenesis (Oswald et al. 2018), increases in hydrogen (pH_2) or CO_2 partial pressures (pCO_2) seem to favour formate production at the expense of acetate, even at low partial pressures (i.e. a few bar). As far as pH_2 is concerned, the higher hydrogen concentration has been assumed to be responsible for the inhibition of enzymes involved in the Wood–Ljungdahl pathway (Braun and Gottschalk 1981, Morinaga and Kawada 1990, Peters et al. 1999, Kantzow and Weuster-Botz 2016, Stoll et al. 2018). Whereas in the case of higher pCO_2, the rise in CO_2 concentration would lead to a decrease in cytoplasmic pH (Oswald et al. 2018, Tarraran et al. 2023). This acidification would reduce membrane potential and thus ATPase activity. This drop in energy yield would lead to the impossibility of finalizing the Wood–Ljungdahl pathway and the accumulation of formate. In experimental simulations of hydrogen injection in three different UGSs in deep aquifers, the formate production was systematic (Haddad et al. 2022, Mura et al. 2024). Once sulfate has been depleted, methanogenic archaea (the family of *Methanothermobacteriaceae*) outcompete sulfate reducers, which then are able to survive due to the dissolution of sulfate minerals (barite) or fermentation. In the context of a massive hydrogen influx, sulfate reduction and methanogenesis both contribute to the alkalinization of the formation water through the consumption of H^+ and CO_2 (Eq. (1) and (2); Berta et al. 2018, Mura et al. 2024). We can therefore design a composite picture of the taxonomic diversity in artificial SLiMEs that might evolve in future UHS in aquifers. This is a community in which a significant proportion of the microorganisms are fermentative organisms involved in recycling necromass. In the presence of sulfate, sulfate reducers dominate the community, cohabiting with acetogens and a minority of methanogens. Under sulfate-limiting conditions, methanogens dominate the community without limiting homoacetogenic activity as long as carbon dioxide is not limiting (Haddad et al. 2022). These sulfate limitations are expected to favour sporulating sulfate reducers of the family of *Peptococcaceae*, particularly those belonging to the genera of *Desulforudis* and *Desulfotomaculum* or the LA-dsrAB taxonomic group (Aüllo et al. 2013, Müller et al. 2015, Berlendis et al. 2016, Karnachuk et al. 2019). Due to the presence of large quantities of hydrogen, it is possible that the taxonomic diversity of microbial communities will be durably impacted in the artificial SLiME. Indeed, microbial lithoautotrophic activities lead to alkalinization of the artificial SLiME, with pH values >9 or even 10 (Mura et al. 2024, Ranchou-Peyruse et al. 2024), resulting in even more harsh environmental conditions for the microbial community. Unlike natural SLiMEs, in which hydrogen of geochemical or biological origin is most often the limiting resource, in the case of artificial SLiMEs, carbon dioxide could become a limiting factor. For example, it has

been suggested that carbon dioxide depletion could lead to the dissolution of carbonate minerals in rocks, such as calcite (Haddad et al. 2022). Subsequently, alkalinization is expected to lead to the reprecipitation of the remaining CO_2 into calcite, resulting in competition for this resource between biotic (i.e. lithoautotrophy) and abiotic (i.e. mineralization) phenomena. Lithoautotrophs benefit from proximity with fermenting microorganisms that feed them with carbon dioxide and hydrogen from microbial necromass.

Conclusion

SLiMEs have been referenced since the mid-1990s, but these ecosystems are likely limited by hydrogen availability from geochemical processes. The creation of UHS enables microbial communities in deep environments to access virtually infinite quantities of hydrogen (i.e. millions of Nm^3). In these artificial SLiMEs, it is clear that this massive injection of an energy source will lead to profound changes in native microbial diversity. One constraint on microbial activity in deep environments will be removed. However, it is likely that near the UHS, hydrogen concentrations will reach an inhibitory threshold for microorganisms, even for hydrogenotrophs. Similarly, carbon dioxide and other nutrients become limiting agents, in turn constraining microbial growth and metabolic activity. Several experiments and models in salt caverns and deep aquifers have shown that intense lithoautotrophy in such poorly renewed ecosystems (i.e. low water flow) will result in alkalinization, leading to even stronger selection of prokaryotes, as well as carbonate precipitation. This leads us to imagine that possible competition exists for carbon dioxide between autotrophs and these geochemical mineralization processes, which will contribute to a lasting physicochemical modification of formation waters in the case of deep aquifers. Under conditions favorable to microbial life, perennial hydrogen storage in salt cavities is entirely feasible. For deep porous geological reservoirs, the question is more complex, and the answer seems intimately linked to the physicochemical conditions of each site (deep aquifers, hydrocarbon-depleted reservoirs). In the case of a deep oligotrophic aquifer with slow water renewal, it seems reasonable to imagine that perennial hydrogen storage could be achieved due to progressive depletion of essential nutrients. In depleted reservoirs, massive hydrogen injection seems to lead to increased methanogenic activity most of the time and opens up the possibility of developing UMRs to reduce our society's dependence on fossil fuels.

