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# HyUSPRe

## Hydrogen Underground Storage in Porous Reservoirs

### **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.)**

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## The HyUSPRe consortium



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## Executive summary

Underground storage of  $H_2$  in depleted porous reservoirs, salt caverns and aquifers presents a viable option, but also harbors challenges due to potential microbial metabolic activities. Microorganisms, particularly those involved in methanogenesis, sulfate reduction and acetogenesis, can impact  $H_2$  storage in various ways, including the consumption of the stored  $H_2$ , the production of contaminating gases like sulfide ( $H_2S$ ) and methane ( $CH_4$ ), the induction of microbial-influenced corrosion and pipe clogging by 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 enables predicting these microbial-influenced risks. One promising approach is to estimate the microbial kinetics, which are linked to a complex interplay between environmental conditions and microbial activity, by modeling to assess risks in the field.

This report elaborates on the effect of temperature (range 35-80°C) and nutrient addition on incubation experiments with reservoir brine samples from a potential  $H_2$  storage site. Microbial  $H_2$  consumption and formation of  $CH_4$  and acetate varied with the temperature (less activity at 80°C) and were boosted by the addition of minerals and vitamins during the incubation experiments. Furthermore, the accumulation of  $CH_4$  and acetate resulted in pH fluctuations, influencing metabolic activities. These observations confirm that analyzing microbiological and geochemical characteristics of potential storage sites and monitoring them continuously is essential.

Data on microbial growth,  $H_2$  consumption and product formation were implemented in numerical simulations to provide insights into microbial growth dynamics by determining the maximum growth rate (1/s), the yield factor (1/mol), and the half velocity constant of  $H_2$  and  $CO_2$  (mol/mol). This helped to parameterize a model for predicting microbial kinetics in subsurface environments. These simulations offered valuable insights into the impact of microbial activity on  $H_2$  storage operations. Hence, modeling microbial growth and reactions based on laboratory studies can offer a comprehensive evaluation of risks associated with subsurface  $H_2$  storage, to enable reaching the European Union's goal of a sustainable energy future.

The raw microbial data 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).

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## About HyUSPRe

# Hydrogen **U**nderground **S**torage in **P**orous **R**eservoirs

The HyUSPRe project researches the feasibility and potential of implementing large-scale underground geological storage of 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.

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## 1. Introduction

The European Union is committed to realizing a zero-emissions energy system across Europe by 2050. To facilitate this transition, widespread adoption of renewable energies, such as wind and solar power, is imperative. However, these techniques are subject to production variations coupled with fluctuations in energy consumption throughout the year. To address this challenge, molecular energy carriers like hydrogen ( $H_2$ ) have been proposed for storage during periods of energy surplus, which can then be converted back into usable energy when needed on the consumption side. Aboveground storage of  $H_2$  is impractical as the volumes necessary to support a hydrogen energy system cannot be economically realized in tanks or pipelines. Consequently, attention has shifted to underground  $H_2$  storage in depleted natural gas or oil reservoirs, aquifers, and salt caverns. However,  $H_2$  can interact in multifaceted ways with the environment of these subsurface storage sites including potential detrimental effects from microorganisms, posing a challenge to the effectiveness of  $H_2$  storage (Dopffel et al., 2021).

$H_2$  serves as an excellent electron donor in anaerobic microbial metabolisms (Claassens et al., 2018), including hydrogenotrophic sulfate reducers, methanogens, and acetogens (Thaysen et al., 2021). Therefore,  $H_2$  storage could stimulate microbial activity of those functional groups, leading to adverse effects on  $H_2$  storage. Through their metabolic activity, microorganisms can cause the loss of the stored  $H_2$  and the production of  $H_2$ -contaminating products such as hydrogen sulfide ( $H_2S$ ) and methane ( $CH_4$ ) (Dopffel et al., 2021). Further side effects are the potential impact of microbial-influenced corrosion (MIC) leading to metal corrosion of gas transport lines and the accumulation of bio-based solids (microbial biomass, extracellular polymeric substances (EPS), metal sulfides, etc.) causing the clogging of the pipelines and the pore spaces of the reservoir rock, and with this, a reduction in gas injectivity (Dopffel et al., 2021). However, these effects can be predicted through an understanding of the limiting environmental parameters and their impact on microbial kinetics.

