Inserti "minimi" per la dignità del dopo. Cimitero nel Vorarlberg (Austria)

Biogeochemical Modeling of High-Pressure/High-Temperature Bioreactor Systems for Enhanced Microbial Risk Assessment in Underground Hydrogen Storage

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Summary

Underground hydrogen storage (UHS) can be a valuable solution for efficient and environmentally friendly energy storage but it introduces complex microbial and geochemical interactions that pose unique challenges. This research leverages advanced biogeochemical modeling to accurately replicate these interactions, reproduced within a laboratory-scale bioreactor system that mimics the high-pressure and high-temperature conditions typical of many underground gas storages. Utilizing a dual-platform approach, we used COMSOL® Multiphysics and CMG-GEM, augmented by supplementary simulation tools like PHREEQC, to perform an in-depth analysis of the evolution of microbial populations and gas and liquid composition, and of the hydrochemical processes in geological formations. Our comparative study demonstrates the effective application of these platforms in modeling the complex dynamics of heat and fluid dynamics, mass transfer, and biochemical reactions. The models were meticulously validated against experimental data, displaying high accuracy in kinetic parameter fitting and the ability to replicate the observed phenomena, including microbial hydrogen consumption rates below 0.05% under specified conditions and no detectable H_2S production at high pressures. The simulation results from COMSOL® and CMG-GEM showed remarkable agreement, with differences in the respective outcomes under 3–5%, confirming the reliability and robustness of the simulations across different computational environments. The research highlights the benefits of integrating multiple simulation platforms to achieve a comprehensive and comparative understanding of biogeochemical processes at various scales. This approach not only enhances our predictive capabilities but also facilitates the transfer of biochemical and geochemical kinetics from bioreactor-scale to reservoir-scale models, to make the implementation of hydrogen storage possible. These findings underscore the potential of the modeling tools to support the assessment and management of microbial risks associated with hydrogen storage, contributing to fully assessing the storage feasibility. By providing a detailed comparison of two leading software platforms, we established an essential methodological framework for advancing the UHS technology toward safe implementation.

Introduction

The transition to a sustainable energy future is a paramount challenge of the 21st century, with the imperative to reduce greenhouse gas emissions and mitigate climate change effects, and it is driving the search for renewable energy sources and efficient storage solutions (Antonicelli et al. 2023; Tarraran et al. 2023; European Commission 2014). In this context, UHS emerges as a pivotal technology, offering a means to balance the intermittent nature of renewable energy sources such as wind and solar (Bo et al. 2021; Jahanbani Veshareh et al. 2022; Salina Borello et al. 2024). UHS not only represents a critical step toward achieving a carbon-neutral energy system but also introduces unique environmental and safety challenges, particularly concerning the interactions between stored hydrogen and geological formations (Benetatos et al. 2021; Yekta et al. 2018).

The feasibility and safety of UHS are contingent upon a comprehensive understanding of the complex microbial and geochemical reactions occurring within potential storage sites (Henkel et al. 2014; Jadhawar and Saeed 2024). These interactions, especially the activities of microbial communities present in geological formations, can significantly influence the integrity and efficiency of hydrogen storage systems. Risk assessment associated with microbial influences, including acetogenic bacteria (AB), sulfate-reducing bacteria (SRB), and methanogenic archaea, necessitates a meticulous examination to ensure the environmental compatibility and operational safety of UHS solutions (Shojaee et al. 2024; Hagemann et al. 2016). The feasibility and safety of UHS are contingent upon a comprehensive understanding of the complex microbial and geochemical reactions occurring within potential storage sites (Henkel et al. 2014). Numerous studies have investigated these dynamics, highlighting the roles of microbial communities, such as AB, SRB, and methanogenic archaea, in influencing reservoir integrity and hydrogen storage stability (Buriánková et al. 2022; Hellerschmied et al. 2024; Haddad et al. 2022; Šmigáň et al. 1990). These microbial interactions and their metabolic pathways can substantially impact fluid chemistry, pH levels, and gas quality within storage reservoirs (Bade et al. 2024; Wang et al. 2023; Saeed and Jadhawar 2024).

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This paper (SPE 220064) was accepted for presentation at the SPE Europe Energy Conference and Exhibition, Turin, Italy, 26–28 June 2024, and revised for publication. Original manuscript received for review 9 August 2024. Revised manuscript received for review 9 November 2024. Paper peer approved 4 December 2024. Supplementary materials are available in support of this paper and have been published online under Supplementary Data at https://doi.org/10.2118/220064-PA. SPE is not responsible for the content or functionality of supplementary materials supplied by the authors.

Modeling plays a crucial role in this regard, offering a powerful tool for predicting and analyzing the complex interactions between microbial communities, geochemical constituents, and hydrogen storage matrices (Saeed and Jadhawar 2024; Feldmann et al. 2016). Previous studies have highlighted the importance of considering both biogeochemical and geochemical reactions in assessing the feasibility and safety of UHS (Bellini et al. 2022; Berta et al. 2018). These reactions encompass a spectrum of processes, including microbial metabolism, mineral dissolution, gas phase equilibria, and fluid-rock interactions, all of which influence the fate and transport of hydrogen within subsurface reservoirs (Jin and Kirk 2016).

From a biogeochemical perspective, the activities of AB, SRB, and methanogenic archaea are of particular interest due to their roles in hydrogen metabolism and gas production. AB, for instance, catalyze the conversion of hydrogen and carbon dioxide (CO_2) into acetate, a process that can impact pH levels and mineral solubility in formation waters (Bonk et al. 2019). Similarly, SRB mediate the reduction of sulfate to sulfide, leading to alterations in fluid chemistry and potential souring issues in gas reservoirs (Chen et al. 2019; George et al. 2021). Conversely, archaea produce methane through the reduction of CO_2 or acetate, further influencing reservoir composition and gas quality (Shojaee et al. 2024; Conrad 1999; Abdel Azim et al. 2023; Wang et al. 2019).

By integrating insights from these studies and leveraging advanced modeling techniques, we aim to elucidate the underlying mechanisms governing microbial interactions and geochemical processes within UHS environments. Through a combination of experimental data and computational simulations, our approach seeks to provide a comprehensive understanding of microbial risks and inform the development of strategies for improving the safety and efficiency of hydrogen storage in subsurface formations.

Advanced simulation tools, including COMSOL® Multiphysics, CMG-GEM, and PHREEQC, have been instrumental in studying UHS at various scales—from the microscale of porous media (Liu et al. 2023; Massimiani et al. 2023) to laboratory-scale experiments (van Houten et al. 1997; Braga Nan et al. 2020; Strobel et al. 2023b), to macroscale reservoir simulations (Shojaee et al. 2024; Saeed and Jadhawar 2024). These tools allow for the in-depth analysis of heat and fluid dynamics, mass transfer, and the intertwined geochemical and biochemical reactions that define the UHS environment (Hemme and Van Berk 2018; Whitmore et al. 2011).

Significant contributions from the scientific community have leveraged these platforms to simulate UHS scenarios, shedding light on the biogeochemical processes that support microbial risk assessments and geochemical stability. For instance, the integration of COMSOL and CMG-GEM, complemented by the geochemical insights from PHREEQC, exemplifies the interdisciplinary approach required to tackle the multifaceted challenges of UHS (Diring et al. 2017; Azad et al. 2016; Nassan and Amro 2019). Usually, COMSOL is known for its flexibility in solving a wide range of partial differential equations, allowing for detailed modeling of biogeochemical phenomena at both laboratory and reservoir scales (Shojaee et al. 2024; Bogdanov et al. 2007; Wang et al. 2019). CMG is widely used for simulating hydrocarbon production and recovery processes, offering advanced tools for modeling fluid-rock interactions and flow dynamics in reservoirs (Tremosa et al. 2023).

The use of different software platforms for modeling reservoirs has garnered interest among researchers as each platform has specific advantages and limitations. Previous studies have conducted comparisons between modeling software platforms, such as COMSOL and CMG, aiming to assess their capabilities in monitoring and simulating biogeochemical processes in underground storages (Azad et al. 2016). These comparisons have been motivated by the need to identify the most suitable platforms to address the challenges associated with modeling the geochemical and biological processes influencing UHS.

Our aim in this paper is to build upon these foundational studies by presenting a comprehensive biogeochemical modeling approach that replicates the intricate microbial and geochemical reactions under reservoir conditions. By performing batch tests and reactor tests in a laboratory-scale bioreactor system that mirrors the high-pressure and high-temperature environment typical of underground gas storages, we obtained essential experimental results. These experimental insights served as a benchmark for our modeling efforts, enabling the accurate simulation of microbial interactions and geochemical processes within UHS contexts. To support this goal, the paper is structured to provide a detailed overview of both the experimental and modeling methodologies, offering a thorough analysis of microbial and geochemical interactions under reservoir conditions. After introducing the methodological approach, the paper presents a comparative evaluation of COMSOL® Multiphysics and CMG-GEM platforms, enriched by PHREEQC's geochemical capabilities, to validate the accuracy and robustness of our approach.

The comparative study of simulation platforms extends beyond theoretical analysis to incorporate practical experimental data, thereby ensuring the reliability and applicability of our findings. By fitting biochemical kinetic parameters from experimental observations into our models, we bridge the gap between laboratory-scale experiments and the multiphysics simulation of hydrogen storage in porous media.

The transfer and implementation of fitted biochemical parameters from one simulation platform to another exemplify the innovative approach of our study. This cross-platform parameter application facilitates a comprehensive understanding of the biogeochemical phenomena influencing hydrogen storage safety and efficiency. It underscores the adaptability and interoperability of modeling tools such as COMSOL® Multiphysics and CMG-GEM, enriched by PHREEQC's geochemical insights, in addressing the multifaceted challenges posed by UHS. Through this integrative modeling strategy, we aim to provide profound insights into the microbial risks associated with hydrogen storage and pose the basis for upscaling laboratory results to reservoir-scale applications, offering a methodological framework to guide the development and implementation of UHS technologies toward ensuring environmental compatibility and operational safety.