A further argument in favour of the possibility of massive hydrogen storage underground is that we have discovered several underground sites with almost pure natural hydrogen. While the processes by which hydrogen is generated are most certainly carried out under physicochemical conditions incompatible with microbial life (i.e. temperature, pH, etc.), this highly mobile molecule can subsequently migrate to surface layers, and increasing evidence indicates the existence of possible reservoirs closer to the surface, in which the conditions could be compatible with microbial life. These natural microbial ecosystems and artificial SLiMEs could therefore be considered analogues.

Acknowledgements

Thanks to Jagoba Malumbres-Olarte for Fig. 1. The autor would like to thank the two anonymous reviewers for their insightful comments and suggestions.

Author contributions

Anthony Ranchou-Peyruse (Conceptualization, Funding acquisition, Investigation, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing)

Conflict of interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was supported by the Agence Nationale de la Recherche (ANR) (ANR-20-CHIN-0001) and ENGIE in the ORHYON industrial chair.

References

Adhikari RR, Glombitza C, Nickel JC *et al.* Hydrogen utilization potential in subsurface sediments. *Front Microbiol* 2016;**7**:8.

Ajayi T, Gomes JS, Bera A. A review of CO_2 storage in geological formations emphasizing modeling, monitoring and capacity estimation approaches. *Pet Sci* 2019;**16**:1028–63.

Alabbadi AA, Obaid OA, AlZahrani AA. A comparative economic study of nuclear hydrogen production, storage, and transportation. *Int J Hydrog Energy* 2024;**54**:849–63.

Al-Yaseri A, Abu-Mahfouz IS, Yekeen N *et al.* Organic-rich source rock/H_2/brine interactions: implications for underground hydrogen storage and methane production. *J Energy Storage* 2023a;**63**:106986.

Al-Yaseri A, Al-Mukainah H, Yekeen N *et al.* Experimental investigation of hydrogen–carbonate reactions via computerized tomography: implications for underground hydrogen storage. *Int J Hydrog Energy* 2023b;**48**:3583–92.

Amirthan T, Perera MSA. Underground hydrogen storage in Australia: a review on the feasibility of geological sites. *Int J Hydrog Energy* 2023;**48**:4300–28.

Anderson RT, Chapelle FH, Lovley DR. Evidence against hydrogen-based microbial ecosystems in basalt aquifers. *Science* 1998;**281**:976–7.

Aüllo T, Ranchou-Peyruse A, Ollivier B *et al.* *Desulfotomaculum* spp. and related gram-positive sulfate-reducing bacteria in deep subsurface environments. *Front Microbiol* 2013;**4**:1–12.

Bagnoud A, Chourey K, Hettich RL *et al.* Reconstructing a hydrogen-driven microbial metabolic network in Opalinus Clay rock. *Nat Commun* 2016;**7**:12770.

Barbier S, Huang F, Andreani M *et al.* A review of H_2, CH_4, and hydrocarbon formation in experimental serpentinization using network analysis. *Front Earth Science* 2020;**8**:209.

Basso O, Lascourreges JF, Le Borgne F *et al.* Characterization by culture and molecular analysis of the microbial diversity of a deep subsurface gas storage aquifer. *Res Microbiol* 2009;**160**:107–16.

Bastin ES, Greer FE, Merritt CA *et al.* The presence of sulfate reducing bacteria in oil field waters. *Science* 1926;**63**:21–4.

Bastin ES. With the Collaboration of, Anderson B, Greer FE, Merreet CA, Moulton G. The problem of the natural reduction of sulfates. *AAPG Bull* 1926;**10**:1270–99.

Bauer S. Underground sun. Storage final report. 2017. https://www.underground-sun-storage.at/fileadmin/bilder/03_NEU_SUNSTORAGE/Downloads/Underground_Sun.Storage_Publizierbarer_Endbericht_English.pdf (5 November 2024, date last accessed).

Bellini R, Bassani I, Vizzarro A *et al.* Biological aspects, advancements and techno-economical evaluation of biological methanation for the recycling and valorization of CO_2. *Energies* 2022;**15**:4064.

Berlendis S, Ranchou-Peyruse M, Fardeau ML *et al.* *Desulfotomaculum aquiferis* sp. nov. and *Desulfotomaculum profundi* sp. nov., isolated from a deep natural gas storage aquifer. *Int J Syst Evol Microbiol* 2016;**66**:4329–38.