The subsurface environment encompasses a range of extreme conditions, including high temperatures, pressures, and salinity (Escudero et al., 2018), as well as limited pore sizes to pass through or to provide space for colonization (Sharma and McInerney, 1994; Fredrickson et al., 1997). Microbial abundance and activity are highly dependent on these environmental factors. Thaysen et al. (2021) conducted a review on the "windows of viability" for microorganisms in the subsurface environment using published data of 532 pure strains of acetogens, SSRM (Sulfur species reducing microorganisms), and methanogens. With this, they established limits to potential risks from microbial activities for subsurface  $H_2$  storage. Their review identified temperature, either alone or in combination with salinity, as the most significant constrain on microbial growth and activity in subsurface environments. In general, sites with temperatures exceeding  $122^\circ C$  are typically considered sterile, while temperatures ranging from  $80^\circ C$  to  $94^\circ C$  generally minimize the likelihood of  $H_2S$  formation. The review illustrated the boundaries of potential growth by plotting critical temperature against NaCl concentrations, indicating that storage sites with temperatures above  $55^\circ C$  and salinity levels surpassing 1.7 M are optimal to mitigate the risk of microbial impact (Thaysen et al., 2021). However, until today no limits for pressure resistance of microbial life could clearly be defined (Dopffel et al., 2021). It is essential to know the limits of microbial life, but also to assess microbial kinetics under potential storage site conditions within the window of microbial viability to broaden site options and to evaluate risks within this range.

Microbial kinetics involves studying metabolic rates of microorganisms and their dependency on various microbiological and environmental factors. The overall kinetics within a community depend on its composition, and even microorganisms with the same metabolism can exhibit significantly different rates under varying environmental conditions. One environmental factor that exerts a profound influence on biological activity is temperature with not only impacting

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enzyme kinetics, but also the thermodynamics of a processes (Jin et al., 2013; Conrad, 2023). Most studies assessing hydrogen consumption kinetics rely on pure cultures growing under optimal conditions in the laboratory, not reflecting the complexity of the environment in view of the microbial community and its conditions (Harris et al., 2007). To better approximate environmental conditions, hydrogenotrophic microbial kinetics can be determined using laboratory experiments with field samples at relevant subsurface conditions. Subsequently, extrapolating to a field simulation through modelling allows for an estimation of the impact of microorganisms on subsurface hydrogen storage in potential sites.

This report elucidates the effect of temperature on the growth, the substrate consumption and the product formation of incubated reservoir brine samples originating from a potential hydrogen storage site. Moreover, the kinetics of hydrogenotrophic methanogenesis were modelled for one temperature from these incubations. The raw data on microbial growth, substrate consumption and product formation, which was 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).



## 2. Measurement of microbial growth, substrate consumption and product formation of subsurface enrichments at different temperatures

To estimate the impact of microorganisms on subsurface H<sub>2</sub> storage, microbial incubation experiments are crucial. With the aim to study the influence of temperature and the addition of nutrients on their metabolic activity, brine samples were incubated and followed over time for their H<sub>2</sub> consumption and the production of CH<sub>4</sub>, H<sub>2</sub>S and acetate.

### 2.1. Experimental set-up

Reservoir brine samples from a potential hydrogen storage site were incubated under two conditions at different temperatures. This environmental sample is referred to as "case 1" henceforth, following the nomenclature used in deliverable D3.3. The first condition simulates hydrogen storage conditions, where the brine samples were incubated with 100% H<sub>2</sub> headspace and in absence of additional nutrients ("H<sub>2</sub>-only"- samples). Under this condition, the microbial community is allowed to grow with only the minerals present in the environmental sample and the supplied H<sub>2</sub>. In the second condition, brine samples were incubated with a 80%H<sub>2</sub>/20%CO<sub>2</sub> headspace, and a surplus of various minerals and vitamins were added to enhance growth ("Nutrient-supplemented"-samples), thus representing the high-impact scenario for hydrogen storage in terms of being a more favorable condition to enhance microbial activities and kinetics. To test the microbial activity, brine samples were cultivated in anaerobic bottles at 1.7 bar, at 35, 50, 65, and 80°C, with three samples and two blanks (sterile controls) per condition (Figure 1). For both conditions, 250 mg/l NaHCO<sub>3</sub>, and 0.48 g/l Na<sub>2</sub>S × 9 H<sub>2</sub>O and 0.5 mg/l resazurin were added. NaHCO<sub>3</sub> was added as buffer and Na<sub>2</sub>S as reducing agent to ensure an anaerobic environment during the cultivation, as indicated colorimetrically by resazurin. Additionally, vitamins and trace elements were added to the "Nutrient-supplemented" samples (Stams et al., 1993; Plugge, 2005). The starting brine sample was analyzed for its chemical composition, samples at the start and at the end were taken for molecular analysis and during the incubation, samples were taken to analyze the gas (H<sub>2</sub>, CH<sub>4</sub>), and the liquid phase composition (pH, H<sub>2</sub>S, SO<sub>4</sub><sup>2-</sup> and acetate).

