Co-funded by the European Union

HyUSPRe

Hydrogen **U**nderground **S**torage in **P**orous **Re**servoirs

Competition dynamics between different microbial metabolisms to validate simulation models

Prepared by: Anne-Catherine Ahn, Wageningen University
Adrian Hidalgo Ulloa, Wageningen University
Bart P. Lomans, Shell
Diana Z. Sousa, Wageningen University

Please cite this report as: Ahn, A.C., Hidalgo Ulloa, A., Lomans, B.P., Sousa, D.Z., 2024: Competition dynamics between different microbial metabolisms to validate simulation models, H2020 HyUSPRe project report. 21 pp.

This report represents HyUSPRe project deliverable number D3.4.

The HyUSPRe consortium

Funded by

Clean Hydrogen Partnership

Co-funded by the European Union

Acknowledgement

This project has received funding from the Fuel Cells and Hydrogen 2 Joint Undertaking (now Clean Hydrogen Partnership) under grant agreement No 101006632. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme, Hydrogen Europe and Hydrogen Europe Research.

Disclaimer

Executive summary

Underground storage of H_2 in depleted hydrocarbon reservoirs, salt caverns, and aquifers presents a promising option for energy storage of renewable energy, but also poses challenges due to potential microbial metabolic activities. Microorganisms, particularly those involved in methanogenesis, sulfate reduction, and acetogenesis, can affect H_2 storage in various ways, including consuming stored H_2, producing gas contaminants like hydrogen sulfide (H_2S) and methane (CH_4), inducing microbial-influenced corrosion, and causing pipe and pore clogging through the accumulation of biomass and bio-based solids. Environmental parameters play a crucial role in limiting or completely inhibiting microbial growth and metabolic activity. Understanding the impact of environmental conditions allows for predicting these microbial-influenced risks. One approach to better assess risks in the field is to study microbial activities and community compositions in lab-based incubations. These studies enable to unravel the complex interplay between environmental conditions and microbial behavior.

This report explores the impact of temperature and nutrient addition on microbial activity and community composition during lab incubation experiments using bicarbonate-buffered reservoir brine samples from a potential H_2 storage site. Microbial consumption of H_2 was associated to the formation of CH_4 and acetate, with primarily acetate at 35°C, and CH_4 at 50, 65 and 80°C being produced in the "H_2-only" incubations, and acetate at 35 and 50°C, and CH_4 at 65 and 80°C in the "H_2/CO_2-nutrient supplemented" incubations. No sulfidogenic activity was detected for the reservoir brine samples studied. The extent of H_2 conversion varied with temperature, peaking at 65°C at both conditions, and was enhanced by the addition of minerals and vitamins during the incubation experiments. Analysis of microbial community composition suggested that CH_4 was produced by the hydrogenotrophic methanogens of the Methanobacteria class. Acetate was likely produced through homoacetogenesis and fermentative pathways by members of the Bacillota phylum, including Clostridia, Thermoanaerobacteria, and Moorelia, as well as candidate phylum Acetothermiia in the "H_2-only" incubations, and by members of Clostridia, Thermanaerobacteria, Moorellia, Thermotogae, and Thermacetogenia, with the later possibly able to also perform syntrophic acetate reduction in combination with the methanogen, in the "H_2/CO_2-nutrient supplemented" incubations. The addition of CO_2 and nutrients led to less complex microbial communities, with certain taxa outcompeting others rapidly. These findings underscore the importance of analyzing the microbiological and geochemical characteristics of potential storage sites and continuously monitoring them during H_2 storage to reduce risks provoked by microorganisms. However, future research should focus on the effects of high partial H_2 pressure to better simulate H_2 storage conditions and to derive valuable insights for potential applications in porous reservoirs.

The raw microbial activity data (H_2 consumption, product formation and pH) collected for this report can be reviewed in the deliverable D3.3 (Database of relevant input parameters from D3.1 and D3.2 for the integrated reservoir and wellbore modelling in WP6), and the modeling of the kinetic parameters from this study is detailed in report D3.2 (Report on the kinetics of microbial growth & activity (specific growth rates, growth yields, Ks values, etc.) as well as generation of biobased solids and their dependences on relevant environmental parameters (e.g. temperature etc.)).

About HyUSPRe

Hydrogen **U**nderground **S**torage in **P**orous **Re**servoirs

The HyUSPRe project researches the feasibility and potential of implementing large-scale underground geological storage for renewable hydrogen in Europe. This includes the identification of suitable porous reservoirs for hydrogen storage, and technical and economic assessments of the feasibility of implementing large-scale storage in these reservoirs to support the European energy transition to net zero emissions by 2050. The project will address specific technical issues and risks regarding storage in porous reservoirs and conduct an economic analysis to facilitate the decision-making process regarding the development of a portfolio of potential field pilots. A techno-economic assessment, accompanied by environmental, social, and regulatory perspectives on implementation will allow for the development of a roadmap for widespread hydrogen storage by 2050, indicating the role of large-scale hydrogen storage in achieving a zero-emissions energy system in the EU by 2050.

This project has two specific objectives. Objective 1 concerns the assessment of the technical feasibility, associated risks, and the potential of large-scale underground hydrogen storage in porous reservoirs for Europe. HyUSPRe will establish the important geochemical, microbiological, flow, and transport processes in porous reservoirs in the presence of hydrogen via a combination of laboratory-scale experiments and integrated modelling; and establish more accurate cost estimates to identify the potential business case for hydrogen storage in porous reservoirs. Suitable storage sites will be identified, and their hydrogen storage potential will be assessed. Objective 2 concerns the development of a roadmap for the deployment of geological hydrogen storage up to 2050. The proximity of storage sites to large renewable energy infrastructure and the amount of renewable energy that can be buffered versus time varying demands will be evaluated. This will form a basis for developing future scenario roadmaps and preparing for demonstrations.

Document information, revision history, approval status

Document information

Title:	Competition dynamics between different microbial metabolisms to validate simulation models
Lead beneficiary:	Wageningen University
Contributing beneficiaries:	Wageningen University
Due date:	17/06/2024
Dissemination level:	Public
Published where:	HyUSPRe website
Recommended citation:	Ahn, A.C., Hidalgo Ulloa, A., Lomans, B.P., Sousa, D.Z., 2024: Competition dynamics between different microbial metabolisms to validate simulation models, H2020 HyUSPRe project report 21 pp.

Revision history

Version	Name	Delivery date	Summary of changes
V01	A.C. Ahn	2024.05.03	First draft
V02	A.C. Ahn, A. Hidalgo Ulloa, D.Z. Sousa	2024.05.15	Draft delivered for the industry partners review
V03	A.C. Ahn	2024.06.15	Revised document with reviews from partners
V04	A.C. Ahn	2024.06.15	Creating censored version of original, confidential report
V05	H. Cremer	2024.06.17	Final, formatted version

Approval status

Role	Name	Delivery date
Deliverable responsible:	D. Z. Sousa	2024.06.15
Task leader:	D. Z. Sousa	2024.06.15
WP leader:	D. Z. Sousa	2024.06.15
HyUSPRe lead scientist	R. Groenenberg	2024.06.17
HyUSPRe consortium manager:	H. Cremer	2024.06.17

Table of Content

1. Introduction

The European Union has set the goal of achieving a zero-emissions energy system across Europe by 2050. To enable this transition, it is crucial to widely adopt renewable energy sources like wind and solar power. However, these sources are susceptible to energy production and consumption variations throughout the day and period of the year. In response to this challenge, molecular energy carriers such as hydrogen (H_2) have been proposed for storage of energy surplus. H_2 can be converted back into usable energy, like electricity, when needed. Aboveground storage of H_2 is impractical due to the large volumes required, and meets with spatial planning and environmental constraints. Consequently, attention has turned to underground H_2 storage in depleted hydrocarbon reservoirs, salt caverns and aquifers. However, H_2 can interact with the environment of these subsurface storage sites in complex ways, including potential detrimental effects from microorganisms, which poses a challenge to the effectiveness of H_2 storage (Dopffel et al., 2021).