Laboratory Setups and Experiments for Microbial Growth Analysis

The specialized equipment and analytical processes used in our studies are described below, highlighting the methods utilized for both low-pressure experiments and high-pressure reactor trials. These methodologies are crucial for obtaining accurate data necessary for the kinetic parameter fitting and for a comprehensive assessment of microbial activity under simulated underground storage conditions, respectively. In all the experiments, formation water sampled from an Italian gas reservoir to be potentially converted into a UHS was used. The water composition is summarized in **Table 1** (Bassani et al. 2023).

To obtain the necessary data needed for the modeling approach, the following workflow was applied:

- Initial metagenomic and hydrochemical characterization of formation fluids to define the presence of microorganisms of interest for UHS application and the chemical environment in which they live.
- Assessment of microbial activity in batch tests performed in serum bottles at low pressure under anaerobic conditions: The batches were inoculated with reservoir formation water and incubated under different gas compositions to evaluate the activity of indigenous microorganisms when cultured either in reservoir hydrochemical conditions, or in enriching conditions by providing key nutrients to enhance the microbial activity.
- Assessment of microbial activity in reactor tests at high pressure to evaluate the activity of indigenous microbial consortia by emulating reservoir chemophysical conditions;

Description	Range		Analytical Method
	Average	St. Dev.	
pH	5.97	0.00	
Conductivity (μS/cm)	97 976	452	APAT CNR IRSA 2030 Man 29 2003
Ammonia nitrogen, NH_3-N (mg/L)	98	5	UNI 11669:2017
Calcium (mg/L)	8550	250	UNI EN ISO 11885:2009
Magnesium (mg/L)	1320	0	
Sodium (mg/L)	21 046	582	
Potassium (mg/L)	383	4	
Lithium (mg/L)	5.10	0.15	
Manganese (mg/L)	4.58	0.36	
Nickel (mg/L)	0.04	0.00	
Strontium (mg/L)	232	1	
Phosphate, PO_4 (mg/L)	<8	0	APAT CNR IRSA 4020 Man 29 2003
Chlorides (mg/L)	45 893	2907	
Sulfates (mg/L)	<50	0	
Bicarbonates (mg/L)	395	8	
Iron (mg/L)	113	8	
Nitrates (mg/L)	<50	0	
Bromides (mg/L)	250	2	
Total organic carbon (mg/L)	2720	40	UNI EN 1484:1999
Total inorganic carbon (mg/L)	75	45	
Total carbon (mg/L)	2795	85	

Table 1—Hydrochemical analysis: Formation water properties.

- Analysis of microbial activity: For both the experimental tests at low and high pressures, the continuous monitoring of pressure, temperature, optical density (OD600), quantification of methanogenic archaea, AB, and SRB by quantitative polymerase chain reaction (qPCR) analysis of mcrA/fhs/dsrB genes, headspace gas composition [e.g., methane (CH_4), hydrogen (H_2), carbon dioxide (CO_2), and hydrogen sulfide (H_2S) by gas chromatography (GC)], pH, and volatile fatty acids (VFAs) using high-performance liquid chromatography (HPLC) analysis were used to assess microbial growth and activity.

Experimental Setup, Performed Tests, and Data Analysis for Low-Pressure Batch Tests. The experimental activity first involved batch culture tests using the formation water from an Italian depleted natural gas reservoir as the inoculum. These tests were designed to study microbial activity under simulated conditions relevant to the UHS. The batch cultures were set up in 158-mL serum flasks, each containing 50 mL of working volume composed of formation water. The flasks were initially flushed with nitrogen to create anaerobic conditions and then sealed with rubber septa to maintain these conditions throughout the experiments. The experiments were conducted under two main conditions—with and without nutrient enrichment in the liquid phase. For the gas phase, four conditions have been assessed: (i) One positive control sparged with nitrogen (N_2) and supplemented with 10 mM glucose solution; (ii) 100% CH_4; (iii) CH_4/H_2 mixture at a 90:10 ratio (%v/v); and (iv) CH_4/H_2 mixture at a 50:50 ratio (%v/v).

Initial tests without nutrient enrichment provided baseline microbial activity data. Subsequent tests included nutrient enrichment to stimulate microbial growth and mimic potentially more critical scenarios. These nutrients included "carbon sources, mineral nutrients, and cofactors such as vitamins and trace elements" (Bassani et al. 2023). The gas phase for these cultures included different mixtures of methane and hydrogen to simulate potential underground storage conditions (Bellini et al. 2024).

Gas compositions in the headspace of the batch cultures were analyzed using GC (MicroGC Fusion, INFICON), which was calibrated to measure H_2, oxygen, N_2, CH_4, CO_2, and H_2S. Samples were taken at the start and end of the cultivation period (23 days) for the assessment of microbial gas consumption and production dynamics. The analysis of VFAs in the liquid phase was conducted using HPLC (Thermo-Fischer Dionex Ultimate 3000) (Bellini et al. 2024), which enabled precise quantification of these acids to understand the metabolic pathways active within the microbial communities. Microbial growth was monitored through optical density measurements at 600 nm, while qPCR was used to quantify functional marker genes associated with methanogens (mcrA), SRB (dsrB), and AB (fhs). This combination of methods provided a comprehensive overview of the microbial dynamics and their functional potential under the tested conditions.

These experimental approaches, combined with rigorous analytical techniques, have enabled a detailed characterization of the microbial consortia present in formation waters and their response to different gas mixtures.

Experimental Setup, Performed Tests, and Data Analysis for High-Pressure Reactor Test. The high-pressure reactor experiments were designed to assess microbial risks associated with UHS under reservoir conditions. The experimental setup utilized the Bio-xplorer system, a customized multireactor assembly capable of simulating high-pressure and high-temperature conditions up to 200 bar and 150°C, respectively. This system includes two 1-L stainless steel bioreactors, which house rock samples and formation water (Vasile et al. 2024).

The reactor tests were performed with the formation water **(Table 1)** and the core samples were collected from an Italian depleted gas reservoir. The rock composition is provided in **Table 2**.

	U.M.	
Quartz	wt%	33.7
Illite	wt%	24.3
Chlorite	wt%	1.8
Kaolinite	wt%	2
Calcite	wt%	22
Dolomite	wt%	24.2
Plagioclase	wt%	6.7
k-Feldspar	wt%	6.9

Table 2—Rock sample compositions used in the simulations coming from mineralogical analysis.

The studies conducted on this reservoir involved a gas mixture with 99% H_2 and 1% CO_2. Gas composition variations were monitored using GC (MicroGC Fusion, INFICON), focusing on H_2, CH_4, CO_2, and other gases. This analysis helped to determine the stability of the gas environment and any microbial-induced changes. The biochemical properties of the liquid phase were analyzed, including VFA concentrations, using HPLC. This was crucial for understanding the metabolic processes occurring within the microbial communities. Microbial density and activity were assessed using qPCR to quantify specific microbial populations, particularly SRB and hydrogenotrophic methanogens (HM). This molecular technique targeted key functional genes, providing insights into the microbial dynamics under high-pressure conditions. The reactors were equipped with sensors for continuous monitoring of pressure, temperature, and other relevant parameters. This comprehensive data collection was essential for correlating physical conditions with microbial responses and ensuring the system operated within the desired parameters.

Mathematical Model Description

The objective of this study was to simulate the thermo-fluid dynamic and biogeochemical processes occurring within the high-pressure, high-temperature bioreactor. This entailed a multistep approach, starting from the fitting of biochemical kinetics derived from experimental data and culminating in the modeling of all relevant reactor phenomena. To achieve this, two prominent software platforms, COMSOL® Multiphysics and CMG-GEM, were utilized. COMSOL® Multiphysics is a finite element modeling program renowned for its versatility in solving partial differential equations, making it suitable for detailed modeling of biogeochemical processes. Conversely, CMG-GEM is specialized in reservoir simulation, offering advanced tools for modeling fluid-rock interactions and flow dynamics. By harnessing the capabilities of both platforms, we aimed to construct a comprehensive model capable of capturing the complex interplay between thermo-fluid dynamics and biogeochemistry within the bioreactor.

To clarify the integration of modeling tools in this study, we present a workflow diagram **(Fig. 1)** illustrating how COMSOL, CMG-GEM, and PHREEQC were combined to simulate the biogeochemical processes relevant to UHS. COMSOL is used primarily for its capability to model detailed biochemical kinetics and transport phenomena in 0D and 3D domains, allowing for accurate simulation of microbial activity and nutrient dynamics under varied conditions. CMG-GEM, on the other hand, is used at the reactor scale to handle reservoir-operation processes, including fluid flow, mass transfer, and interactions within porous media. PHREEQC, integrated with CMG-GEM platforms, provides essential data for geochemical equilibria, specifically the solubility and reaction constants for geochemical reactions and various gases (H_2, CH_4, CO_2, and H_2S) under different conditions.

This workflow enables us to establish continuity between experimental low-pressure and high-pressure systems, with biochemical kinetics fitted initially in COMSOL's 0D model, then validated in 3D COMSOL, and CMG-GEM reactor models. PHREEQC's databases contribute equilibrium data directly into CMG-GEM for accurate representation of gas solubilization and geochemical reactions. This multiplatform approach ensures that all relevant physical, chemical, and biological interactions are captured across the range of pressures and conditions examined in this study.