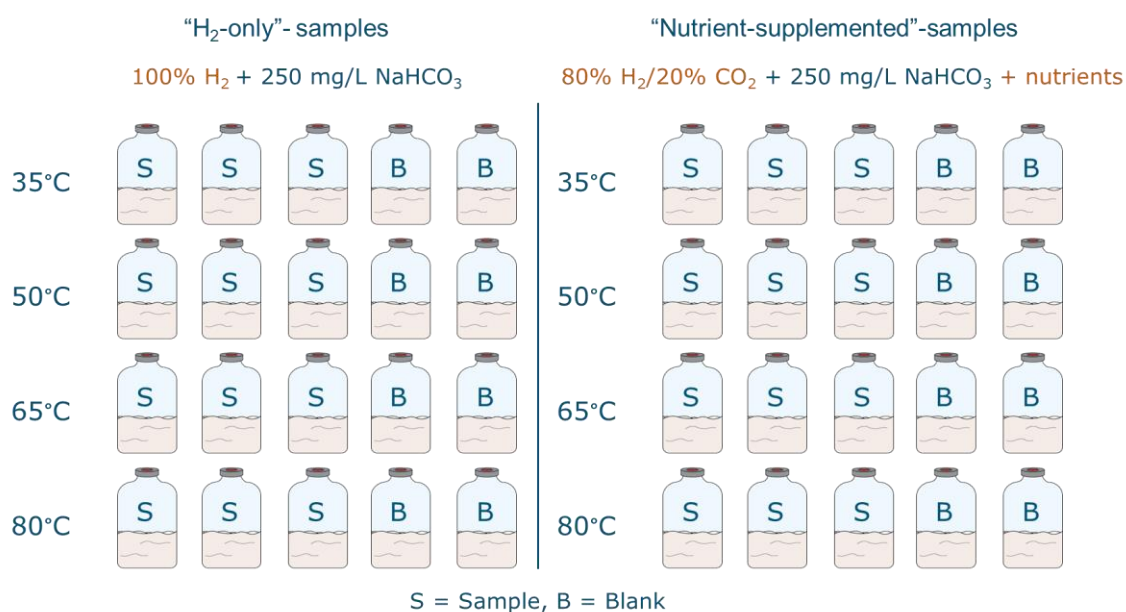


Figure 1. Schematic representation of experimental set-up.

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<sub>2</sub> and CH<sub>4</sub> in the gaseous phase were measured by gas chromatography with a thermal conductivity detector (GC-TCD) on a CompactGC4.0 (Interscience, The Netherlands). H<sub>2</sub>S concentrations in the liquid phase were quantified using the methylene blue method (Standard methods for the examination of water and wastewater, 2017). SO<sub>4</sub><sup>2-</sup> 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). The initial and final cell counts of methanogens were incorporated into the growth model, reflecting methanogenesis as the predominant microbial process throughout the incubation period. Therefore, absolute quantification of methanogens, was performed by qPCR (quantitative polymerase chain reaction), targeting the *mcrA* gene, which encodes the  $\alpha$ -subunit of the methyl-coenzyme M reductase catalyzing the final step in the methanogenesis, leading to methane production. The primer pairs used to amplify this gene are mlas-mod and mcrA-rev (Angel et al., 2011). The qPCR was conducted using a CFX384 Real-Time PCR System (BioRad, USA).