Hydrogen plays an essential role as an electron donor in anaerobic microbial processes (Claassens et al., 2018), involving H_2-utilizing sulfate reducers, methanogens, and acetogens (Thaysen et al., 2021). As a result, the storage of H_2 underground may trigger the activity of these microbial groups, potentially leading to detrimental effects on H_2 storage. Microbes, through their metabolic processes, can cause depletion of stored H_2 and the formation of contaminating reaction products like hydrogen sulfide (H_2S) and methane (CH_4) (Dopffel et al., 2021). Moreover, microbial-influenced corrosion (MIC) can occur, causing the deterioration of gas transport infrastructure and the buildup of organic solids (including microbial biomass, extracellular polymeric substances (EPS), and metal sulfides). These solids can obstruct pipelines and the pore spaces of reservoir rocks, diminishing gas injection capacity (Dopffel et al., 2021). A better understanding of the key environmental factors influencing microbial behavior and community composition can facilitate the prediction of these outcomes.

The subsurface environment harbors various extreme conditions, including elevated temperatures, pressures, and salinity (Escudero et al., 2018), as well as restricted pore sizes for passage or colonization (Sharma and McInerney, 1994; Fredrickson et al., 1997). Microbial abundance and activity heavily rely on these environmental parameters. Thaysen et al. (2021) conducted an analysis of the "windows of viability" for microorganisms in subsurface environments using data from 532 different pure strains of acetogens, SSRMs (sulfur species reducing microorganisms), and methanogens. Through this analysis, they established thresholds for potential risks associated with microbial activities in subsurface H_2 storage. Their examination highlighted temperature, either alone or in combination with salinity, as the most significant limiting factor for microbial growth and activity in subsurface environments. Typically, sites with temperatures exceeding 122°C are considered sterile, while temperatures between 80°C and 94°C generally reduce the likelihood of H_2S formation. The analysis demonstrated the boundaries of potential growth by plotting critical temperatures against NaCl concentrations, indicating that storage sites with temperatures above 55°C and salinity levels exceeding 1.7 M are optimal for mitigating the risk of microbial impact (Thaysen et al., 2021). However, clear limits for microbial pressure resistance have not yet been defined (Dopffel et al., 2021). It is crucial to understand the thresholds of microbial life, and to evaluate microbial kinetics and the impact on the microbial community composition under various conditions within the window of microbial viability. This approach enables to define site options and allows for risk assessment within this range.

Interest in subsurface H_2 storage is increasing, yet our understanding of the microbial community inhabiting these environments and how they will be influenced by the H_2 storage remains restricted. Currently, little is known about the structure and function of microbial communities present in these habitats. Microbial community analysis plays a crucial role in

shedding light on this issue by identifying the microorganisms and quantifying their relative abundance, allowing us to study their functional composition, diversity, and interactions with each other and their surroundings. The composition of microbial communities is highly influenced by environmental conditions, which are linked to the individual growth kinetics and adaptations of each microorganism. Several studies have examined the environmental microbiomes of underground gas storage sites in various locations, including porous reservoirs (Šmigáň et al., 1990; Ivanova et al., 2007; Buriánková et al., 2022), aquifers (Basso et al., 2009; Gulliver et al., 2019; Ranchou-Peyruse et al., 2019, 2021; Kadnikov et al., 2020), and salt caverns (Bordenave et al., 2013; Schwab et al., 2022), filled with different types of gases such as natural gas, town gas, or CO_2. However, there is a scarcity of studies focusing on the effect of H_2 storage on microbial communities in subsurface environments. For this aim, Dohrmann and Krüger (2022) investigated the impact of H_2 injection and withdrawal cycles and temperature variations on a porous reservoir, while Schwab et al. (2023) examined the effect of varying salt concentrations and carbon sources on H_2 oxidation in mixed cultures enriched from materials sourced from various hypersaline aquifers and caverns. However, it is also essential to recognize that the subsurface environment under H_2 storage conditions carries the risk of introducing allochthonous organisms from the surface, as well as surface gases and drilling fluids (Heinemann et al., 2021), which can lead to changes in the microbial community during the operation period. Therefore, understanding the community composition and the effect of different H_2 storage conditions is of tremendous importance for inferring competitive interactions and better monitoring the microbial impact of subsurface H_2 storage.

This report investigates the impact of temperature and the addition of nutrients on the composition of the microbial community associated with the microbial consumption of H_2, and the formation of products in brine samples from a potential H_2 storage site. Amplicon sequence analysis based on the 16S rRNA gene was used to identify the microbial key players in the community composition and to determine which functional group (methanogens, sulfate reducers and acetogens) has advantages over others under certain conditions. The original data regarding microbial H_2 consumption and product formation collected for this report can be found in deliverable D3.3. (Database of relevant input parameters from D3.1 and D3.2 for the integrated reservoir and wellbore modelling in WP6), while the modeling of the kinetic parameters from this study is detailed in report D3.2 (Report on the kinetics of microbial growth & activity (specific growth rates, growth yields, Ks values, etc.) as well as generation of biobased solids and their dependences on relevant environmental parameters (e.g. temperature etc.).

2. Experimental set-up

Reservoir brine samples from a potential hydrogen storage site were incubated under two conditions ("H_2-only" and "H_2/CO_2-nutrient supplemented") at four different temperatures (35, 50, 65, and 80°C). The studied reservoir corresponds to "case 1", following the nomenclature used in deliverable D3.3. The reservoir of case 1 has an average temperature of 83°C and samples were retrieved from a sand trap, which was bleed off prior the sampling. The first condition simulates H_2 storage conditions, where the brine was incubated with 100% H_2 and in absence of additional nutrients ("H_2-only" condition). Under this condition, the microbial community only has access to minerals and organics already present in the brine. In the second condition, the brine was incubated with 80%H_2/20%CO_2, and a surplus of various minerals and vitamins was provided ("H_2/CO_2-nutrient supplemented" condition). This condition represents the high-impact scenario for H_2 storage, as it creates a more favorable condition to enhance microbial activities and kinetics. Brine samples were incubated in anaerobic bottles at 1.7 bar, at 35, 50, 65, and 80°C in triplicates, and additionally two blanks (sterilized brine) were added, per condition (Figure 1). For both conditions, 250 mg/l $NaHCO_3$ (buffer), and 0.48 g/l Na_2S . 9 H_2O (reducing agent) and 0.5 mg/l resazurin (redox indicator) were added. Additionally, nutrients, vitamins and trace elements prepared as previously described (Stams et al., 1993; Plugge, 2005) were added to the "H_2/CO_2-nutrient supplemented" incubations. The starting brine sample was analyzed for its chemical composition, and liquid samples at the start and at the end of incubations were preserved for microbial community composition analysis. Throughout the incubation, headspace samples were analyzed for H_2 and CH_4 quantification, and liquid samples for pH, H_2S, SO_4^{2-} and acetate.