The methodology used in this study involved a multistage approach. Initially, the main equations describing the biogeochemical reactions potentially occurring in the presence of microorganisms in porous media where H_2 is injected were defined. These equations, used in both software platforms, are listed in **Table 3**.

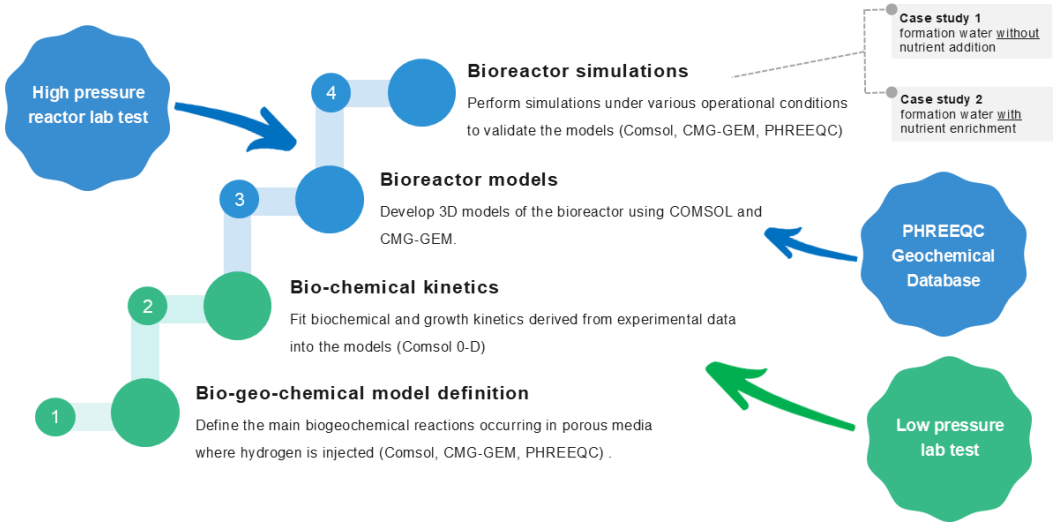

Fig. 1—Modeling workflow and approach on integration between COMSOL, CMG, and PHREEQC platforms and experimental data.

Sulfate-reducing reactions	$4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$
	$Acetate^- + SO_4^{2-} \rightarrow 2\ HCO^- + HS^-$
	$Propionate^- + 0.75\ SO_4^{2-} \rightarrow Acetate^- + HCO_3^- + 0.75\ HS^- + 0.25\ H^+$
	$Butyrate^- + 0.5\ SO_4^{2-} \rightarrow 2\ Acetate^- + 0.5\ HS^- + 0.5\ H^+$
Acetogenic reactions	$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$
	$Glucose \rightarrow 3\ Acetate^- + 3H^+$
	$Propionate^- + 3\ H_2O \rightarrow Acetate^- + HCO_3^- + H^+ + 3\ H_2$
	$Butyrate^- + 2\ H_2O \rightarrow 2\ Acetate^- + H^+ + 2\ H_2$
Methanogenic and homoacetogenic reactions	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
	$Acetate^- + H_2O \rightarrow CH_4 + HCO_3^-$
	$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$
	$4\ H_2 + 2\ HCO_3^- + H^+ \rightarrow Acetate^- + 4\ H_2O$
Geochemical reactions	$CaCO_3 = CO_3^{2-} + Ca^{2+}$
	$CaMg(CO_3)_2 = Ca^{2+} + Mg^2 + 2CO_3^{2-}$
CO_2/bicarbonate equilibrium	$CO_{2,g} + H_2O \rightleftarrows CO_{2,liq} + H_2O \rightleftarrows H^+ + HCO^{3-}$
H^+/H_2 equilibrium	$8H^+ + 8e^- \rightarrow 4\ H_{2,liq}$
HS^-/H_2S equilibrium	$H_2S \rightleftarrows HS^- + H^+$
	$HS^- \rightleftarrows S_2^- + H^+$
	$S_2^- + 2H^+ \rightleftarrows H_2S$
Species mass balance	$\frac{d(c_j V_r)}{dt} = V_r R_i$
	$R_i = \sum_j v_{ij} r_j$
Ideal gas law	$p = R_g T \sum c_j$
Mass action law	$r_j = k_j^f \prod_{i \in \text{react}} c_j^{-v_{ij}}$
Arrhenius expression	$k^f = A^f \left(\frac{T}{T_{\text{ref}}}\right)^{n^f} \exp\left(\frac{-E^f}{R_g T}\right)$

Table 3—Main equations implemented in the model.

Energy balance	$\rho Cp\partial T\partial t + \rho Cpu \cdot \nabla T = \nabla \cdot (k\nabla T) + Q$
Mass transport	$\frac{\partial}{\partial t}\left(\rho\omega_i\right) + \nabla \cdot \left(\rho\omega_i u\right) = -\nabla \cdot j_i + R_i$
Fluid dynamic in porous media	$\phi \frac{\partial\left(\rho_g c_g^k S_g + \rho_w c_w^k S_w\right)}{\partial t} + \nabla \cdot \left(\rho_w c_w^k S_w + J_w^k + \rho_g c_g^k S_g + J_g^k\right) = q^k$
	$\left(1-\phi\right)\frac{\partial\left(\rho_s c_s^k\right)}{\partial t} = q^k$
	$P_c\left(S_w\right) = P_g - P_w = P_e S_{we}^{\frac{-1}{\lambda}}$
	$S_{we} = \frac{S_w - S_{we}}{1 - S_{wr} - S_{gr}}$
	$S_g + S_w = 1 \quad \sum_k c_w^k = 1 \quad \sum_k c_g^k = 1$
Mass transfer: Two-film theory	$\frac{\partial \phi_g \rho_g}{\partial t} + \nabla \cdot N_{\rho_g \phi_g} = -m_{gl}$
	$c^* = \frac{p + p_{\text{ref}}}{H}$
	$N = k_L\left(c^* - c\right)$
	$m_{gl} = k\left(c^* - c\right)Ma$
	$\frac{\partial c}{\partial t} + \nabla \cdot \left(cu_l\right) = \nabla \cdot \left(D\nabla c\right) + \frac{m_{gl}}{M}$
Microbial growth	$\Psi^{\text{growth}} = \frac{1}{t_e}\frac{n}{1+\frac{n^2}{n_{\max}^2}}\left(\frac{c^S}{\alpha + c^S}\right)$
	$\Psi_{M-A-S}^{\text{growth}} = \Psi_{M-A-S,\max}^{\text{growth}} \prod_i \left(\frac{c_w^{H_2,CO_2,C_2H_4O_2,C_6H_{12}O_6,SO_4}}{\alpha_{M-A-S,i} + c_w^{H_2,CO_2,C_2H_4O_2,C_6H_{12}O_6,SO_4}}\right)$

Table 3 (continued)—Main equations implemented in the model.

Then, the biochemical kinetics were fitted using experimental data obtained from batch experiments conducted at low pressure, as detailed in the study of Bellini et al. (2024). These fitted kinetics were subsequently incorporated into 3D models of the bioreactor using COMSOL® Multiphysics and CMG-GEM. Additionally, mathematical models capturing thermo-fluid dynamics, including porous media effects, were developed within these software platforms. Subsequently, simulations were performed on the reactor models using both software to compare results and assess the feasibility of transferring information between platforms, particularly at the laboratory scale of the reactor.

COMSOL General Overview and Model Definition. A multiscale model was developed using the COMSOL® Multiphysics platform, a finite element modeling program designed to solve a vast array of partial differential equations (PDEs), which has emerged as an innovative and successful approach to groundwater modeling (Liu and Liu 2017; Sainz-Garcia et al. 2017; Azad et al. 2016; Jin et al. 2011; Jin and Kirk 2016). The model allows for the determination of growth kinetics and gas/VFA production and consumption, accounting for the concentration in moles of target chemicals and their presence in both gas and liquid phases. Additionally, it considers microbial growth occurring in the liquid phase. Governing equations for the "four main microbial processes, represented by the reaction equations outlined in **Table 3**, are implemented in the model" (Tremosa et al. 2023; Jin and Bethke 2005; Delattre et al. 2020; Conrad 1999; Feldmann et al. 2016; Strobel et al. 2023b; Berta et al. 2018; Hoehler et al. 1998). In the model, we did not consider iron-reducing bacteria reactions, as they were not detected in the metagenomic analyses (Bassani et al. 2023), and there were no relevant sources of reactants for their metabolisms in the formation water **(Table 1)**. These equations are based on fundamental biochemical principles and are calibrated using experimental data.

The model factors in equilibrium reactions for "gas-water interactions focused on potential increases in dissolved carbonate and sulfate in reservoir water due to bacterial and archaea reactions"(Gholami 2023). Equilibrium calculations were based on the mass action law, considering all the species used in this study and their corresponding equilibrium constants (Christina 2019; Louca et al. 2019; Gelencsér et al. 2023; Gholami 2023). The inclusion of equilibrium reactions provided a more comprehensive understanding of the chemical speciation and distribution within the reservoir system.

The Monod model, a standard approach for "modeling microbial growth under substrate-limited conditions, was used to capture the dependence of microbial growth and decay on substrate and electron acceptor availability" (Hagemann 2018; Strobel et al. 2023b). To

address this dependency, the "modified Monod model" was proposed, which accounts for multiple limiting factors and their interactions (Hagemann et al. 2016; Jin and Kirk 2016; Strobel et al. 2023a). By incorporating these models into our simulations, we could accurately predict microbial dynamics and their impact on hydrogen storage processes.