Berta M, Dethlefsen F, Ebert M *et al.* Geochemical effects of millimolar hydrogen concentrations in groundwater: an experimental study in the context of subsurface hydrogen storage. *Environ Sci Technol* 2018;**52**:4937–49.

Berta M. Experimental investigation of hydrogeochemical consequences of gas leakages into shallow aquifers. Ph.D. Thesis, Kiel University, 2017.

Bonchosmolovskaya E, Miroshnichenko M. Effect of molecular-hydrogen and elemental sulfur on metabolism of extremely thermophilic archaebacteria of the genus Thermococcus. *Microbiology* 1994;**63**:433–6.

Boon M, Hajibeygi H. Experimental characterization of H_2/water multiphase flow in heterogeneous sandstone rock at the core scale relevant for underground hydrogen storage (UHS). *Sci Rep* 2022;**12**:14604.

Bordenave S, Chatterjee I, Voordouw G. Microbial community structure and microbial activities related to CO_2 storage capacities of a salt cavern. *Int Biodeter Biodegr* 2013;**81**:82–7.

Boschee P. Comments: the new "gold rush" hunts for subsurface hydrogen. *J Petrol Tech* 2023;**75**:10–1.

Boston PJ, Ivanov MV, McKay C. On the possibility of chemosynthetic ecosystems in subsurface habitats on Mars. *Icarus* 1998;**95**:300–8.

Bourg IC, Beckingham LE, DePaolo DJ. The nanoscale basis of CO_2 trapping for geologic storage. *Environ Sci Technol* 2015;**49**:10265–84.

Boyd ES, Schut GJ, Adams MW *et al.* Hydrogen metabolism and the evolution of biological respiration. *Microbe* 2014;**9**:361–7.

Braun K, Gottschalk G. Effect of molecular hydrogen and carbon dioxide on chemo-organotrophic growth of *Acetobacterium woodii* and *Clostridium aceticum*. *Arch Microbiol* 1981;**128**:294–8.

Cabrol L, Marone A, Tapia-Venegas E *et al.* Microbial ecology of fermentative hydrogen producing bioprocesses: useful insights for driving the ecosystem function. *FEMS Microbiol Rev* 2017;**41**:158–81.

Cazier EA, Trably E, Steyer JP *et al.* Biomass hydrolysis inhibition at high hydrogen partial pressure in solid-state anaerobic digestion. *Bioresour Technol* 2015;**190**:106–13.

Cedigaz. Underground gas storage in the world—2022 status. 2023. https://www.cedigaz.org/underground-gas-storage-in-the-world-2022-status/ (4 November 2024, date last accessed).

Chapelle FH, O'Neill K, Bradley PM *et al.* a hydrogen-based subsurface microbial community dominated by methanogens. *Nature* 2002;**415**:312–5.

Crespo-Medina M, Twing KI, Kubo MDY *et al.* Insights into environmental controls on microbial communities in a continental serpentinite aquifer using a microcosm-based approach. *Front Microbiol* 2014;**5**:604.

Davoodi S, Al-Shargabi M, Wood DA *et al.* Review of technological progress in carbon dioxide capture, storage, and utilization. *Gas Sci Eng* 2023;**117**:205070.

Dohrmann AB, Kruüger M. Microbial H_2 consumption by a formation fluid from a natural gas field at high-pressure conditions relevant for underground H_2 storage. *Environ Sci Technol* 2023;**57**:1092–102.

Donadei S, Schneider GS. Compressed air energy storage in underground formations. In: *Storing Energy*. Amsterdam: Elsevier Inc., 2016, 113–33.

Dopffel N, An-Stepec BA, Bombach P *et al.* Microbial life in salt caverns and their influence on H_2 storage—current knowledge and open questions. *Int J Hydrog Energy* 2024;**58**:1478–85.

Dopffel N, An-Stepec BA, de Rezende JR *et al.* Editorial: microbiology of underground hydrogen storage. *Front Energy Res* 2023a;**11**:1242619.

Dopffel N, Jansen S, Gerritse J. Microbial side effects of underground hydrogen storage—knowledge gaps, risks and opportunities for successful implementation. *Int Journal Hydrog Energy* 2021;**46**:8594–606.

Dopffel N, Mayers K, Kedir A *et al.* Microbial hydrogen consumption leads to a significant pH increase under high-salinity-conditions–implications for hydrogen storage in salt caverns. *Sci Rep* 2023;**13**:10564.

Dupraz S, Stephant S, Perez A *et al.* Patagonia Wind–using methanogenesis to store hydrogen on large scales. In: *22st World Hydrogen Energy Conference-WHEC 2018*. 2018, 17–22 June, Rio de Janeiro, Brazil.