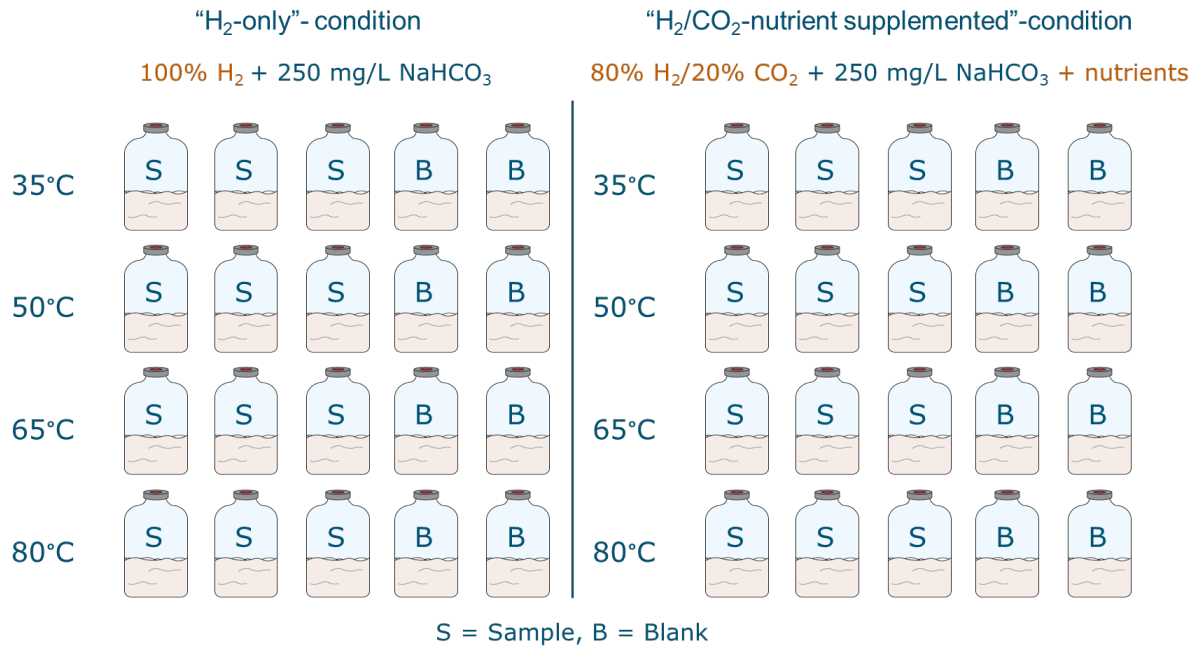

Figure 1. Schematic representation of experimental set-up.

Chemical analysis of metal concentrations of the starting brine sample was determined using an ICP-OES (Perkin Elmer Avio 500, USA). During incubation, the concentrations of H_2 and CH_4 in the gaseous phase were measured by gas chromatography with a thermal conductivity detector (GC-TCD) on a CompactGC4.0 (Interscience, The Netherlands). HS^- concentrations in the liquid phase were quantified using the methylene blue method (Standard methods for the examination of water and wastewater, 2017). These values (and the pH) were used to calculate 'total sulfide' concentration, corresponding to the sum of dissolved and gas-phase

sulfide (referred to as H_2S throughout this report). SO_4^{2-} was measured using ion exchange chromatography Dionex ICS-2100 (Dionex, USA) equipped with a Dionex IonPac AS17 column (Dionex). Concentrations of acetate and other volatile fatty acids were determined using High-Performance Liquid Chromatography (HPLC) on a Shimadzu Prominence LC2030C Plus (Shimadzu Corporation, Japan) equipped with a UV/Vis detector. Genomic DNA was extracted from samples for molecular analysis with the DNeasy PowerSoil Pro Kit (Qiagen, The Netherlands) with 0.1 mm G2 DNA/RNA extraction enhancer beads (Ampliqon, Denmark). Amplicon sequencing was performed at Novogene sequencing the 16S rRNA gene V4 region (2 x 250 bp) with the universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') on an Illumina NovaSeq 6000 platform (Illumina, San Diego, USA). Within QIIME2 (version 2023.9), amplicon sequence variants (ASVs) were determined using the DADA2 algorithm and taxonomy was assigned to representative ASVs based on the Silva 138 99% OTUs 16S rRNA gene database.

3. Results

3.1 Physical and chemical conditions of the subsurface reservoir

Case 1's reservoir is at an average temperature of 83°C, with an initial pressure of 116 bar, and the brine sample had an average pH upon receipt of 7.34. The chemical composition includes 0.049 mM SO_4^{2-}, 1 mM acetate, and no formate. The elemental composition of the brine showed 104.65 mg/L sodium, 3.68 mg/L of sulfur, and 0.69 mg/L of boron (see Table 1). Other dissolved elements examined in this analysis were below the quantification limit.

Table 1. Elemental composition of the initial case 1 sample.

Element	Concentration (mg/L)
S	3.68
Al	< 2
Ba	< 0.2
Ca	< 20
Cd	< 2
Co	< 0.2
Cr	< 2
Cu	< 2
Fe	< 2
K	< 20
Li	< 2
Mg	< 2
Mn	< 2
Na	104.65
Ni	< 2
Pb	< 0.2
Zn	< 2
B	0.69
P	< 0.2

3.2 Microbial activity and community composition

Brine incubations were performed under two conditions. "H_2-only" incubations were cultivated with a 100% H_2 headspace and in the absence of additional nutrients, whereas "H_2/CO_2-nutrient supplemented" incubations were cultivated with an 80% H_2/20% CO_2 headspace, and a surplus of various minerals and vitamins to enhance growth. In both incubation conditions, consumption of H_2 and production of CH_4 and acetate were observed. However, the rates of H_2 consumption and the proportions of products varied depending on the temperature and condition. This variation is also reflected in the microbial community composition, which allows inferring the microorganisms potentially responsible for the different metabolic activities.

For the microbial community composition, it is important to consider that taxonomic assignments at lower levels may be uncertain. Assignments at the class level are more reliable, but the report also discusses functional properties at the genus level, which should be approached with caution.

3.2.1 "H_2-only" condition

The "H_2-only" incubations were cultivated with $NaHCO_3$ and 100% H_2. At 35, 50, and 65°C, H_2 consumption occurred along with the generation of CH_4 and acetate. No significant H_2 consumption was detected at 80°C, compared to sterile controls. The observed loss in H_2 in the incubations and in the sterile controls is likely due to the escape of gas during sampling and through the stopper during the incubation. CH_4 production increased at higher temperatures, particularly between 50 and 80°C. Acetate was primarily generated at 35°C and to a lesser extent at higher temperatures, except for 80°C. No significant H_2S production was observed at any temperature for the "H_2-only" incubations.