The model also accounts for changes in key components across both gas and liquid phases. The concentration of "components within the liquid phase, or solubility, is generally represented using Henry's Law" (Sander 2015). A crucial assumption here is that there exists an equilibrium between concentrations in both phases, which is upheld by mass transfer processes occurring at the liquid-gas interface. Additionally, "the mathematical model is based on the two-film theory, where the 'film' between the phases serves as the resistance against mass transfer" (Li 2017; Liu and Liu 2017). The mass transport between the liquid and gas phases is described using a generalized approach, which accounts for molecular diffusion and convective flow (Feldmann et al. 2016; Hagemann et al. 2016). By combining mass transfer with microbial reactions, we derived the equations for the production and consumption of H_2, H_2S, CO_2, and CH_4 within the liquid phase.

To enhance the mathematical model and align it with the observed physical realities in bioreactors, it was crucial to consider the dynamics of two-phase flows. Mass transport between phases is governed by a set of equations that describe mass conservation for each chemical component, considering both advective and dispersive/diffusive transport. The model incorporates the hydraulic properties of the porous medium, such as capillary pressure and relative permeability, to describe how saturation affects the flow properties. Fick's laws for molecular diffusion, combined with the Stefan-Maxwell law for diffusion in gases, are used to calculate mass flows between phases.

In the COMSOL model, heat transfer was simulated using the Heat Transfer in Porous Media module, which allowed us to capture thermal conductivity and temperature gradients across the reactor. The mass transfer was modeled with the Transport of Diluted Species and Chemistry modules, where a modified Monod model and film theory were applied to simulate gas-liquid interactions and microbial kinetics. Diffusion coefficients and Henry's constants were calibrated on materials database to match reservoir conditions (temperature and pressure) and ensure realistic mass transfer dynamics. In the 0D model, the Reaction Engineering module was used to integrate the various reactions within the software platform. For the 3D version, the Chemistry module, coupled with the Transport of Diluted Species, Free and Porous Media Flow, and Heat Transfer in Porous Media modules, was used to couple the biogeochemical reactions with the reactor's thermo-fluid dynamics.

The modeling of thermal behavior and biochemical reactions within the reactor also requires an accurate energy balance. Its equation accounts for specific heat, heat flows, and heat production or consumption due to biochemical reactions.

Integration of CMG-GEM and PHREEQC for Reactor Modeling. In addition to the use of COMSOL® Multiphysics for detailed biogeochemical modeling, our study extensively used CMG-GEM alongside PHREEQC to simulate the dynamic interactions within the reactor environment under conditions analogous to UHSs. This combined modeling approach enhances our ability to capture the nuanced interactions between microbial activity and geochemical processes that significantly influence the feasibility and safety of hydrogen storage.

CMG-GEM, a sophisticated reservoir simulation tool, is particularly adept at handling complex fluid dynamics and multiphase flow in porous media (Shojaee et al. 2024). In our reactor simulations, CMG-GEM was used to model the hydrodynamic and thermodynamic behavior of the system, providing a robust platform for simulating the large-scale interactions typically observed in geological formations.

To complement the hydrodynamic modeling capabilities of CMG-GEM, PHREEQC was utilized for its strong geochemical analysis features. PHREEQC allows for the detailed simulation of chemical equilibria, kinetic reactions, and transport of dissolved species (Saeed et al. 2023; Jacquemet et al. 2020). In our study, PHREEQC was used to model the geochemical reactions likely to occur in the reactor, such as mineral dissolution and precipitation, ion exchange, and complexation reactions. These reactions are pivotal for understanding how microbial processes can alter the geochemical stability of the storage site, as evidenced by their impact on carbonate and sulfate dissolution in scenarios modeled in previous studies (Tremosa et al. 2023; Jakobsen 2007). In this study, we used the preconfigured geochemical model structure of PHREEQC within the CMG-GEM software, which allows the user to directly input the gas equilibrium reactions (involving CH_4, H_2, CO_2, and H_2S) using the PHREEQC integrated databases to accurately simulate the relevant geochemical equilibrium reactions.

The integration of CMG-GEM and PHREEQC in our simulations involved the fluid flow and transport equations as the Navier-Stokes equations, coupled with mass conservation equations. These equations facilitated the simulation of how gases and liquids move through the porous matrix of the reactor. Then, PHREEQC handled the detailed chemistry of the system, simulating reactions under equilibrium and kinetic controls. It modeled the transport of chemical species through advection, diffusion, and dispersion mechanisms, essential for understanding how chemical changes impact the overall reactor environment.

In CMG-GEM, heat transfer was simplified by assuming a constant temperature, as the batch and fed-batch conditions and the small volume in our simulations, along with the absence of gas injection or extraction, justified this approach. For mass transfer, CMG-GEM's built-in solubility modules and databases provided Henry's constants, diffusion coefficients, and gas solubility data across various temperatures and pressures, enabling accurate modeling of gas-liquid interactions.

The combination of CMG-GEM and PHREEQC allowed for a comprehensive understanding of the physical, chemical, and biological dynamics within the reactor. By modeling the production and consumption of key gases (H_2, CO_2, and H_2S), we assessed their impact on reactor stability, ensuring a holistic understanding of the processes that govern UHS viability.

Biochemical Kinetics Fitting Procedure. The validation of the model involved verifying its operational efficacy through comparisons between simulated and experimental data under identical conditions using the COMSOL® platform in 0D simulations. The model-fitting procedure entailed adjusting the values of "unknown parameters, particularly the biochemical kinetics constants, to minimize discrepancies between the model predictions and the experimental observations" (Bellini et al. 2024). A detailed comparative analysis was conducted through statistical error analysis to evaluate the precision of the model.

This validation method used a constrained search procedure, which necessitated the specification of lower and upper limits for the parameters. These limits were based on the range of values typically reported in the relevant literature. As outlined in the previous sections, extensive "knowledge of mass transfer and bacterial growth kinetics was fully integrated into the formulation of the modeling equations, leading to the model implemented within the COMSOL® computing platform" (Bellini et al. 2024).

To derive "accurate parameter estimates essential for the mathematical representation, we relied on either experimentally determined values or on an inference procedure that fitted the model simulations to the observed data" (Vasile et al. 2021). Comparisons between "simulated and experimental data were performed using a statistical error analysis based on the Levenberg-Marquardt method, further

refined by a second-order least-squares regression technique to minimize residuals" (Vasile et al. 2021). The exploration of the "parameter space continued until the model simulation optimally aligned with the experimental data. In the initial phase of the modeling work, the model was used to verify its operational efficacy by examining the agreement between simulation estimates and experimental measurements for growth rate, VFAs, methane, carbon dioxide, hydrogen sulfide, and hydrogen evolution" derived from laboratory tests, as exposed in the "Results" section. In Table S-1 of the Supplementary Materials, we list the matching parameters and unknown parameters used in the model calibration process. The matching parameters include gas phase composition and pressure, liquid phase composition (VFA), and cell count for each microbial cluster. These parameters serve as reference points to evaluate the model's accuracy by comparing simulated outputs with experimental data. The unknown parameters, estimated through a fitting procedure, consist of biochemical reaction kinetics and Monod parameters, which govern microbial growth dynamics, pressure, gas, and liquid phase compositions. The equations used for these fitted parameters (mass action law and microbial growth) are shown in **Table 3**. Temperature (kept constant in the experiments) and pressure have indirect effects on the unknown parameters by influencing gas solubility and availability within the system. Both the quantity and rate of gas solubilization impact the amount of gas that remains in the gas phase and the availability of nutrients such as hydrogen and CO_2 for microbial reactions. This availability, in turn, affects the fitted Monod parameters and biochemical reaction kinetics, as these parameters are closely linked to nutrient presence and gas composition in the system.

Model Assumptions and Boundary Conditions. To effectively address mass balances, fluid dynamics, and kinetic equations, various boundary conditions and model-specific assumptions were made. Mass transfer between the liquid culture and fed gas was considered, with velocities in the inlet/outlet areas set to zero in batch configurations where no liquid exchange occurs. Model parameters such as "temperature, inlet gas quantity, volume fractions, initial pressures, microbial types, and nutrient concentrations were incorporated based on experimental data" (Bellini et al. 2024; Vasile et al. 2024) and metagenomic analyses (Bassani et al. 2023).

Boundary Conditions.

- Mass transfer was considered between the liquid culture and the fed gas.
- Velocities in the inlet/outlet areas were zero in the batch configuration, given that there was no liquid exchange.
- Model parameters included "temperature, inlet gas quantity, volume fractions, initial pressures, microbial and nutrient types, and the initial amount for both" (Bellini et al. 2024).
- Operational "data from reactor experiments were incorporated to set the initial and working parameters of the models" (Vasile et al. 2024).
- "Metagenomics analysis" (Bassani et al. 2023) and "low-pressure batch tests with formation water informed initial microbial compositions and active species" (Bellini et al. 2024).
- "Biochemical kinetics for the main metabolisms in the reservoir were derived from experimental data" (Bellini et al. 2024).

Model Assumptions.

- "Only active microorganisms in batch tests with the formation waters of the reservoir were considered" (Bassani et al. 2023).
- For the study cases involving the addition of essential nutrients in the liquid phase, continuous addition was considered.
- The results from "the hydro-chemical analyses of formation waters for the reservoir were used to inform the model about bicarbonate, sulfate, organic carbon amounts, and pH values" (Bassani et al. 2023; Bellini et al. 2024).
- "Equilibrium reactions were simulated to account for possible dissolved carbonate/sulfate presence in the formation water for archaea and bacteria biochemical reactions" (Bellini et al. 2024).
- "The liquid phase was saturated with CO_2 and H_2, and the initial amounts matched the solubility limit for each gas at operating pressures" (Tawil et al. 2024; see also Reitenbach et al. 2015; De Lucia et al. 2015; Duan et al. 1992; Zhan et al. 2024).
- CO_2 was considered for both liquid and gas phases.
- The model considered "the influence of operational conditions on bacterial growth rates" (Bellini et al. 2024).