## 2.2. Physical and chemical conditions of the subsurface reservoir

The reservoir of case 1 has a temperature of 83 °C, with an initial pressure of 116 bar, and the average pH of the starting brine sample is 7.34. For the initial chemical composition, 0.049 mM SO<sub>4</sub><sup>2-</sup>, 1 mM acetate and no formate were detected. ICP analysis revealed as dissolved elements 104.65 mg/L sodium, 3.68 mg/L sulfur, and 0.69 mg/L of boron (Table 1). Other dissolved elements analyzed by ICP were below the quantification limit.

**Table 1. Results ICP analysis of dissolved elements of case 1.**

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

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## 2.3. Microbial growth, substrate consumption and product formation

Both incubation conditions, “H<sub>2</sub>-only”-samples and “Nutrient-supplemented”-samples, show consumption of H<sub>2</sub> and production of CH<sub>4</sub> and acetate. However, different rates and product proportions are obtained depending on the temperature.

### 2.3.1. “H<sub>2</sub>-only”-samples

For the “H<sub>2</sub>-only”-samples at 35, 50, and 65°C, which were incubated with NaHCO<sub>3</sub> and 100% H<sub>2</sub>, H<sub>2</sub> consumption was accompanied by CH<sub>4</sub> and acetate generation. No H<sub>2</sub> consumption was measured at 80°C compared to sterile controls. The amount of CH<sub>4</sub> formed increased with higher temperatures, mostly between 50 and 80°C. Acetate was predominantly formed at 35°C and in smaller amounts at higher temperatures, except for at 80°C. No significant H<sub>2</sub>S production was observed at any temperature for the “H<sub>2</sub>-only”-samples.

### 2.3.2. “Nutrient-supplemented”-samples

For the “Nutrient-supplemented”-samples, which were incubated with 80% H<sub>2</sub>/20% CO<sub>2</sub>, NaHCO<sub>3</sub> and nutrients to enhance microbial growth, H<sub>2</sub> consumption rates were significantly higher as compared to “H<sub>2</sub>-only” incubations with rates being again highest at 65°C. Compared to “H<sub>2</sub>-only” incubations, acetate formation was much more pronounced and in this case highest at 50°C (instead of 35°C).

Growth parameters were implemented into a model to predict the maximum growth rate (1/s), the yield factor (1/mol), and the half velocity constant of H<sub>2</sub> and CO<sub>2</sub> (mol/mol). For this purpose, data from case 1 of the “Nutrient-supplemented”-samples at 65°C after the last H<sub>2</sub> refilling of this incubation were chosen.. No fluctuation in pH and no variations in the amount of acetate were observed during this incubation period.

### 3. Determination of growth parameters by matching laboratory observations with numerical simulations

In general, batch experiments are helpful to assess the risk of biochemical reactions during UHS. Estimations of the potential impact of these reactions on the operation of UHS can be obtained by reproducing these experiments with numerical simulations and upscaling the simulation model to field scale. In the following, a numerical model for biochemical reactions during UHS is introduced, and afterwards, the relevant growth kinetics are obtained by a model fitting process to the previously described experimental data from batch experiments.

#### 3.1. Overview of relevant growth model

Various models are available for modeling microbial growth, coming with their individual pros and cons. For the application of biochemical reactions during UHS, the model of (Hagemann, 2018) showed promising results. In this model, the microbial population size is controlled by the continuous growth and decay of microorganisms:

$$\frac{\partial \phi(n \cdot S_w)}{\partial t} = (\psi^{\text{growth}} - \psi^{\text{decay}}) \cdot n \cdot S_w \cdot \phi$$

where  $n$  is the microbial density in  $1/\text{m}^3$ ,  $\phi$  is the porosity (dimensionless),  $S_w$  is the water saturation (dimensionless),  $\psi^{\text{growth}}$  is the growth rate in  $1/\text{s}$ , and  $\psi^{\text{decay}}$  is the decay rate in  $1/\text{s}$ .

The growth depends on the quantity of available substrates, as described by a double Monod (Monod, 1949) equation:

$$\psi^{\text{growth}} = \psi_{\text{max}}^{\text{growth}} \left( \frac{c_w^{S1}}{\alpha^{S1} + c_w^{S1}} \right) \left( \frac{c_w^{S2}}{\alpha^{S2} + c_w^{S2}} \right)$$

where  $\psi_{\text{max}}^{\text{growth}}$  is the maximum growth rate in  $1/\text{s}$ ,  $c_w^S$  is the mole concentration of substrate  $S$  in the aqueous phase and  $\alpha^S$  is the half velocity constant of the respective substrate.