The microbial community composition at the beginning and end of the "H_2-only" incubations at the different temperatures identified the microorganisms potentially responsible for the observed activities. Due to low quality, the sequencing data from the incubations at 80°C were excluded from this report.

The initial composition of the incubations revealed a predominant relative abundance of the archaeal class Methanobacteria, along with bacteria of the genus *Thermolithobacter* (classified as Incertae Sedis within the Bacillota), the candidate phylum Acetothermiia, and the class Bacilli. Additionally, the microbial community also comprised members from classes such as Clostridia, Thermoanaerobacteria, Moorellia, Thermotogae, Thermacetogenia, and Bacteroidia, albeit in smaller proportions.

Based on the sequencing results, CH_4 production was attributed in all sequenced temperature conditions of the "H_2-only" incubations to hydrogenotrophic methanogenesis by Methanobacteria. In this process, the methanogens utilized H_2 and HCO_3^- (as CO_2) provided to the culture or H_2 and CO_2 produced through fermentation during the incubation. However, acetate production occurred via homoacetogenesis and fermentative pathways likely carried out by members of the Bacillota phylum, including Clostridia, Thermoanaerobacteria, and Moorellia, and those from the candidate phylum Acetothermiia, encountered in those samples. Clostridia, predominantly represented by the genus *Eubacterium* at 35°C, was probably the primarily responsible for the acetate production at this temperature, as species of this genus are known for its acetogenic capacities with most species favouring growth at a temperature range between 30 and 45°C (Wade, 2011). Conversely, lower levels of acetate produced at 50°C and 65°C were likely the result of activity from strains of the Thermoanaerobacteria and the Moorellia class, and the Acetothermiia phylum.

Methanobacteria members were present at all three temperatures. The largest proportion in the relative abundance analysis was observed at 50°C, but these methanogens composed the majority of the community at both 50 and 65°C. Methanobacteria are typically encountered in deep subsurface high-pressure environments (Kotelnikova and Pedersen, 1997; Miettinen et al., 2015; Ranchou-Peyruse et al., 2021). To this class, the amplicon analysis assigned only the genus *Methanothermobacter*. This genus is known to be thermophilic, with the fastest growth observed between 55 and 65°C, and they are known to perform methanogenesis using H_2 and CO_2. Some strains can also use formate as an electron donor (Wasserfallen et al., 2000; Shima and Thauer, 2021). *Methanothermobacter* also includes isolates originating from petroleum reservoirs, and it has been suggested that in these environments, methanogenesis by *Methanothermobacter* is coupled with syntrophic acetate oxidation (Cheng et al., 2011).

Clostridia comprise the majority of the community at 35°C, at which the highest amounts of acetate were produced, with only minor proportions at 50 and 65°C. The analysis primarily detected in the incubations members of the genus *Eubacterium* and, to a lesser extent, *Soehngenia*. Members of the genus *Eubacterium* are heterogeneous (Wade, 2006), with some

isolates previously identified as homoacetogenic strains within an underground gas storage (UGS) environment (Tarasov et al., 2011; Vigneron et al., 2017). Conversely, the genus *Soehngenia* includes fermenters capable of producing acetate, H_2, and CO_2, and also includes representatives isolated from oilfields (Parshina et al., 2003; Nazina et al., 2020).

Members of the class Thermoanaerobacteria increased in proportion at higher temperatures and constituted the second largest microbial proportion at 65°C. At 35°C, this class is primarily represented by the genus *Thermoanaerobacterium*, while at 50 and 65°C, it includes mainly the genera *Thermoanaerobacter* and *Caldanaerobacter*. The genera *Thermoanaerobacter* and *Thermoanaerobacterium* comprise thermophilic, obligate anaerobic bacteria that ferment polysaccharides and carbohydrates, commonly found in deep subsurface environments. They mainly produce L-lactic acid, acetic acid, ethanol, CO_2, and H_2 (Shaw et al., 2010). Similarly, the genus *Caldanaerobacter* consists of fermentative thermophilic bacteria, isolated from oil reservoirs. Members of this genus are obligate organotrophs and ferment various sugars, peptides, and polysaccharides, generating acetate, L-alanine, H_2, and CO_2 as the main products (Fardeau et al., 2004; Kozina et al., 2010).

In minor proportions, the class Moorellia is found in the community with the highest percentage in the relative abundance composition at 50°C, followed by 65°C. Members of the genus *Moorella* are known acetogenic bacteria, including thermophilic strains previously detected in oil reservoirs. These strains have been shown to grow on various carbon sources, including H_2/CO_2, CO, sugars, formate, and methanol, serving as substrates (Redl et al., 2020; Hidalgo et al., 2021; Jia et al., 2023).

The class designation Incertae Sedis signifies that the broader relationships are unknown or undefined, under which the genus *Thermolithobacter* was consistently associated with the incubations at all temperatures. This genus currently comprises only two described thermophilic and lithotrophic species isolated from terrestrial hot springs, and its closest family is Incertae Sedis XVI. These strains are capable of oxidizing H_2 coupled with the reduction of iron(III) oxide to magnetite at temperatures ranging from 50 to 75°C. Additionally, hydrogenic oxidation of CO has been observed in this genus (Sokolova et al., 2007; Sokolova and Wiegel, 2014). A strain closely related to this genus was also identified in an incubation study of an oil field (Cheng et al., 2013).

The candidate phylum Acetothermiia is detected at similar proportions at the different temperatures, but little is known about their physiology and ecological importance. Members of this phylum are thermophilic and have also been detected in the deep biosphere. Based on genome analysis, an anaerobic chemoheterotrophic lifestyle has been proposed, in which the bacterium obtains its energy and carbon through the fermentation of peptides, amino acids, and simple sugars, resulting in the formation of acetate, formate, and H_2 (Hao et al., 2018; Bergsten et al., 2021).

3.2.2 “H_2/CO_2-nutrient supplemented” condition

In the "H_2/CO_2-nutrient supplemented" incubations, which were cultured with 80% H_2/20% CO_2, $NaHCO_3$, and additional nutrients to boost microbial growth, H_2 consumption rates were higher compared to the "H_2-only" incubations, with the highest rates observed notably at 65°C. In contrast to the "H_2-only" incubations, acetate production was higher, peaking at 50°C instead of 35°C.

The microbial community structure unveiled the microorganisms responsible for the various processes observed in the “H_2/CO_2-nutrient supplemented” incubations at varying temperatures. Samples for community analysis were taken at the start of the incubation and

at the end of each cycle before the headspace of the incubation bottles was gas exchanged again with 80% H_2/20% CO_2. The sequencing data from the incubation samples at 80°C was of poor quality and thus omitted from this report.

The relative abundance composition at the start of the "H_2/CO_2-nutrient supplemented" incubations resembled the one for the "H_2-only" incubations. The initial makeup showed a significant presence of the archaeal class Methanobacteria, alongside bacteria from the genus *Thermolithobacter* (categorized as Incertae Sedis within the Bacillota), the candidate phylum Acetothermiia, and the class Bacilli. Moreover, the microbial community also included classes like Clostridia, Thermoanaerobacteria, Moorellia, Thermotogae, Thermacetogenia, and Bacteroidia, although in smaller amounts.