Modeled Domains. The bioreactor is entirely custom-built, integrating all necessary domains for comprehensive simulations. Constructed in Type 316 stainless steel, the reactor houses a cylindrical rock sample approximately 8 cm in diameter. This sample is securely held within the reactor by a tubular polymer-made inflatable adapter, ensuring stability and alignment. For samples that are poorly consolidated or tend to crumble, an additional mesh bag is used, encasing the rock sample to provide enhanced stabilization (Vasile et al. 2024). The bioreactor schematic and picture are shown in **Fig. 2**.

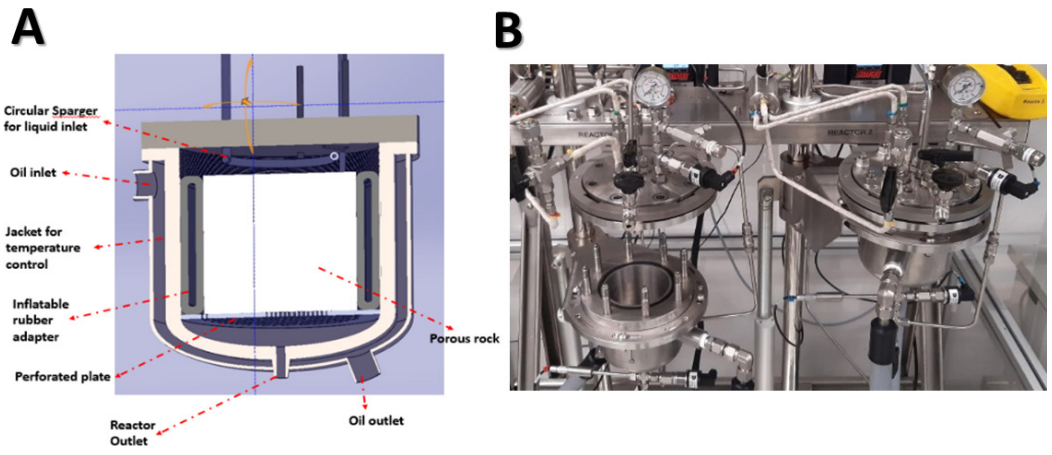

Fig. 2—Domains modeled for the simulations: (a) 3D section and (b) real High Pressure Bioreactor (HPB) configuration.

In terms of computational modeling, two different meshing strategies were utilized to cater to the specific requirements of each simulation software. COMSOL® Multiphysics employs a free tetrahedral meshing approach, which is particularly effective for handling complex geometries and ensuring detailed representation of the domains. The mesh used to simulate the reactor comprises 2.31×10^6

tetrahedral elements (mesh sensitivity analysis reported in **Fig. S-1** of the Supplementary Materials), chosen to prevent inaccuracies and ensure a high degree of convergence in the results. Conversely, CMG uses a radial grid mesh, which is ideally suited for simulating phenomena that are radially symmetric, typical in cylindrical reactor settings. This mesh type simplifies the computational load while still capturing the essential features and dynamics expected in the reactor's operation. The model we set up accounts for 10,000 elements in the grid. The adopted grids are shown in **Fig. 3**.

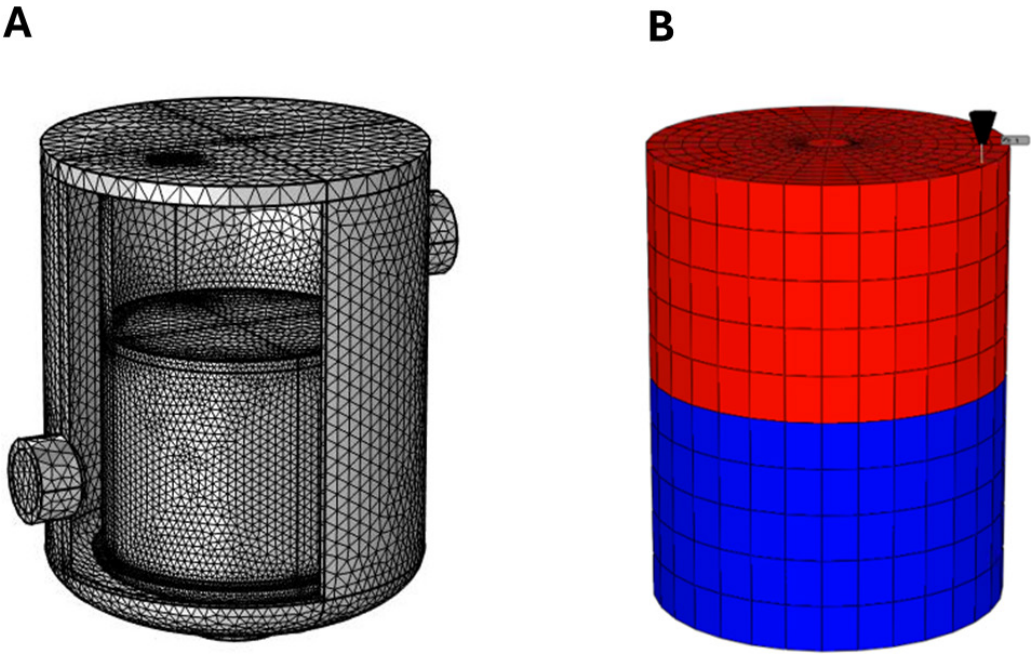

Fig. 3—Domains modeled for the simulations: (a) tetrahedral COMSOL mesh and (b) CMG-GEM grid.

The modeled domains in both COMSOL and CMG include the headspace, the porous rock, and the formation water, which are crucial for accurate simulation of the reactor environment. In addition, COMSOL's model incorporates specific domains related to the reactor vessel, as outlined in **Fig. 2**, providing a more detailed analysis of the interactions between the reactor components and the biological and chemical processes occurring within than with CMG-GEM. This comprehensive setup enabled the simulation of a range of scenarios, from chemical reactions and microbial growth within the rock and formation water to gas exchange dynamics in the headspace. The different meshing strategies and detailed domain modeling ensured that both macroscopic and microscopic phenomena were accurately captured, thereby enhancing the predictive capabilities and reliability of the bioreactor simulations. It is important to underline that at the laboratory reactor scale, the CMG software requires careful tuning of the numerical parameters for computational convergence and accuracy. This optimization is necessary due to the software's typical use in larger domains, requiring adjustments to handle significant concentration, pressure, and saturation variations within a small domain. Fine-tuning of parameters such as time, saturation, and pressure steps (and similar) ensured effective simulation convergence also at the bioreactor scale. COMSOL and CMG-GEM have very different computational times due to their different mesh resolutions and functionalities. COMSOL, with its capacity for high-resolution modeling, uses a fine mesh to capture detailed reaction-transport processes at a laboratory or small-scale domains. This allows for a precise coupling of biogeochemical reactions with fluid dynamics and heat transfer, though at the expense of long computational times. In contrast, CMG-GEM is optimized for large-scale reservoir simulations as it can use a coarse mesh, allowing for faster runtime compared with COMSOL. This makes CMG-GEM more suitable for field-scale applications where computational efficiency is essential. Therefore, COMSOL is recommended for detailed laboratory-scale analyses, while CMG-GEM is preferred for larger, fieldwide simulations where computational speed is a priority.

Performed Simulations and Operational Conditions. Our simulation strategy involved two key approaches: initial 0D simulations for kinetic parameter fitting and subsequent 3D simulations to replicate reactor operations under realistic (no nutrients) and hypothetical (abundance of nutrients) conditions. The 0D simulations were critical for fitting the biochemical kinetics based on real experimental data, as reported in the "Results" section. These simulations allowed us to fine-tune the parameters necessary for accurately representing the biochemical processes within the reactor, thereby ensuring that our 3D simulations could reliably predict the dynamics under various operational conditions.

Following the parameter fitting, comprehensive 3D simulations were conducted using COMSOL and CMG-GEM to model the experiments at the reactor scale. These simulations were performed according to the operational parameters listed in **Table 4**, encompassing a detailed representation of the reactor's working volume, headspace, liquid volume, and rock sample, all set within a controlled environment of "50°C and a total pressure of 150 bar" (Vasile et al. 2024). The gas mixture, comprising 1% CO_2 and 99% H_2, was simulated under batch conditions over a period ranging from 1 to 3 years.

	U.M.	Values
Reactor working volume	L	1
Headspace volume	L	0.3
Liquid volume	L	0.35
Rock sample	kg	0.35
Temperature	°C	50
Total pressure	bar	150
Gas mixture	vol%	1%CO_2–99% H_2
Working condition		Batch
Simulated time	year	1–3
Case Study 1, liquid phase		Formation water
Case Study 2, liquid phase		Formation water +nutrients addition

Table 4—Working process parameters for biochemical model and reactor operation.

Two distinct case studies were explored to understand the system performance under different liquid phase compositions:

- Case Study 1: Real formation water, identical to those tested experimentally, to validate the model against known conditions.
- Case Study 2: A hypothetical conservative scenario where continuous nutrient addition was imposed, aiming to eliminate growth limitations due to the absence of basic nutrients and potentially accelerate the biochemical reactions.

These scenarios helped in assessing the robustness of our reactor design and the adaptability of the biochemical processes within varied environments. The different liquid compositions in the case studies allowed for an evaluation of how nutrient levels affect bacterial growth.