To mimic the decay of microorganisms, which enables a reduction in population size, the decay term is modelled as follows:

$$\psi^{\text{decay}} = b \cdot n$$

where  $b$  is the decay factor in  $\text{m}^3/\text{s}$ .

Based on the growth, the substrate is metabolized and consequently converted. This is considered in the source term of the molar balance equation:

$$q_{\text{bio}}^k = \phi \gamma_{\text{bio}}^k \frac{\psi^{\text{growth}}}{Y} n \cdot S_w$$

where  $\gamma_{\text{bio}}^k$  is the stoichiometric factor and  $Y$  is the yield factor in  $1/\text{mol}$ . In general, the smaller the yield factor, the faster is the reaction speed.

A more extensive description of the model can be found in Hagemann (2018) and Hogeweg et al. (2023b).

## 3.2. Implementation of batch reactor experiments into the open-source simulator DuMu<sup>x</sup>

To determine the kinetic growth parameters, the experiments were reproduced by numerical simulations. As suitable simulation tools, various options are available, ranging from open source (e.g. Python scripts) (Strobel et al., 2023) to commercial solutions such as COMSOL Multiphysics. In this study, a simplified model was used in the open-source simulator DuMu<sup>x</sup> (Flemisch et al., 2011; Koch et al., 2021), which focuses on the modeling of transport processes in porous media. The advantages of utilizing DuMu<sup>x</sup> are the existing implementation of the fluid systems, including the microbial growth model, and the potential usage on field scale.

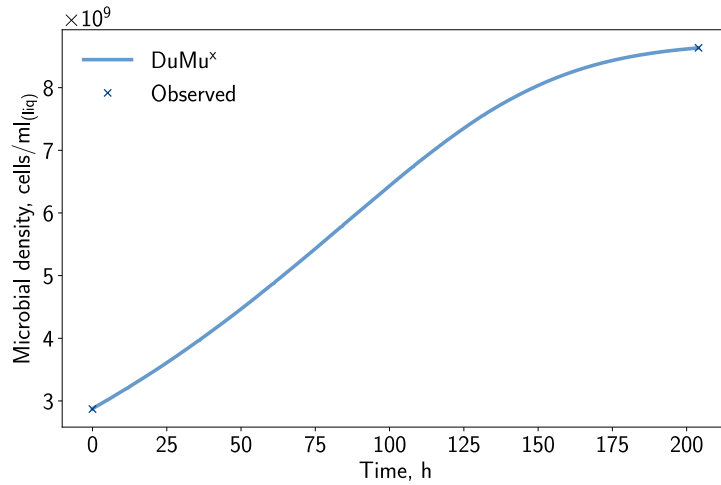
The implementation in DuMu<sup>x</sup> considers, in general, two phases (gas and water) composed of multiple components. For the specific scenario, the fluid system includes the reaction-specific components for methanation and sulfate-reduction: water, methane, hydrogen, carbon dioxide, sulfate, and hydrogen sulfide. As microbial species, which are implemented as a pseudo component, the methanogenic archaea and the sulfate-reducing bacteria are considered. The growth model is realized, as elucidated in the previous section. At every point in time in space, thermodynamic equilibrium by Henry's Law (Henry, 1803) is assumed, thereby describing the distribution of the chemical components in the phases.

Focusing on the definition of the simulation scenario, the reactors were represented by a single grid cell containing the corresponding volume and the porosity of unity. Further parameters, such as phase saturations and gas composition, were defined following the experimental procedure. Samples from the headspace and the liquid phase were taken during the experiment. To consider this in the matching, the corresponding liquid volume is extracted from the grid cell, and in addition, the cell pressure decreases by the measured gas pressure drop during sampling.