With the addition of supplementary nutrients, methanogens of the class Methanobacteria appeared to be outcompeted at 35°C by acetogenic members of the Clostridia class, mainly associated with the genera *Sporobacterium* and *Eubacterium*, as well as by the class Thermoanaerobacteria, mainly represented by the genus *Thermoanaerobacterium*. This reflects the absence of CH_4 production and the abundant formation of acetate at this temperature. Based on the composition of the acetogens, it can be hypothesized that acetate was partly produced by homoacetogenesis by *Eubacterium* species and potentially through the fermentative pathway involving *Sporobacterium* and *Thermoanaerobacterium* (assuming the presence of organics in the brine).

The competition between acetogens and methanogens at 50°C followed a similar pattern, but occurred more gradually, with methanogens still abundantly present at T1 (end of first incubation cycle), but almost absent at T2 (end of second incubation cycle). This change in the community reflects a low CH_4 production observed until T1, but almost no CH_4 production at T2 as methanogens decreased in abundance and were largely replaced by acetogens from the Thermoanaerobacteria. These acetogens likely outcompeted the Clostridia at this temperature. Thermoanaerobacteria were primarily composed of fermentative acetogens from the genus *Thermoanaerobacterium*, followed in proportion by *Thermoanaerobacter* and *Caldanaerobacter.* Small proportions in the community were also occupied by the classes Moorellia and Thermacetogenia, both possibly capable of homoacetogenesis and the fermentative acetogenic pathway. Thermacetogenia may also perform syntrophic acetate oxidation (Hattori et al., 2000; Keller et al., 2019), along with the genus *Tepidanaerobacter* within the class Thermovenabulia (Sekiguchi et al., 2006; Westerholm et al., 2011). In cases where syntrophic acetate oxidation occurs, acetate is converted to H_2 and CO_2, which are subsequently transformed to CH_4 by hydrogenotrophic methanogens.

At 65°C, the community composition stabilized from T2 onwards. Throughout, Methanobacteria predominantly comprised the community, reflecting the robust CH_4 production, which was observed at this temperature. Following Methanobacteria, Thermotogae were the primary acetogens, with Thermacetogenia and Thermoanaerobacter (genera *Caldanaerobacter* and *Thermoanaerobacterium*) present in smaller amounts. All three are capable of performing the fermentative acetogenic pathway, and Thermacetogenia can also perform syntrophic acetate oxidation, potentially explaining the decrease in acetate after T1. However, all three acetogens decreased further in abundance from T2 onwards.

At 35°C, the class Clostridia is predominantly composed of the genera *Sporobacterium* and *Eubacterium*. *Sporobacterium* consists of strictly chemo-organotrophic, anaerobic bacteria reported to perform fermentative acetogenesis using crotonate, methanol, and a wide range of aromatic compounds. They thrive optimally between 25 and 45°C. Currently, the genus comprises only one species, which was isolated from an olive mill wastewater treatment digester (Mechichi et al., 1999; Mechichi and Patel, 2015).

Members of the sulfate-reducing bacteria belonging to the *Desulfofundulus* genus within the Desulfomatoculia were also detected at 50°C, despite the absence of detected sulfide. They are capable of utilizing sulfate, sulfite, and thiosulfate as electron acceptors, coupled with growth either chemoorganoheterotrophically on organic acids, fatty acids, benzoate, and alcohols to produce acetate or CO_2, or chemolithoautotrophically using H_2/CO_2 and formate. This genus includes thermophilic strains, and has been previously found in oil reservoirs and the deep subsurface (Nazina et al., 1988; Watanabe et al., 2018, 2020).

The class Thermotogae comprised according to the sequencing data, the second largest relative portion of the community at 65°C, with the genera *Fervidobacterium* and *Pseudothermotoga* present in similar proportions. The phylum Thermotogae encompasses extremely thermophilic anaerobic bacteria, with optimal growth temperatures reaching up to 80°C. Both genera engage in fermentative metabolism and have been identified in various geothermal environments, including oil reservoirs (Javier-Lopez et al., 2023). *Fervidobacterium* are thermophiles (60-80°C) with a glycolytic metabolism that utilizes a wide range of carbohydrates such as glucose, sucrose, starch, and lactose, producing lactate, acetate, H_2, and CO_2 as end products (Patel et al., 1985; Javier-Lopez et al., 2023). Similarly, *Pseudothermotoga* are thermophiles that grow around 60-70°C and ferment sugars, primarily yielding acetate, CO_2, and H_2 as end products (Bhandari and Gupta, 2014; Farrell et al., 2021).

At 50 and 65°C, small relative portions of the community were attributed to the newly identified family Thermacetogeniaceae. These anaerobic thermophiles, represented by the genus *Thermacetogenium*, possess a dual metabolic capability. They can produce acetate through the fermentation of substrates like formate or by reducing CO_2 with H_2. Additionally, they can also function as acetate-oxidizing bacteria. This can occur either alone by coupling acetate oxidation to sulfate or thiosulfate reduction, or in a syntrophic relationship with a methanogen under methanogenic conditions. In this syntrophic process, acetate is converted to H_2 and CO_2, which the methanogen then utilizes to produce CH_4 (Hattori et al., 2000; Keller et al., 2019).

The class Thermovenabulia, to which the genus *Tepidanaerobacter* was assigned, was present in the incubations at T1 of 50°C. Bacteria of *Tepidanaerobacter* grow thermotolerant in a range of 20-55°C and chemoorganotrophically on various carbohydrates. In co-culture with a hydrogenotrophic methanogen, strains are either capable of degrading alcohol and lactate synthrophically or performing syntrophic acetate-oxidation (Sekiguchi et al., 2006; Westerholm et al., 2011).

3.2.3 Comparison of community composition between "H_2-only" and "H_2/CO_2-nutrient supplemented" conditions

The addition of supplementary nutrients generally resulted in a decrease in the overall diversity evenness in the samples, while certain classes experienced a significant boost, allowing them to outcompete others. At 35 and 50°C, the nutrient supplementation stimulated the growth of Thermoanaerobacteria, leading to a reduction in the relative abundance of the methanogens from Methanobacteria. However, at 65°C, members of Methanobacteria dominated over all other strains present in the "H_2-only" incubations, including Thermoanaerobacteria, Moorellia, *Thermolithobacter* (Incertae Sedis), and Acetothermia, leaving only Thermatogae to establish themselves in this incubation. The class Acetothermia was outcompeted in all samples with added nutrients.

Comparing the starting culture to those of the incubations, it is evident that at all three temperatures, both in the "H_2-only" and the "H_2/CO_2-nutrient supplemented" incubations, strains of the class Bacilli were outcompeted by hydrogenotrophic archaea and bacteria.

4. Conclusions

This report investigates the impact of temperature and nutrient supplementation on microbial activity and community composition using incubation experiments with HCO_3-buffered brine samples from a potential H_2 storage site. Incubations under both "H_2-only" and "H_2/CO_2-nutrient supplemented" conditions revealed microbial activity, including H_2 consumption and the production of CH_4 and acetate, with no detectable H_2S. However, rates and proportions of these processes varied with temperature and nutrient availability. Interestingly, this case study showed an interplay between methanogenesis and acetogenesis. Linking the chemical analysis with the microbial community composition analysis allowed us to hypothesize that CH_4 was likely produced by the hydrogenotrophic methanogens from the genus *Methanothermobacter*, while acetate was likely produced via homoacetogenesis and fermentative pathways, leading to a disbalance in stoichiometry of H_2 consumption when only hydrogenotrophic pathways were considered.