Results and Discussion

Biochemical Kinetics and Microorganism Growth: Experiments and Simulation Results. Due to the effects that "indigenous microbial communities could have on the stored gases, the variations of headspace pressure and gas concentrations/partial pressures (i.e., CH_4, H_2, CO_2, and H_2S) were considered along with possible pressure drops due to liquid and gas sampling" (Bellini et al. 2024). The results of the simulations on "headspace gas compositions, pressures, and volatile fatty acids (VFA) production" are shown in **Figs. 4 and 5** (Bellini et al. 2024).

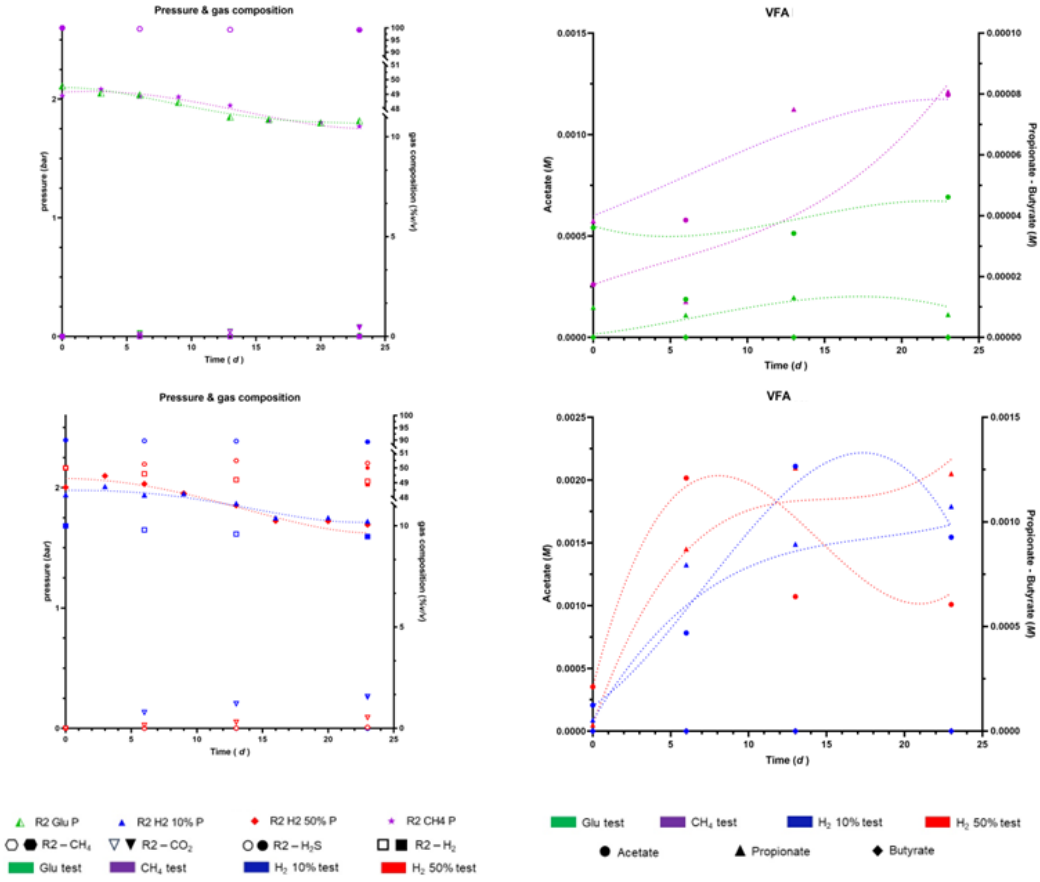

Fig. 4—Experimental (solid symbols) and modeled trends (dashed lines for VFA and pressure and no-filled symbols for the gas phase) of gas composition, VFA, and pressure during batch cultivation of bacteria in formation waters fed with various CH_4/H_2 gas mixtures.

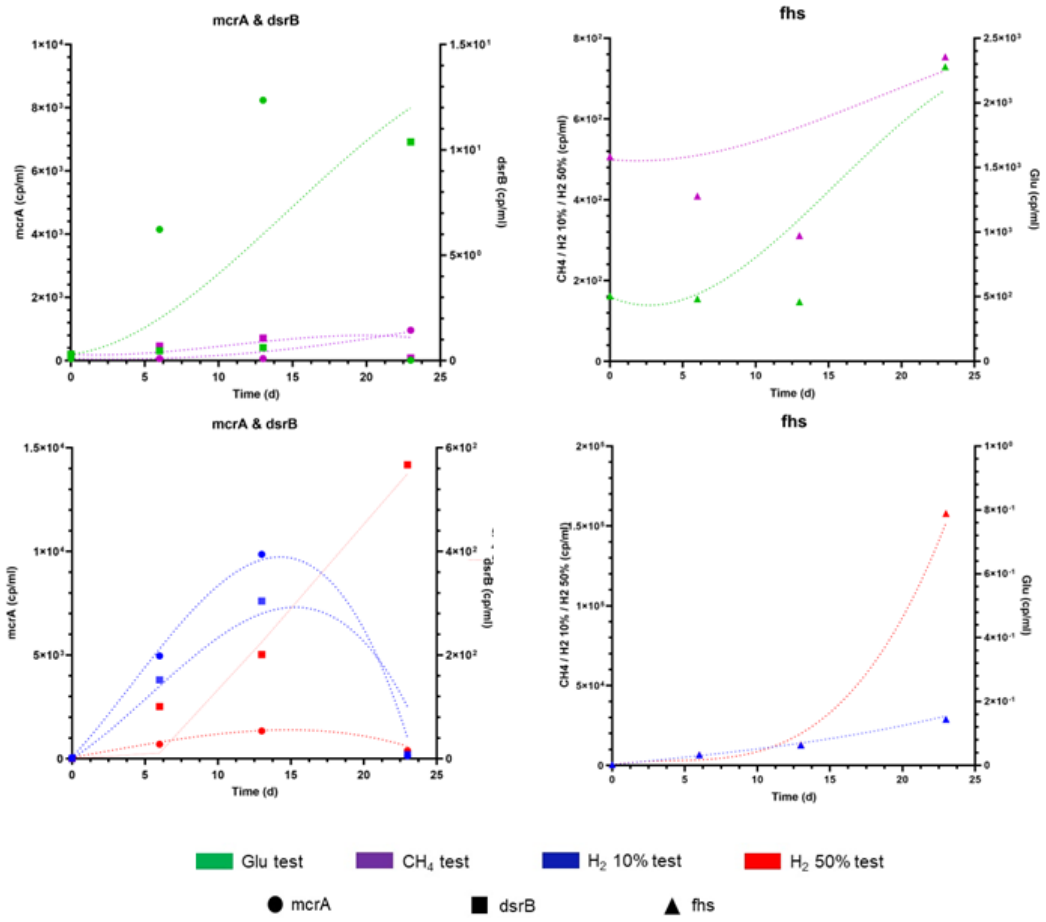

Fig. 5—Experimental (solid symbols) and simulated trends (dashed lines) of copies/mL of mcrA, dsrB, and fhs during batch cultivation of bacteria in formation waters fed with various CH_4/H_2 gas mixtures.

The results of the simulations show that the pressure decrease is similar in almost all the analyzed cases and is "mainly due to gas and liquid sampling and the dissolution of gases in the liquid culture" (Bellini et al. 2024). The model also provides information on the variation of moles present in the gas phase for each component. "Slight consumption of hydrogen was recorded for all the cultures when H_2 was initially injected in the headspace" (Bellini et al. 2024), then it was almost negligible (< 0.1%) for H_2 10%. Conversely, in the cases with H_2 50%, the simulations report hydrogen consumption of less than 0.2%. As for CH_4, the simulations did not show any production in all the case studies. Similar trends were observed when analyzing the growth kinetics of HM, AB, and SRB modeled with the growth model. The data reported in **Fig. 5** show the modeled trends of cell number growth for the three considered microbial classes.

In the batch tests conducted, no measurable concentrations of H_2S were detected. This undetectable H_2S level is likely due to the limited activity of SRB, influenced by the specific environmental conditions of the formation water, including low sulfate availability. According to EU gas standards, the maximum allowable concentration of H_2S for safe pipeline injection is 5 mg/m³ ("IGU 2023). The absence of detectable H_2S in the batch tests indicates that, under the tested conditions, the risk of H_2S generation is minimal and comfortably within the established safety limits for UHS applications.

The simulation results align with those obtained experimentally, emphasizing that AB are the most active microbial cluster in formation waters. Low microbes activity and growth of cell number were also found for SRB, which showed even lower levels than HM. The "discrepancy in the growth levels of microbial classes seemingly points out minimal possible HM and SRB activity in the investigated reservoir" (Bellini et al. 2024; Bassani et al. 2023).

Dominant presence of AB was observed, and it relates to an increased production of several acidic compounds, such as acetate. This predominance of "acetogenic metabolisms is made more evident when analyzing the quantities of VFA produced, along with their mass balances, carbon and hydrogen uptake, and the selectivity of carbon and hydrogen distribution metabolized by microbes in various products, mainly CH_4, VFA, CO_2, and H_2S, generated by the three selected microbial classes" (Bellini et al. 2024). From the simulation results, the main part of carbon source is utilized to produce VFA, while the residual part is used for CO_2 and biomass production. The model reproduces a "similar type of partitioning for hydrogen, as most of the uptaken hydrogen is utilized for the production of VFA" (Bellini et al. 2024). Higher uptake of carbon and hydrogen was observed when H_2 was initially present in the headspace gas mixture.

The observed minimal hydrogen consumption and stable pressure within the reservoir environment can be attributed to multiple factors limiting microbial activity. First, the low sulfate concentration in the reservoir brine and formation water likely restricts SRB activity, reducing their capacity for hydrogen consumption (Bellini et al. 2024). In addition, the moderate salinity and near-neutral to slightly alkaline pH further constrain microbial growth by lowering microbial activity and limiting the metabolic efficiency of SRBs.