Ebrahimiyekta A. Characterization of geochemical interactions and migration of hydrogen in sandstone sedimentary formations: application to geological storage. Ph.D. Thesis, Université d'Orléans, 2017.

Edlmann K, Heinemann N, Mabon L *et al.* HyStorPor shows geological storage of hydrogen is possible. University of Edinburgh, 2023. https://era.ed.ac.uk/bitstream/handle/1842/40956/HyStorPor%20%20brochure%20FINAL.pdf?sequence=3 (4 November 2024, date last accessed).

Ehinger S, Seifert J, Kassahun A *et al.* Predominance of *Methanolobus* spp. and *Methanoculleus* spp. in the archaeal communities of saline gas field formation fluids. *Geomicrobiol J* 2009;**26**:326–38.

Ekzertsev VA, Kuznetsov SI. Issledovanie mikroflory neftenosny kh mestorozhdenii Vtorogo Baku (studies of the microflora of oil fields in Baku II). *Mikrobiologiya* 1954;**23**:3–14.

Ember. Global electricity review, 2023. https://ember-climate.org/app/uploads/2023/04/Global-Electricity-Review-2023.pdf (4 November 2024, date last accessed).

Foh S, Novil M, Rockar E *et al.* 1979. Underground hydrogen storage—final report. https://doi.org/10.2172/6536941 (4 November 2024, date last accessed).

Fry NK, Fredrickson JK, Fishbain S *et al.* Population structure of microbial communities associated with two deep, anaerobic, alkaline aquifers. *Appl Environ Microb* 1997;**63**:1498–504.

Gleeson T, Befus KM, Jasechko S *et al.* The global volume and distribution of modern groundwater. *Nat Geosci* 2016;**9**:161–7.

Gniese C, Bombach P, Rakoczy J *et al.* Relevance of deep-subsurface microbiology for underground gas storage and geothermal energy production. *Adv Biochem Eng Biotechnol* 2014:**142**;95–121.

Gold T. The deep, hot biosphere. *Proc Natl Acad Sci USA* 1992;**89**:6045–9.

Greening C, Biswas A, Carere CR *et al.* Genomic and metagenomic surveys of hydrogenase distribution indicate H_2 is a widely utilised energy source for microbial growth and survival. *The ISME J* 2016;**10**:761–77.

Greening C, Cook GM. Integration of hydrogenase expression and hydrogen sensing in bacterial cell physiology. *Cur Opin Microbiol* 2014;**18**:30–8.

Gregory S, Barnett M, Field L *et al.* Subsurface microbial hydrogen cycling: natural occurrence and implications for industry. *Microorganisms* 2019;**7**:53.

Guidehouse. Extending the European hydrogen backbone. A European hydrogen infrastructure vision covering 21 countries. 2021. https://gasforclimate2050.eu/sdm_downloads/extending-the-european-hydrogen-backbone (4 November 2024, date last accessed).

Haddad PG, Ranchou-Peyruse M, Guignard M *et al.* Geological storage of hydrogen in deep aquifers—an experimental multidisciplinary study. *Energy Environ Sci* 2022;**15**:3400–15.

Hagemann B, Rasoulzadeh M, Panfilov M *et al.* Mathematical modeling of unstable transport in underground hydrogen storage. *Environ Earth Sci* 2015;**73**:6891–8.

Hassanpouryouzband A, Adie K, Cowen T *et al.* Geological hydrogen storage: geochemical reactivity of hydrogen with sandstone reservoirs. *ACS Energy Let* 2022;**7**:2203–10.

Hassanpouryouzband A, Joonaki E, Edlmann K *et al.* Offshore geological storage of hydrogen: is this our best option to achieve net-zero?. *ACS Energy Let* 2021;**6**:2181–6.

Haveman SA, Pedersen K. Distribution of culturable microorganisms in Fennoscandian Shield groundwater. *FEMS Microbiol Ecol* 2002;**39**:129–37.

Hawkes FR, Hussy I, Kyazze G *et al.* Continuous dark fermentative hydrogen production by mesophilic microflora: principles and progress. *Int J Hydrog Energy* 2007;**32**:172–84.

Hellerschmied C, Schritter J, Waldmann N *et al.* Hydrogen storage and geo-methanation in a depleted underground hydrocarbon reservoir. *Nature Energy* 2024;**9**:333–44.

Helm-Clark CM, Rodgers DW, Smith RP. Borehole geophysical techniques to define stratigraphy, alteration and aquifers in basalt. *J Applied Geophys* 2004;**55**:3–38.

IEA. The future of hydrogen, Report prepared by the IEA for the G20, Japan. Seizing Today's Opportunities, 2019.