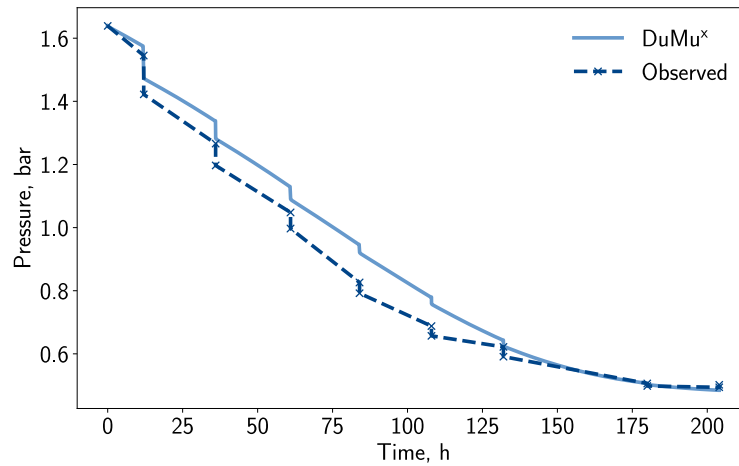
More information about the particular implementation in DuMu<sup>x</sup> can be found in Hogeweg et al. (2023b), and the corresponding source code can be found in Hogeweg and Hagemann (2022) and Hogeweg et al. (2023a).

## 3.3. Results of the matching procedure

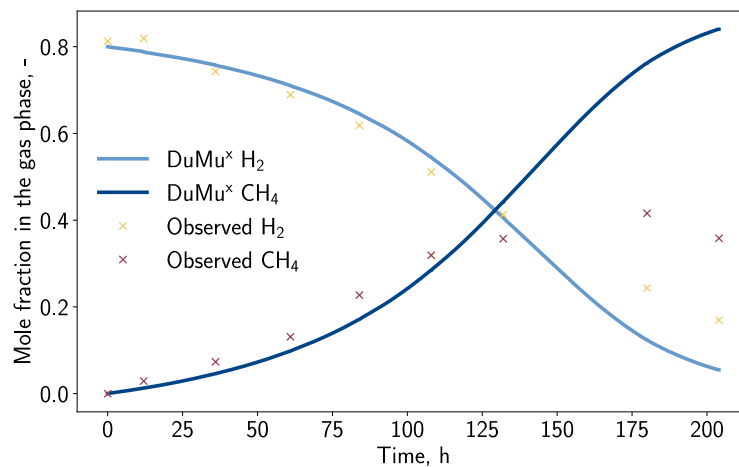
A selection of experiments targeting methanogenic archaea was reproduced by modelling to determine the growth parameters. Best matches were achieved in an iterative process by adjusting the parameters of maximum growth rate, yield factor, and the half velocity constants. Matched experimental parameters are the final microbial density, the pressure drop in the gas phase, and the concentrations of methane and hydrogen in the gaseous phase. The best match for case 1 (experiment A) at 65°C with added nutrients is depicted in Figure 2 to Figure 4. During the experiment, the initial substrate supply allows the microbial population to grow, coming with the metabolization from hydrogen and carbon dioxide to methane and water. This reaction furthermore induces a pressure drop as five mole of gas are converted into two mole of liquid and one of gas. The extractions of liquid and gas samples are visible, in particular in the pressure, resulting in a step-wise characteristic. Concerning the gas composition, the match in the beginning is satisfying, while after strong changes in the gas composition occur, the quality worsens. Weaknesses regarding the calibration of the gas chromatograph can explain this.



**Figure 2. Microbial density in the liquid phase versus time of case 1 (experiment A) at 65°C with added nutrients.**



**Figure 3. Pressure drop in the batch reactor versus time of case 1 (experiment A) at 65°C with added nutrients.**



**Figure 4. Gas composition in the gaseous phase measured and simulated versus time of case 1 (experiment A) at 65°C with added nutrients.**

The best-fitted growth parameters for the particular experiments are depicted in Table 2.

**Table 2. Matched growth parameters for a selection of three experiments of case 1 at 65°C with added nutrients.**

Parameter	A	B	C	Mean
Initial microbial density, 1/ml	$2.87 \cdot 10^9$	$6.38 \cdot 10^9$	$3.52 \cdot 10^9$	$4.26 \cdot 10^9$
Maximum growth rate, 1/s	$3.30 \cdot 10^{-6}$	$2.80 \cdot 10^{-7}$	$2.75 \cdot 10^{-6}$	$2.11 \cdot 10^{-6}$
Yield factor, 1/mol	$4.15 \cdot 10^{13}$	$5.70 \cdot 10^{12}$	$5.00 \cdot 10^{13}$	$4.26 \cdot 10^{13}$
Half velocity constant H <sub>2</sub> , mol/mol	$4.00 \cdot 10^{-6}$	$4.00 \cdot 10^{-6}$	$4.00 \cdot 10^{-6}$	$4.00 \cdot 10^{-6}$
Half velocity constant CO <sub>2</sub> , mol/mol	$2.00 \cdot 10^{-8}$	$2.00 \cdot 10^{-8}$	$2.00 \cdot 10^{-8}$	$2.00 \cdot 10^{-8}$