Moreover, the activity of other hydrogen-consuming microbial metabolisms, such as methanogenesis and acetogenesis, is also highly limited in this reservoir environment. High concentrations of inhibitory ions such as calcium and magnesium interfere with the membrane integrity and enzymatic processes of methanogens and acetogens, further reducing their hydrogen uptake and conversion capabilities (Bellini et al. 2024). Finally, the low solubility of hydrogen at the relatively low pressures considered in this study inherently limits the availability of dissolved hydrogen for microbial metabolism, which impacts SRBs, methanogens, and acetogens alike. This combination

of environmental factors supports the observed trends of limited hydrogen consumption and stable pressure, reinforcing the low-risk profile of this UHS environment.

For what concerns the tests which do not account for the addition of nutrients to the liquid phase, the "simulation data are in line with the experimental results, underlining the absence of activity and growth for the considered microbial clusters (i.e., AB; HM; SRB)" (Bellini et al. 2024).

Case Study 1: Experiments and Simulation Results. The experimental activities in the bioreactor were obtained with the "Bio-xplorer system, capable of maintaining the required high operational temperatures and pressures for the tests" (Vasile et al. 2024). The formation water from the reservoir was characterized by lower salinity and a higher content of carbon sources **(Table 1)**, providing a unique environment for assessing the microbiological impact on hydrogen storage. The formation water had unique chemical attributes that influenced the experimental outcomes. Lower salinity levels and richer organic content in the formation water could potentially support a broader range of microbial processes, which was a key focus of the study to understand how such conditions might impact hydrogen storage.

The experiments maintained a constant temperature of 50°C and a pressure of 150 bar. A challenging environment was created using a gas mixture of 99% H_2 and 1% CO_2, chosen to simulate potential reservoir conditions and assess the hydrogen's impact on microbial activity. The reactor was charged with the gas mixture to test the resilience of microbial communities to high hydrogen levels. Despite the conditions potentially conducive to microbial activity (Heinemann et al. 2021; Jahanbani Veshareh et al. 2022), the tests indicated minimal activation of microbial populations, including SRB and HM. This was an important observation, suggesting that even potentially supportive environments might not lead to significant microbial risks. The experimental results indicating a low risk of microbiological activation, evidenced by the absence of significant changes in pressure level and in microbial populations, and the stability of gas and VFA compositions, are shown in **Fig. S-2** of the Supplementary Material and **Fig. 6**.

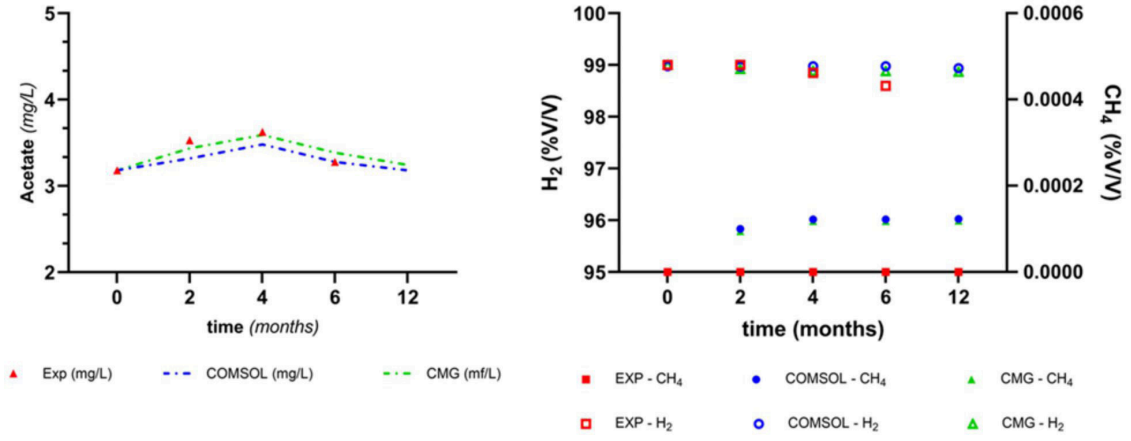

Fig. 6—Comparison between experimental (red—solid and empty symbols) and simulated trends for COMSOL (blue—line and symbols) and CMG-GEM (green—line and symbols) model data for gas composition and VFA during 1 year high-pressure reactor cultivation of bacteria in formation waters fed with an H_2/CO_2 gas mixture.

The tests showed limited occurrence of SRB and HM, indicating a low microbiological risk in the storage environment. No significant change in the headspace pressure and gas composition was detected throughout the experiment, suggesting that microbial communities did not substantially consume or produce hydrogen or methane. The production of VFAs was monitored as a direct indicator of microbial metabolism. Consistent with the low microbial activity, there was no significant production of VFAs, reinforcing the conclusion of minimal microbial interference under the tested conditions.

Furthermore, no H_2S was detected in the gas phase during the testing period. This result aligns with the observed low density of SRB populations in the reservoir, as confirmed by qPCR analysis, where SRB gene markers (e.g., dsrB) were undetectable following incubation in hydrogen-rich conditions. The absence of H_2S in these high-pressure tests reinforces the low-risk profile for in-situ H_2S generation, indicating that the conditions within the considered reservoir meet the safety standards for UHS, with H_2S levels well below the maximum allowable limit of 5 mg/m³ set by EU gas regulations (IGU 2023).

When compared with other works, the present case study does not report significant H_2 losses. Previous research on UHS has observed microbial hydrogen consumption rates ranging from 20% to 40% of the initial gas volume, particularly in environments with low salinity (<10 g/L) and neutral to mildly alkaline pH levels (7–8.5) (Hellerschmied et al. 2024; Buriánková et al. 2022; Šmigáň et al. 1990; Haddad et al. 2022). In contrast, hydrochemical analyses of the investigated reservoir indicate higher salinity levels (>40 g/L) and slightly acidic pH values (5.5–6). These two factors alone are known to significantly restrict microbial abundance, diversity, and activity, which in turn can greatly reduce H_2 consumption (Bagaria et al. 2013; Jaafar et al. 2009; Muhammed et al. 2022). In addition, studies on similar formations report that moderate salinity and low pH inhibit hydrogenotrophic microbial communities, including methanogens, SRB, and acetogens, by disrupting microbial membrane integrity and enzyme functionality (Bellini et al. 2024; Bassani et al. 2023). Accordingly, formation fluids from the studied reservoir exhibit reduced microbial concentrations and lower microbial diversity compared with those in reservoirs with lower salinity and higher pH. These conditions likely contribute to the limited or negligible hydrogen consumption observed in our case study, contrasting with the more substantial hydrogen losses reported in other studies under different reservoir conditions.

The comparison between the experimental data and the simulation outputs from COMSOL and CMG-GEM reveals a notable congruence, particularly in the variation of gas composition at the reactor headspace and the production of VFAs. **Fig. 6** illustrates this alignment, underscoring the effectiveness of both modeling platforms in accurately replicating the experimental phenomena This good agreement between experimental and modeled data highlights the robustness of the models in both platforms, where both COMSOL and CMG-GEM report similarly low or nearly absent activation of the metabolic pathways of interest. These findings validate the models' capability to

faithfully reproduce the lack of significant microbial activity under the stated operational conditions, confirming the model's utility in predicting microbial behavior in scenarios mimicking real operation of hydrogen storage environments.

Case Study 2: Simulation Results. Considering the results obtained from the initial simulations using zero dimensions on COMSOL and three dimensions on both COMSOL and CMG-GEM, we conducted a second set of simulations to explore the effects of the presence of nutrients on hydrogen consumption linked to methanogenic, dsrB, and acetogenic biochemical reactions within a simulated process environment. These simulations focused on the thermo-fluid dynamics and biochemical operating conditions within the reactor, and we present the main findings regarding microbiological activity, such as the consumption of reactants (i.e., H_2), the generation of products (i.e., CH_4, H_2S, $C_2H_4O_2$, etc.), and their combinations, as representative of the phenomena occurring under the selected operational conditions. First, the simulations were conducted in a batch mode for 1 year, under the operating pressure and temperature conditions specified in **Table 4**. **Fig. 7** illustrates the trends in methane, hydrogen, and acetate levels over a year of the simulated process, employing the kinetics fitted from the 0D model. The simulations indicate productive microbial activity predominantly during the first part of the simulated period. This activity is primarily due to the assumptions inherent to the models, which limit the amount of carbon available for the bioprocesses to that initially present in the gas and liquid phases. The initial presence of CO_2 in the headspace, in addition to the existing carbon sources in the formation water, leads to an increase in CH_4 and acetate production, mainly in the first 6 months. During this period, there is an observed increase in the activity of AB and a slight production of CH_4, which results in higher hydrogen consumption, as shown in **Fig. 7**. This is largely attributable to the CO_2 available at the start of the simulated bioprocesses. In the analyzed reservoir, there is still a lower risk of activation of methanogens and acetogens, mainly due to the lower activation observed in the batch laboratory tests (Bellini et al. 2024), which is consistently replicated in the simulations, even over periods longer than those tested in low-pressure tests.