IEA. Global hydrogen review 2023, IEA, Paris, 2023. https://www.iea.org/reports/global-hydrogen-review-2023 (4 November 2024, date last accessed).

INERIS, Institut national de l'environnement industriel et des risques, Livrable L6.3 du projet ROSTOCK-H, Verneuil-en-Halatte : Ineris–159826–v1.0, 2021.

Ivanova AE, Borzenkov IA, Tarasov AL *et al.* a microbiological study of an underground gas storage in the process of gas injection. *Mikrobiologiia* 2007;**76**:515–23.

Jaakkola ST, Ravantti JJ, Oksanen HM *et al.* Buried alive: microbes from ancient halite. *Trends Microbiol* 2016;**24**:148–60.

Jensen MB, Strübing D, de Jonge N *et al.* Stick or leave—pushing methanogens to biofilm formation for *ex situ* biomethanation. *Bioresour Technol* 2019;**291**:121784.

Kantzow C, Weuster-Botz D. Effects of hydrogen partial pressure on autotrophic growth and product formation of *Acetobacterium woodii*. *Bioprocess Biosyst Eng* 2016;**39**:1325–30.

Karnachuk OV, Frank YA, Lukina AP *et al.* Domestication of previously uncultivated *Candidatus Desulforudis audaxviator* from a deep aquifer in Siberia sheds light on its physiology and evolution. *ISME J* 2019;**13**:1947–59.

Khajooie S, Gaus G, Dohrmann AB *et al.* Methanogenic conversion of hydrogen to methane in reservoir rocks: an experimental study of microbial activity in water-filled pore space. *Int J Hydrog Energy* 2024;**50**:272–90.

Khanal SK, Chen WH, Li L *et al.* Biological hydrogen production: effects of pH and intermediate products. *Int J Hydrog Energy* 2004;**29**:1123–31.

Kleinitz W, Böhling E. Underground gas storage in porous media—operating experience with bacteria on gas quality. In *67th European Association of Geoscientists and Engineers, EAGE Conference and Exhibition, incorporating SPE EUROPEC 2005–Extended Abstracts*. 2005.

Kotelnikova S, Pedersen K. Evidence for methanogenic Archaea and homoacetogenic bacteria in deep granitic rock aquifers. *FEMS Microbiol Rev* 1997;**20**:339–49.

Krumholz LR, McKinley JP, Ulrich GA *et al.* Confined subsurface microbial communities in cretaceous rock. *Nature* 1997;**386**:64–6.

Kuznetsov SI. Examination of the possibility of contemporary methanogenesis in gas-and petroleum-bearing facies of the Saratov and Buguruslan province. *Mikrobiologiya* 1950;**19**:193–202.

Labat N. Rôle de particularités sédimentaires et structurales sur le comportement des sables sous-molassiques soumis aux fluctuations induites par les stockages souterrains de gaz. *Application à L'étude De Leur Influence Sur L'hydrodynamisme Des émergences Locales*, Vol. 3. Ph.D. Thesis, Bordeaux, 1998, 228.

Larson WP, Hartzell TB, Diehl HS. The effect of high pressures on bacteria. *J Infect Dis* 1918;**22**:271–9.

Lin L-H, Hall J, Lippmann-Pipke J *et al.* Radiolytic H_2 in continental crust: nuclear power for deep subsurface microbial communities. *Geochem Geophys* 2005;**6**:1–13.

Lollar BS, Onstott TC, Lacrampe-Couloume G *et al.* The contribution of the Precambrian continental lithosphere to global H_2 production. *Nature* 2014;**516**:379–82.

Londe LF. Four ways to store large quantities of hydrogen. In: *Abu Dhabi International Petroleum Exhibition and Conference, SPE*, 2021, D022S189R001.

Lovley DR, Chapelle FH. Deep subsurface microbial processes. *Rev Geophys* 1995;**33**:365.

Lowenstein TK, Schubert BA, Timofeeff MN. Microbial communities in fluid inclusions and long-term survival in halite. *GSA Today* 2011;**21**:4–9.

Lysyy M, Ersland G, Fernø M. Pore-scale dynamics for underground porous media hydrogen storage. *Adv Water Resour* 2022;**163**:104167.

Magnabosco C, Lin L-H, Dong H *et al.* The biomass and biodiversity of the continental subsurface. *Nature Geosci* 2018;**11**:707–17.

Meckenstock RU. Fermentative toluene degradation in anaerobic defined syntrophic cocultures. *FEMS Microbiol Let* 1999;**177**:67–73.

Ménez B, Pasini V, Brunelli D. Life in the hydrated suboceanic mantle. *Nat Geosci* 2012;**5**:133–7.

Molíková A, Vítězová M, Vítěz T *et al.* Underground gas storage as a promising natural methane bioreactor and reservoir? *J Energy Storage* 2022;**47**:103631.