In the “H_2-only” incubations, limited H_2 consumption occurred at 35, 50, and 65°C, accompanied by CH_4 and acetate production, albeit with variations. Additionally, CH_4 production led to a significant increase in pH, reasonably limiting further microbial activity. *Methanothermobacter* members (class Methanobacteria) are potentially responsible for CH_4 production, while acetate production occurred via homoacetogenesis and fermentative pathways likely by members of the Bacillota phylum, including Clostridia, Thermoanaerobacteria, and Moorellia, as well as candidate phylum Acetothermiia. It has to be emphasized that these “H_2-only” incubations were HCO_3-buffered and therefore the microbial community could have used the bicarbonate as carbon source for the methanogenesis or homoacetogenesis in the brine samples.

In the "H_2/CO_2-nutrient supplemented" incubations, H_2 consumption rates were notably higher than in the “H_2-only” incubations, especially at 65°C. Acetate production increased significantly, particularly at 35 and 50°C, resulting in a decrease in pH and inhibition of further microbial activity. CH_4 production was prominent at 65°C, which corresponded with changes in the relative abundance of the microbial community composition at different temperatures. However, CO_2 and nutrient supplementation led to less complex microbial communities, with certain taxa outcompeting others rapidly. *Methanothermobacter* members were again dominant and potentially responsible for hydrogenotrophic methanogenesis, while acetate was predominantly formed through homoacetogenesis and fermentative pathways by members of Clostridia, Thermoanaerobacteria, Moorellia, Thermotogae, and Thermacetogenia. The latter, particularly prominent at 65°C, could possibly perform syntrophic acetate reduction in combination with *Methanothermobacter*, explaining the reduction of acetate over time at this temperature.

Translating these results to a potential H_2 storage application in Case 1's reservoir, which has a temperature of 83°C, we can hypothesize that the microbial risks of H_2 consumption would be very low. This is based on the observation that no H_2 consumption occurred during a four-month period in the "H_2-only" incubations at 80°C. However, it is expected that CH_4 will form *in situ*, as observed in those incubations, and its concentrations will likely continue to increase due to the absence of immediate substrate limitations, given the large surface areas in the reservoir's pore systems. The higher H_2 consumption and product formation rates in incubations with CO_2 and nutrient addition highlight the importance of analyzing the chemical composition of the reservoir’s brine and rock beforehand. Including these data in the decision-making process for selecting a potential H_2 storage site is crucial.

Overall, the results highlight the complex interplay between environmental conditions, nutrient availability, and microbial community composition. Understanding these interactions is crucial

for predicting subsurface ecosystem dynamics and potential applications such as subsurface H_2 storage. Further research into the metabolic capabilities of dominant microbial groups and their response to environmental changes is required for a comprehensive understanding of subsurface microbial ecosystems. Additionally, future studies should also focus on the effects of high partial H_2 pressure to better simulate H_2 storage conditions and to derive valuable insights for potential applications in porous reservoirs. Despite its limitations, this report emphasizes the importance of comprehending microbial activities and community compositions in subsurface environments to estimate risks and optimize H_2 storage strategies, in line with the European Union's objective of realizing a sustainable energy future.

5. References

Basso, O., Lascourreges, J. F., Le Borgne, F., Le Goff, C., and Magot, M. (2009). Characterization by culture and molecular analysis of the microbial diversity of a deep subsurface gas storage aquifer. *Res. Microbiol.* 160, 107–116. doi: 10.1016/j.resmic.2008.10.010

Bergsten, P., Vannier, P., Klonowski, A. M., Knobloch, S., Gudmundsson, M. T., Jackson, M. D., et al. (2021). Basalt-hosted microbial communities in the subsurface of the young volcanic island of Surtsey, Iceland. *Front. Microbiol*. 12: 728977. doi: 10.3389/fmicb.2021.728977

Bhandari, V., and Gupta, R. S. (2014). Molecular signatures for the phylum (class) Thermotogae and a proposal for its division into three orders (Thermotogales, Kosmotogales ord. nov. and Petrotogales ord. nov.) containing four families (Thermotogaceae, Fervidobacteriaceae fam. nov., Kosmotogaceae fam. nov. and Petrotogaceae fam. nov.) and a new genus *Pseudothermotoga* gen. nov. with five new combinations. *Antonie van Leeuwenhoek* 105, 143–168. doi: 10.1007/s10482-013-0062-7

Bordenave, S., Chatterjee, I., and Voordouw, G. (2013). Microbial community structure and microbial activities related to CO_2 storage capacities of a salt cavern. *Int. Biodeterior. Biodegradation* 81, 82–87. doi: 10.1016/j.ibiod.2012.08.001

Buriánková, I., Molíková, A., Vítězová, M., Onderka, V., Vítěz, T., Urbanová, I., et al. (2022). Microbial communities in underground gas reservoirs offer promising biotechnological potential. *Fermentation* 8, 251. doi: 10.3390/fermentation8060251

Cheng, L., Dai, L., Li, X., Zhang, H., and Lu, Y. (2011). Isolation and characterization of *Methanothermobacter crinale* sp. nov., a novel hydrogenotrophic methanogen from the Shengli oil field. *Appl. Environ. Microbiol*. 77, 5212–5219. doi: 10.1128/AEM.00210-11

Cheng, L., He, Q., Ding, C., Dai, L. R., Li, Q., and Zhang, H. (2013). Novel bacterial groups dominate in a thermophilic methanogenic hexadecane-degrading consortium. *FEMS Microbiol. Ecol*. 85, 568–577. doi: 10.1111/1574-6941.12141

Claassens, N. J., Sánchez-Andrea, I., Sousa, D. Z., and Bar-Even, A. (2018). Towards sustainable feedstocks: A guide to electron donors for microbial carbon fixation. *Curr. Opin. Biotechnol.* 50, 195–205. doi: 10.1016/j.copbio.2018.01.019

Dohrmann, A. B., and Krüger, M. (2023). 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.* 57, 1092-1102. doi: 10.1021/acs.est.2c07303

Dopffel, N., Jansen, S., and Gerritse, J. (2021). Microbial side effects of underground hydrogen storage – Knowledge gaps, risks and opportunities for successful implementation. *Int. J. Hydrogen Energy* 46, 8594–8606. doi: 10.1016/j.ijhydene.2020.12.058

Escudero, C., Oggerin, M., and Amils, R. (2018). The deep continental subsurface: the dark

biosphere. *Int. Microbiol*. 21, 3–14. doi: 10.1007/s10123-018-0009-y

Fardeau, M. L., Bonilla Salinas, M., L'Haridon, S., Jeanthon, C., Verhé, F., Cayol, J. L., et al. (2004). Isolation from oil reservoirs of novel thermophilic anaerobes phylogenetically related to *Thermoanaerobacter subterraneus*: reassignment of *T. subterraneus*, *Thermoanaerobacter yonseiensis*, *Thermoanaerobacter tengcongensis* and *Carboxydibrachium pacificum* to *Caldanaerobacter subterraneus* gen. nov., sp. nov., comb. nov. as four novel subspecies. *Int. J. Syst. Evol. Microbiol*. 54, 467–474. doi: 10.1099/ijs.0.02711-0