The performed simulations allow to parameterize the implemented model with good accuracy by reproducing the laboratory observations. However, it can be expected that the microbial growth behaves less optimally *in situ*, yielding lower reaction rates. Furthermore, the implemented Monod model assumes a substrate-limited growth, which may be valid for the contact zone between stored and cushion gas. However, in regions close to the injection well, other limitations such as space limitations may be dominating due to the continuous supply of substrate. Nevertheless, the growth parameters taken can be used to estimate the potential risk on field scale.

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## 4. Conclusions

This report explores the influence of temperature and nutrient supplementation on microbial activity using incubation experiments with brine samples from a potential hydrogen storage site. The results indicate differences in  $H_2$  consumption and in production of  $CH_4$  and acetate depending on the temperature and the nutrient condition. Interestingly, this case study showed an interplay between methanogenesis and acetogenesis, in which acetate and  $CH_4$  are not only produced via a hydrogenotrophic pathway. Moreover, it was also observed that strong acetate production provoked a decrease in pH, whereas strong  $CH_4$  formation led to an increase in pH, both limiting further microbial metabolic reactions. The microbial growth parameters derived from these experiments were used to develop numerical simulations to predict microbial reactions in subsurface environments, aiding in the estimation of their impact on hydrogen storage operations. The simulation results provided insights into microbial growth dynamics and helped parameterize the model accurately, though it acknowledges potential variations in microbial behavior *in situ*.

One limitation of this study is that the tests were only conducted at low total and partial pressures of  $H_2$ . Previously, the influence of partial  $H_2$  pressure on reservoir brine incubations at high pressure has been explored by Dohrmann and Krüger (2022). Their findings indicated an absence of microbial activity during reactor incubations at 100 bar with 4.0 – 4.6%  $H_2$  over a three-month period. However, upon reducing the  $H_2$  proportion to 0.6% and 2%, hydrogenotrophic activity became evident. Conversely, Liu et al. (2023) observed hydrogenotrophic sulfate reduction in a pore-scale system incubated at 35 bar with 100%  $H_2$ , revealing that an interplay between partial  $H_2$  pressure and total pressure might be involved in regulating microbial activity and growth. Based on a mixed homoacetogenesis culture, Sivalingam et al. (2021) evaluated the effect of elevated hydrogen partial pressure between 1-25 bar. They found that 15 bars were the optimal pressure in terms of gas uptake rates and that partial  $H_2$  pressures above this threshold would induce inhibition, leading to lowered gas uptake rates and product synthesis.

A second limitation of the study is the absence of reservoir rock matrix, which prevents observations of interactions between microbes and the geological substrate. Incorporating rock samples into microbial incubations could potentially alter outcomes by providing additional surfaces for microbial colonization and influencing the geochemical environment. For example, microbial interactions with carbonate rock could lead to mineral dissolution, releasing trapped nutrients. Moreover, carbonate-rich rocks could provide buffering capacity and possibly mitigate the observed pH fluctuations due to microbial activity. However, the extent to which such processes would influence overall microbial hydrogenotrophic activity in the context of  $H_2$  subsurface storage requires further investigation. The inclusion of rock substrates in future experimental designs would not only illuminate these interactions, but also enhance the applicability of laboratory findings to real-world subsurface conditions.

Modeling microbial growth parameters provides a novel tool for assessing initial risks based on laboratory studies, enabling a more comprehensive evaluation of microbial impact on subsurface  $H_2$  storage in specific reservoirs under varying environmental conditions. Despite its limitations, this report emphasizes the importance of comprehending microbial kinetics in subsurface environments to accurately assess risks and optimize hydrogen storage strategies effectively, in line with the European Union's objective of realizing a sustainable energy future.



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