Morinaga T, Kawada N. The production of acetic acid from carbon dioxide and hydrogen by an anaerobic bacterium. *J Biotechnol* 1990;**14**:187–94.

Motamedi M, Pedersen K. Isolation and characterisation of a mesophilic sulfate-reducing bacterium, *Desulfovibrio aespoeensis* sp. nov. from deep ground water at Äspö Hard Rock Laboratory, Sweden. *Int J Syst Bacteriol* 1998;**48**:311–5.

Muhammed NS, Haq MB, Al Shehri DA *et al.* Hydrogen storage in depleted gas reservoirs: a comprehensive review. *Fuel* 2023;**337**:127032.

Müller AL, Kjeldsen KU, Rattei T *et al.* Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi) sulfite reductases. *ISME J* 2015;**9**:1152–65.

Mura J, Ranchou-Peyruse M, Guignard M *et al.* Comparative study of three H_2 geological storages in deep aquifers simulated in high-pressure reactors. *Int J Hydrog Energy* 2024;**63**:330–45.

Nealson KH, Inagaki F, Takai K. Hydrogen-driven subsurface lithoautotrophic microbial ecosystems (SLiMEs): do they exist and why should we care? *Trends Microbiol* 2005;**13**:405–10.

Neumann F, Zeyen E, Victoria M *et al.* The potential role of a hydrogen network in Europe. *Joule* 2023;**7**:1793–817.
Oren A. Thermodynamic limits to microbial life at high salt concentrations. *Environ Microbiol* 2011;**13**:1908–23.
Oswald F, Stoll IK, Zwick M *et al.* Formic acid formation by *Clostridium ljungdahlii* at elevated pressures of carbon dioxide and hydrogen. *Front Bioeng Biotechnol* 2018;**6**:6.
Pankhurst ES. *The Growth and Occurrence of Sulfate-reducing Bacteria, with Particular Reference to Their Importance in the Gas Industry*. Ph.D. Thesis, Univ. London, Great Britain (Imperial College), 1967.
Pappenreiter PA, Zwirtmayr S, Mauerhofer LM *et al.* Development of a simultaneous bioreactor system for characterization of gas production kinetics of methanogenic archaea at high pressure. *Eng Life Sci* 2019;**19**:537–44.
Park JS, Vreeland RH, Cho BC *et al.* Haloarchaeal diversity in 23, 121 and 419 MYA salts. *Geobiology* 2009;**7**:515–23.
Pedersen K. Evidence for a hydrogen-driven, intra-terrestrial biosphere in deep granitic rock aquifers. In: *Proceedings of the 8th International Symposium on Microbial Ecology Bell CR*. Atlantic Canada Society for Microbial Ecology, Halifax, 1998.
Pedersen K. Exploration of deep intraterrestrial microbial life: current perspectives. *FEMS Microbiol Let* 2000;**185**:9–16.
Pedersen K. Microorganisms and their influence on radionuclide migration in igneous rock environments. *J Nucl Radioch Sci* 2005;**6**:11–5.
Pérez A, Pérez E, Dupraz S *et al.* Patagonia wind-hydrogen project: underground storage and methanation. In: *21st World Hydrogen Energy Conference. 13–16 June*, Zaragoza, 2016.
Peters V, Janssen PH, Conrad R. Transient production of formate during chemolithotrophic growth of anaerobic microorganisms on hydrogen. *Curr Microbiol* 1999;**38**:285–9.
Postec A, Quéméneur M, Bes M *et al.* Microbial diversity in a submarine carbonate edifice from the serpentinizing hydrothermal system of the Prony Bay (New Caledonia) over a 6-year period. *Front Microbiol* 2015;**6**:857.
Pumphrey GM, Ranchou-Peyruse A, Spain JC. Cultivation-independent detection of autotrophic hydrogen-oxidizing bacteria by DNA stable-isotope probing. *App Environ Microbiol* 2011;**77**:4931–8.
Ranchou-Peyruse M, Auguet JC, Mazière C *et al.* Geological gas-storage shapes deep life. *Environ Microbiol* 2019;**21**:3953–64.
Ranchou-Peyruse M, Guignard M, Chiquet P *et al.* Assessment of the *in situ* biomethanation potential of a deep aquifer used for natural gas storage. *FEMS Microbiol Ecol* 2024;**100**:fiae066.
Ranchou-Peyruse M, Guignard M, Haddad PG *et al.* A deep continental aquifer downhole sampler for microbiological studies. *Front Microbiol* 2023;**13**:1012400.
Rice DD, Claypool GE. Generation, accumulation, and resource potential of biogenic gas. *AAPG Bul* 1981;**65**:5–25.
Ritchie H, Roser M, Rosado P. Renewable energy. *Our World In Data* 2020. https://ourworldindata.org/renewable-energy.