Farrell, A., Nesbø, C. L., Zhaxybayeva, O., and L'Haridon, S. (2021). "Pseudothermotoga," in Bergey's Manual of Systematics of Archaea and Bacteria, (Wiley), 1–12. doi: 10.1002/9781118960608.gbm01861

Fredrickson, J. K., McKinley, J. P., Bjornstad, B. N., Long, P. E., Ringelberg, D. B., White, D.C., et al. (1997). Pore-size constraints on the activity and survival of subsurface bacteria in a late cretaceous shale-sandstone sequence, northwestern New Mexico. *Geomicrobiol. J.* 14, 183–202. doi: 10.1080/01490459709378043

Gulliver, D., Lipus, D., Ross, D., and Bibby, K. (2019). Insights into microbial community structure and function from a shallow, simulated CO_2-leakage aquifer demonstrate microbial selection and adaptation. *Environ. Microbiol. Rep*. 11, 338–351. doi: 10.1111/1758-2229.12675

Hao, L., McIlroy, S. J., Kirkegaard, R. H., Karst, S. M., Fernando, W. E. Y., Aslan, H., et al. (2018). Novel prosthecate bacteria from the candidate phylum Acetothermia. *ISME J*. 12, 2225–2237. doi: 10.1038/s41396-018-0187-9

Hattori, S., Kamagata, Y., Hanada, S., and Shoun, H. (2000). *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int. J. Syst. Evol. Microbiol*. 50, 1601-1609. doi: 10.1099/00207713-50-4-1601

Heinemann, N., Alcalde, J., Miocic, J. M., Hangx, S. J. T., Kallmeyer, J., Ostertag-Henning, C., et al. (2021). Enabling large-scale hydrogen storage in porous media-the scientific challenges. *Energy Environ. Sci*. 14, 853–864. doi: 10.1039/d0ee03536j

Hidalgo, K. J., Sierra-Garcia, I. N., Zafra, G., and de Oliveira, V. M. (2021). Genome-resolved meta-analysis of the microbiome in oil reservoirs worldwide. *Microorganisms* 9. doi: 10.3390/microorganisms9091812

Ivanova, A. E., Borzenkov, I. A., Tarasov, A. L., Milekhina, E. I., and Belyaev, S. S. (2007). A microbiological study of an underground gas storage in the process of gas extraction. *Microbiology* 76, 461–468. doi: 10.1134/S0026261707040121

Javier-Lopez, R., Mandolini, E., Dzhuraeva, M., Bobodzhanova, K., and Birkeland, N. K. (2023). *Fervidobacterium pennivorans* subsp. *keratinolyticus* subsp. nov., a novel feather-degrading anaerobic thermophile. *Microorganisms* 11: 22. doi: 10.3390/microorganisms11010022

Jia, D., Deng, W., Hu, P., Jiang, W., and Gu, Y. (2023). Thermophilic *Moorella thermoacetica* as a platform microorganism for C1 gas utilization: physiology, engineering, and applications. *Bioresour. Bioprocess* 10: 61. doi: 10.1186/s40643-023-00682-z

Kadnikov, V. V., Mardanov, A. V., Beletsky, A. V., Karnachuk, O. V., and Ravin, N. V. (2020). Microbial life in the deep subsurface aquifer illuminated by metagenomics. *Front. Microbiol.* 11: 572252. doi: 10.3389/fmicb.2020.572252

Keller, A., Schink, B. and Müller, N. (2019) Energy-conserving enzyme systems active during syntrophic acetate oxidation in the thermophilic bacterium *Thermacetogenium phaeum*. *Front. Microbiol*. 10:2785. doi: 10.3389/fmicb.2019.02785

Kotelnikova, S., Pedersen, K. (1997). Evidence for methanogenic Archaea and homoacetogenic Bacteria in deep granitic rock aquifers. *FEMS Microbiol. Rev* 20, 339-349. doi: 10.1016/S0168-6445(97)00016-8

Kozina, I. V., Kublanov, I. V., Kolganova, T. V., Chernyh, N. A., and Bonch-Osmolovskaya, E. A. (2010). *Caldanaerobacter uzonensis* sp. nov., an anaerobic, thermophilic, heterotrophic bacterium isolated from a hot spring. *Int. J. Syst. Evol. Microbiol*. 60, 1372–1375. doi: 10.1099/ijs.0.012328-0

Mechichi, T., Labat, M., Garcia, J. L., Thomas, P., and Patel, B. K. C. (1999). *Sporobacterium olearium* gen. nov., sp. nov., a new methanethiol-producing bacterium that degrades aromatic compounds, isolated from an olive mill wastewater treatment digester. *Int. J. Syst. Bacteriol*. 49, 1741–1748. doi: 10.1099/00207713-49-4-1741.

Mechichi, T., and Patel, B. K. C. (2015). "*Sporobacterium*", in Bergey's Manual of Systematics of Archaea and Bacteria, (Wiley), 1–5. doi: 10.1002/9781118960608.gbm00654

Miettinen, H., Kietäväinen, R., Sohlberg, E., Numminen, M., Ahonen, L. and Itävaara M (2015). Microbiome composition and geochemical characteristics of deep subsurface high-pressure environment, Pyhäsalmi mine Finland. *Front. Microbiol*. 6:1203. doi: 10.3389/fmicb.2015.01203

Nazina, T. N., Bidzhieva, S. K., Grouzdev, D. S., Sokolova, D. S., Tourova, T. P., Parshina, S. N., et al. (2020). *Soehngenia longivitae* sp. nov., a fermenting bacterium isolated from a petroleum reservoir in Azerbaijan, and emended description of the genus *Soehngenia*. *Microorganisms* 8, 1967. doi: 10.3390/microorganisms8121967

Nazina, T. N., Ivanova, A. E., Kanchaveli, L. P., and Rozanova, E. P. (1988). A new sporeforming thermophilic methylotrophic sulfate-reducing bacterium, *Desulfotomaculum kuznetsovii* sp. nov. *Microbiology* 57, 823–827.

Parshina, S. N., Kleerebezem, R., Sanz, J. L., Lettinga, G., Nozhevnikova, A. N., Kostrikina, N. A., et al. (2003). *Soehngenia saccharolytica* gen. nov., sp. nov. and *Clostridium amygdalinum*

sp. nov., two novel anaerobic, benzaldehyde-converting bacteria. *Int. J. Syst. Evol. Microbiol.* 53, 1791–1799. doi: 10.1099/ijs.0.02668-0

Patel, B. K. C., Morgan, H. W., and Daniel, R. M. (1985). *Fervidobacterium nodosum* gen. nov. and spec. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. *Arch. Microbiol.* 141, 63–69. doi: 10.1007/BF00446741

Plugge, C. M. (2005). Anoxic media design, preparation, and considerations. *Methods Enzymol.* 397, 3–16. doi: 10.1016/S0076-6879(05)97001-8

Ranchou-Peyruse, M., Auguet, J. C., Mazière, C., Restrepo-Ortiz, C. X., Guignard, M., Dequidt, D., et al. (2019). Geological gas-storage shapes deep life. *Environ. Microbiol*. 21, 3953–3964. doi: 10.1111/1462-2920.14745

Ranchou-Peyruse, M., Guignard, M., Casteran, F., Abadie, M., Defois, C., Peyret, P., et al. (2021). Microbial diversity under the influence of natural gas storage in a deep aquifer. *Front. Microbiol.* 12: 688929. doi: 10.3389/fmicb.2021.688929

Redl, S., Poehlein, A., Esser, C., Bengelsdorf, F. R., Jensen, T., Jendresen, C. B., et al. (2020). Genome-based comparison of all species of the genus *Moorella*, and status of the species *Moorella thermoacetica* and *Moorella thermoautotrophica*. *Front. Microbiol.* 10: 3070. doi: 10.3389/fmicb.2019.03070

Schwab, L., Popp, D., Nowack, G., Bombach, P., Vogt, C., and Richnow, H. H. (2022). Structural analysis of microbiomes from salt caverns used for underground gas storage. *Int. J. Hydrogen Energy* 47, 20684–20694. doi: 10.1016/j.ijhydene.2022.04.170

Schwab, L., Prinsen, L., Nowack, G., Popp, D., Noll, M., Vogt, C., et al. (2023). Sulfate reduction and homoacetogenesis at various hypersaline conditions: Implications for H_2 underground gas storage. *Front. Energy Res*. 11: 1125619. doi: 10.3389/fenrg.2023.1125619

Sekiguchi, Y., Imachi, H., Susilorukmi, A., Muramatsu, M., Ohashi, A., Harada, H., et al. (2006). *Tepidanaerobacter syntrophicus* gen. nov., sp. nov., an anaerobic, moderately thermophilic, syntrophic alcohol- and lactate-degrading bacterium isolated from thermophilic digested sludges. *Int. J. Syst. Evol. Microbiol*. 56, 1621–1629. doi: 10.1099/ijs.0.64112-0

Sharma, P. K., and McInerney, M. J. (1994). Effect of grain size on bacterial penetration, reproduction, and metabolic activity in porous glass bead chambers. *Appl. Environ. Microbiol*. 60, 1481–1486. doi: 10.1128/aem.60.5.1481-1486.1994.

Shaw, A. J., Hogsett, D. A., and Lynd, L. R. (2010). Natural competence in *Thermoanaerobacter* and *Thermoanaerobacterium* species. *Appl. Environ. Microbiol.* 76, 4713–4719. doi: 10.1128/AEM.00402-10

Shima, S., and Thauer, R. K. (2021). *Methanothermobacter* – Biokatalysator für die Energiewende. *BIOspektrum* 27, 14–17. doi: 10.1007/s12268-021-1530-8

Šmigáň, P., Greksák, M., Kozánková, J., Buzek, F., Onderka, V., and Wolf, I. (1990). Methanogenic bacteria as a key factor involved in changes of town gas stored in an underground reservoir. *FEMS Microbiol. Lett*. 73, 221–224. doi: 10.1016/0378-1097(90)90733-7

Sokolova, T., Hanel, J., Onyenwoke, R. U., Reysenbach, A. L., Banta, A., Geyer, R., et al. (2007). Novel chemolithotrophic, thermophilic, anaerobic bacteria *Thermolithobacter ferrireducens* gen. nov., sp. nov. and *Thermolithobacter carboxydivorans* sp. nov. *Extremophiles* 11, 145–157. doi: 10.1007/s00792-006-0022-5

Sokolova, T., and Wiegel, J. (2014). "The family Thermolithobacteriaceae", in *The Prokaryotes - Firmicutes and Tenericutes*, ed. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt and F. Thompson. (Berlin-Heidelberg: Springer), 427–431. doi: 10.1007/978-3-642-30120-9_220

Stams, A. J. M., Van Dijk, J. B., Dijkema, C., and Plugge, C. M. (1993). Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. *Appl. Environ. Microbiol*. 59, 1114–1119. doi: 10.1128/aem.59.4.1114-1119.1993

Standard methods committee of the American public health association, American water works association, and water environment federation (2017). "4500-S_2^- sulfide," in: *Standard methods for the examination of water and wastewater*, ed. W. C. Lipps, T. E. Baxter, E. Braun-Howland. (Washington DC: APHA Press). doi: 10.2105/SMWW.2882.096

Tarasov, A. L., Borzenkov, I. A., Chernykh, N. A., and Belyayev, S. S. (2011). Isolation and investigation of anaerobic microorganisms involved in methanol transformation in an underground gas storage facility. *Microbiology* 80, 172–179. doi: 10.1134/S0026261711020147

Thaysen, E. M., McMahon, S., Strobel, G. J., Butler, I. B., Ngwenya, B. T., Heinemann, N., et al. (2021). Estimating microbial growth and hydrogen consumption in hydrogen storage in porous media. *Renew. Sustain. Energy Rev.* 151, 111481. doi: 10.1016/j.rser.2021.111481

Vigneron, A., Alsop, E. B., Lomans, B. P., Kyrpides, N. C., Head, I. M., and Tsesmetzis, N. (2017). Succession in the petroleum reservoir microbiome through an oil field production lifecycle. *ISME J*. 11, 2141–2154. doi: 10.1038/ismej.2017.78

Wade, W.G. (2006). "The Genus *Eubacterium* and related genera", in: *The Prokaryotes*, ed. M. Dworkin,S. Falkow, E. Rosenberg, K. H. Schleifer, E. Stackebrandt, (New York: Springer), 823–835. doi: 10.1007/0-387-30744-3_28

Wade, W. G. (2015). "*Eubacterium*", in Bergey's Manual of Systematics of Archaea and Bacteria, (Wiley), 1–36. doi: 10.1002/9781118960608.gbm00629

Wasserfallen, A., Nölling, J., Pfister, P., Reeve, J., and Conway de Macario, E. (2000). Phylogenetic analysis of 18 thermophilic *Methanobacterium* isolates supports the proposals to create a new genus, *Methanothermobacter* gen. nov., and to reclassify several isolates in three species, *Methanothermobacter thermautotrophicus* comb. nov., *Methanothermobacter wolfeii* comb. nov., and *Methanothermobacter marburgensis* sp. nov.. *Int. J. Syst. Evol. Microbiol.* 50, 43–53. doi: 10.1099/00207713-50-1-43

Watanabe, M., Fukui, M., and Kuever, J. (2020). "*Desulfofundulus*", in Bergey's Manual of Systematics of Archaea and Bacteria, (Wiley), 1–8. doi: 10.1002/9781118960608.gbm01780

Watanabe, M., Kojima, H., and Fukui, M. (2018). Review of *Desulfotomaculum* species and proposal of the genera *Desulfallas* gen. nov., *Desulfofundulus* gen. nov., *Desulfofarcimen* gen. nov. and *Desulfohalotomaculum* gen. nov. *Int. J. Syst. Evol. Microbiol*. 68, 2891–2899. doi: 10.1099/ijsem.0.002915

Westerholm, M., Roos, S., and Schnürer, A. (2011). *Tepidanaerobacter acetatoxydans* sp. nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from two ammonium-enriched mesophilic methanogenic processes. *Syst. Appl. Microbiol*. 34, 260–266. doi: 10.1016/j.syapm.2010.11.018