Sainz-Garcia A, Abarca E, Rubi V *et al.* Assessment of feasible strategies for seasonal underground hydrogen storage in a saline aquifer. *Int J Hydrogen Energy* 2017;**42**:16657e66.
Sambo C, Dudun A, Samuel SA *et al.* A review on worldwide underground hydrogen storage operating and potential fields *Int J Hydrog Energy* 2022;**47**:22840–80.
Schink B. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol* 1997;**61**:262–80.
Schroeder S, Ranchou-Peyruse A, Ranchou-Peyruse M *et al.* Reverse-transcriptase quantitative PCR method to detect uptake of hydrogen produced from cyanobacteria by *Alcaligenes hydrogenophilus*, an aerobic hydrogen-oxidising bacterium. *Arch Microbiol* 2011;**193**:687–92.
Schwab L, Popp D, Nowack G *et al.* Structural analysis of microbiomes from salt caverns used for underground gas storage. *Int J Hydrog Energy* 2022;**47**:20684–94.
Schwartz E, Fritsch J, Friedrich B. H_2-metabolizing prokaryotes. In: Rosenberg E, DeLong EF, Lory S (eds.), *The Prokaryotes: Prokaryotic Physiology and Biochemistry*. Berlin, Heidelberg: Springer, 2013, 119–99.
Seck GS, Hache E, Sabathier J *et al.* Hydrogen and the decarbonization of the energy system in Europe in 2050: a detailed model-based analysis. *Renew Sustain Energy Rev* 2022;**167**:112779.
Sekar LK, Kiran R, Okoroafor ER *et al.* Review of reservoir challenges associated with subsurface hydrogen storage and recovery in depleted oil and gas reservoirs. *J Energy Storage* 2023;**72**:108605.
Sharshir SW, Joseph A, Elsayad MM *et al.* A review of recent advances in alkaline electrolyzer for green hydrogen production: performance improvement and applications. *Int J Hydrog Energy* 2023;**49**:458–88.
Shojaee A, Ghanbari S, Wang G *et al.* Interplay between microbial activity and geochemical reactions during underground hydrogen storage in a seawater-rich formation. *Int J Hydrog Energy* 2024;**50**:1529–41.
Silver BJ, Onstott TC, Rose G *et al.* *In situ* cultivation of subsurface microorganisms in a deep mafic sill: implications for SLiMEs. *Geomicrobiol J* 2010;**27**:329–48.
Šmigáň P, Greksak M, Kozánková J *et al.* Methanogenic bacteria as a key factor involved in changes of town gas stored in an underground reservoir. *FEMS Microbiol Ecology* 1990;**6**:221–4.
Stephenson M, Stickland LCH. Hydrogenase: the bacterial formation of methane by the reduction of one-carbon compounds by molecular hydrogen. *Biochem J* 1933;**27**:1517–27. https://doi.org/10.1042/bj0271517.
Stevens TO, McKinley JP. Lithoautotrophic microbial ecosystems in deep basalt aquifers. *Science* 1995;**270**:450–5.
Stoll IK, Herbig S, Zwick M *et al.* Fermentation of H_2 and CO_2 with Clostridium ljungdahlii at elevated process pressure—first experimental results. *Chem Eng Trans* 2018;**64**:151–6.
Stolten D, Emonts B. *Hydrogen Science and Engineering, 2 Volume Set: materials, Processes, Systems, and Technology*, Vol. **1**. John Wiley & Sons, 2016.
Strobel G, Hagemann B, Huppertz TM *et al.* Underground biomethanation: concept and potential. *Renew Sustain Energy Rev* 2020;**123**:109747.
Sukhanova NI, Lysak LV, Kiryushin AV. Influence of hydrogen fluid on plants in natural conditions and a model experiment. *Moscow Univ. Soil Sci Bull* 2019;**74**:208–13.
Takai K, Gamo T, Tsunogai U *et al.* Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea hydrothermal field. *Extremophiles* 2004;**8**:269–82.
Takai K, Mormile MR, McKinley JP *et al.* Shifts in archaeal communities associated with lithological and geochemical variations in subsurface cretaceous rock. *Environ Microbiol* 2003;**5**:309–20.
Takai KEN, Nakamura K, Suzuki K *et al.* Ultramafics-hydrothermalism-hydrogenesis-HyperSLiME (UltraH3) linkage: a key insight into early microbial ecosystem in the archean deep-sea hydrothermal systems. *Paleontol Res* 2006;**10**:269–82.
Tarkowski R. Underground hydrogen storage: characteristics and prospects. *Renew Sustain Energy Rev* 2019;**105**:86–94.

Tarraran L, Agostino V, Vasile NS *et al.* High-pressure fermentation of CO_2 and H_2 by a modified *Acetobacterium woodii*. *J CO_2 Utilization* 2023;**76**:102583.

Taylor J, Alderson JEA, Kalyanam KM *et al.* Technical and economic assessment of methods for the storage of large quantities of hydrogen. *Int J Hydrogen Energy* 1986;**11**:5–22.

Thaysen EM, Armitage T, Slabon L *et al.* Microbial risk assessment for underground hydrogen storage in porous rocks. *Fuel* 2023;**352**:128852.

Thaysen EM, McMahon S, Strobel GJ *et al.* Estimating microbial growth and hydrogen consumption in hydrogen storage in porous media. *Renew Sustain Energy Rev* 2021;**151**:111481.

Truche L, Berger G, Destrigneville C *et al.* Kinetics of pyrite to pyrrhotite reduction by hydrogen in calcite buffered solutions between 90 and 180 C: implications for nuclear waste disposal. *Geochim Cosmochim Acta* 2010;**74**:2894–914.

Truche L, Bourdelle F, Salvi S *et al.* Hydrogen generation during hydrothermal alteration of peralkaline granite. *Geochim Cosmochim Acta* 2021;**308**:42–59.

Truche L, Donzé FV, Goskolli E *et al.* A deep reservoir for hydrogen drives intense degassing in the Bulqizë ophiolite. *Science* 2024;**383**:618–21.

Tsai CH, Chen KT. Production of hydrogen and nano carbon powders from direct plasmalysis of methane. *Int J Hydrog Energy* 2009;**34**:833–8.

Tyne RL, Barry PH, Lawson M *et al.* Rapid microbial methanogenesis during CO_2 storage in hydrocarbon reservoirs. *Nature* 2021;**600**:670–4.

Fuel Cells and Hydrogen Joint Undertaking. Hydrogen roadmap Europe: a sustainable pathway for the European energy transition. 2019. https://www.fch.europa.eu/sites/ default/files/Hydrogen% 20Roadmap% 20Europe_Report.pdf.

Vasile NS, Bellini R, Bassani I *et al.* Innovative high pressure/high temperature, multi-sensing bioreactors system for microbial risk assessment in underground hydrogen storage. *Int J Hydrog Energy* 2024;**51**:41–50.

Ver Eecke HC, Butterfield DA, Huber JA *et al.* Hydrogen-limited growth of hyperthermophilic methanogens at deep-sea hydrothermal vents. *Proc Natl Acad Sci USA* 2012;**109**:13674–9.

Veshareh MJ, Thaysen EM, Nick HM. Feasibility of hydrogen storage in depleted hydrocarbon chalk reservoirs: assessment of biochemical and chemical effects. *App Energy* 2022;**323**:119575.

Vítězová M, Onderka V, Urbanová I *et al.* *In situ* field experiment shows the potential of methanogenic archaea for biomethane production from underground gas storage in natural rock environment. *Environ Technol Innov* 2023;**32**:103253.

Vogt C, Kleinsteuber S, Richnow HH. Anaerobic benzene degradation by bacteria. *Microb Biotechnol* 2011;**4**:710–24.

Vreeland RH, Piselli AF, McDonnough S *et al.* Distribution and diversity of halophilic bacteria in a subsurface salt formation. *Extremophiles* 1998;**2**:321–31.

Wang G, Pickup G, Sorbie K *et al.* Bioreaction coupled flow simulations: impacts of methanogenesis on seasonal underground hydrogen storage. *Int J Hydrog Energy* 2024;**55**:921–31.

Wang X, Ouyang Z, Zhuo S *et al.* Serpentinization, abiogenic organic compounds, and deep life. *Sci China Earth Sci* 2014;**57**:878–87.

Wankel SD, Germanovich LN, Lilley MD *et al.* Influence of subsurface biosphere on geochemical fluxes from diffuse hydrothermal fluids. *Nature Geosci* 2011;**4**:461–8.

Watson IA, Oswald SE, Mayer KU *et al.* Modeling kinetic processes controlling hydrogen and acetate concentrations in an aquifer-derived microcosm. *Environ Sci Technol* 2003;**37**:3910–9.

Zeng L, Keshavarz A, Xie Q *et al.* Hydrogen storage in Majiagou carbonate reservoir in China: geochemical modelling on carbonate dissolution and hydrogen loss. *Int J Hydrog Energy* 2022;**47**:24861–70.

Zgonnik V. The occurrence and geoscience of natural hydrogen: a comprehensive review. *Earth Sci Rev* 2020;**203**:103140.

Received 25 March 2024; **revised** 9 July 2024; **accepted** 23 October 2024