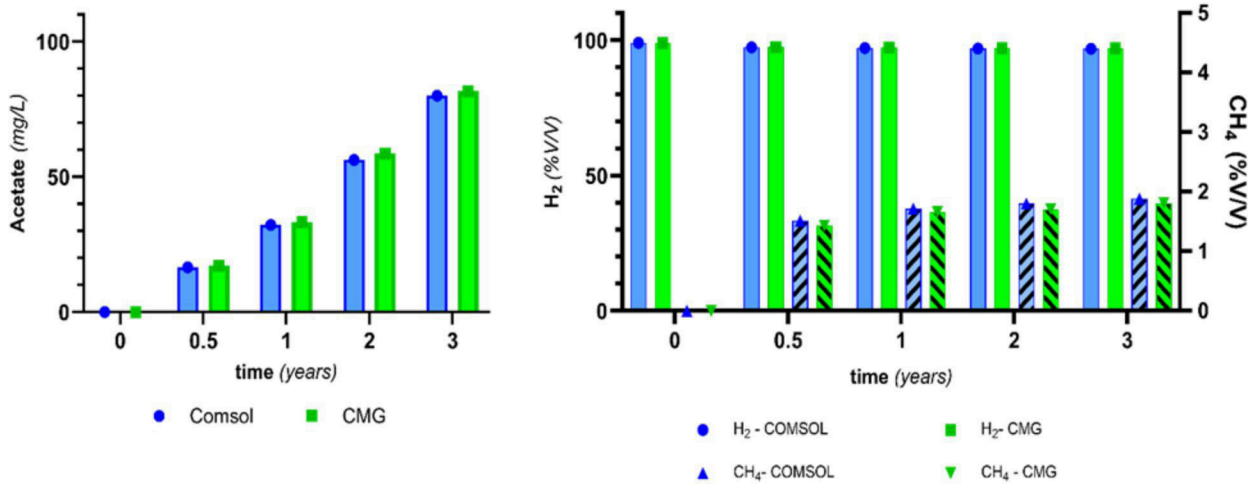

Fig. 7—Simulated trends of headspace gas composition and VFA during 3 years of high-pressure reactor cultivation of bacteria in formation waters with nutrient addition and fed with an H_2/CO_2 gas mixture; comparison between COMSOL (blue bars) and CMG-GEM (green bars) model results.

The simulation duration was extended to 3 years to evaluate whether the bioreactive system might face limitations in reactants such as CO_2, sulfates, and hydrogen. This extended simulation provided insights into the potential long-term behavior of the bioprocesses, including the impact of depletion of reagent sources. The continuous production of acetate mainly by acetogens was observed over the 3 years, facilitated by the high presence of organic carbon and bicarbonates in the formation water, and by the initial amount of 1% of CO_2. In **Fig. 7**, we report a hydrogen consumption of around 0.3% after 3 years in the headspace, as well as acetate production reaching a concentration of approximately 82 mg/L in the liquid phase. The risk of activation of SRB bacteria is again almost negligible.

Similar performances were observed between COMSOL and CMG-GEM, with minimal differences in the simulation outcomes—generally under 3–5%. This consistency is particularly notable when examining the results after 1 year of process simulation. However, a deeper analysis over 3 years reveals that the differences between the two software platforms further diminish. This convergence is primarily attributed to the saturation of production, which inevitably occurs due to the limiting factor of the process—the availability of carbon sources. As the simulation extends over 3 years, the system approaches a plateau in biochemical activity. Initially, available carbon in the formation waters and the additional carbon introduced through the gas mixture are gradually consumed by microbial processes. Once this carbon is exhausted, the key bioprocesses of acetogenesis and methanogenesis begin to decelerate significantly, eventually coming to a halt. The depletion of carbon sources leads to a cessation in microbial activity, effectively capping the potential for further biochemical transformations within the simulated environment.

This extended analysis underscores the critical role of carbon as a fundamental resource in sustaining microbial activity in bioreactor systems and thus in perspective underground reservoirs and highlights the importance of considering long-term carbon availability in the design and management of bioprocesses.

Conclusions

This research has demonstrated the effective application of biogeochemical modeling in understanding and managing the microbial risks associated with UHS. Utilizing both COMSOL® Multiphysics and CMG-GEM, complemented by PHREEQC, the study successfully replicated complex microbial and geochemical interactions under simulated reservoir conditions. The models provided a robust platform for simulating the dynamic interplay of biochemical reactions and hydrochemical processes at both the bioreactor and reservoir scales. The simulations carried out using COMSOL and CMG-GEM have underscored the versatility and precision of these tools in predicting

the behavior of microbial communities and their impact on hydrogen storage systems. The comparative analysis of results from COMSOL and CMG-GEM revealed minimal differences, highlighting the consistency and reproducibility of the simulations across different platforms. This consistency is critical for cross-verifying findings and ensuring that the models are robust across various computational environments.

The use of modeling has proved essential in comprehending the nuanced interactions within UHS environments, offering a predictive tool that is very valuable for risk assessment and management. The models were rigorously validated against experimental data, showing excellent agreement in terms of kinetic parameter fittings and the replication of observed phenomena. This validation not only confirms the accuracy of the models but also enhances the reliability of the simulations in predicting real-world outcomes. The close match between modeled and experimental results, especially in the absence of nutrient enhancement, attests to the models' capability to reflect natural microbial activities accurately.

The outcomes of the experimental and modeling work have significant implications for the feasibility and optimization of UHS. The presence of HM and SRB in the formation water points to the possibility of microbial hydrogen consumption and H_2S production, which could impact both the retention and purity of the stored hydrogen. These findings highlight the importance of including a microbial risk assessment in screening of reservoirs for UHS, as the metabolic potential of the indigenous microbial populations is crucial in predicting and managing hydrogen loss or contamination risks.

The results from the batch and high-pressure/high-temperature reactor tests further underscore the need for specific design and operational measures tailored to the reservoir conditions. Controlling environmental factors such as accidental nutrient influx and maintaining pressure and temperature within certain thresholds could help inhibit microbial activity, thereby preserving hydrogen quality and minimizing microbial alterations to the reservoir.

The gas consumption and production dynamics observed in various gas compositions tested in our experiments also provide valuable guidance for injection and production strategies. Injecting hydrogen with controlled ratios of methane or other inert gases may prove beneficial in stabilizing microbial activity. Moreover, regular monitoring of microbial indicators, such as hydrogen and H_2S levels, during gas withdrawal is essential for promptly identifying and managing any increase in microbial activity.

Furthermore, the formation of biofilms and subsequent bio-geochemical alterations also play a crucial role in the dynamic characteristics of UHS sites. Biofilm growth by hydrogen-consuming microorganisms can decrease permeability by clogging pore spaces, which alters hydrogen flow pathways and influences injection and withdrawal efficiency. Additionally, these microbial biofilms interact with the surrounding geochemistry, potentially inducing mineral precipitation reactions that further reduce porosity and permeability, leading to localized flow restrictions and impacting operational strategies for well injection and withdrawal.

The insights gained from this study not only enhance our understanding of microbial interactions in UHS but also pave the way for the development of advanced models that can simulate and predict the behavior of complex biogeochemical systems at a larger scale. Looking forward, the focus will shift toward scaling these bioprocesses to reservoir scale by using the CMG-GEM platform. Despite the insights gained, this study is subject to limitations inherent to 0D/laboratory scale modeling. While this model's scale facilitated an effective performance comparison between COMSOL and CMG-GEM, it does not account for spatial variations or flow dynamics that would be present in a full-scale reservoir. Real-environment applications would require 2D and 3D dynamic models, where transport equations capture the flow and dispersion of hydrogen through porous media, providing a more comprehensive risk assessment. As we move forward, future work will involve transitioning to reservoir-scale 2D and 3D models to incorporate these spatial complexities. This will enable us to account for the heterogeneous distribution of microbial populations and variable geochemical conditions over larger volumes, which may influence the biotic reaction rates and hydrogen stability in the subsurface. By extending this modeling approach to higher dimensions, we aim to bridge the gap between laboratory-scale observations and field-scale predictions, advancing the reliability of biogeochemical models for UHS and supporting safe, efficient, and scalable storage solutions. Examples of these challenges include scaling the kinetics of microbial reactions that may not behave linearly when transitioning from laboratory-scale models to the vast and complex environments of geological reservoirs.

By continuing to refine these models and expand their applicability, we can advance our knowledge and move closer to realizing safe and efficient UHS solutions that are compatible with the goals of a sustainable energy future.

Nomenclature

A = area, m^2
c_d = mass fraction of dispersed phase, kg kg^{-1}
C = concentration, mol m^{-3}
C_p = specific heat at constant pressure, J m^{-3} K^{-1}
D = diffusion coefficients, m^2 s^{-1}
D_{md} = turbulent dispersion coefficient, m^2 s^{-1}
e = enthalpy flux density, J m^{-2} s^{-1}
EA = activation energy, J mol^{-1}
F = force term, kg m^{-2} s^{-2}
G = incident light radiation, W m^{-2}
$hj(T)$ = enthalpies heat flux densities, J m^{-2} s^{-1}
I = incident light intensity, W m^{-2}
I_b = black body radiation, Wm^{-2}
J = diffusion vector
k = turbulent kinetic energy, m^2s^{-3}
k_c = effective thermal conductivity coefficient, W m^{-1} K^{-1}
K_r = reaction rate constant, m^2s^{-1}
m = mass of species, kg
m_{dc} = mass transfer from dispersed to continuous phase, kg $m^{-3}s^{-1}$
M = molar mass, kg mol^{-1}
n = flux density, mol m^{-2} s^{-1}
n_d = relative mass flux, mol m^{-2} s^{-1}
p = pressure, Pa
q = heat flux densities, W m^{-2}
Q = volumetric charge density, C m^{-3}

Q_r = radiative flux, W m^{-2}
R = universal gas constant, J K^{-1} mol^{-1}
T = temperature, K
u = velocity vector, m s^{-1}
u_c = continuous phase velocity vector, m s^{-1}
u_d = dispersed phase velocity vector, m s^{-1}
u_{slip} = slip velocity vector, m s^{-1}
v = stoichiometric coefficients
V_F = convective velocity, m s^{-1}
w = volume fraction
x = mass fraction
β = extinction coefficient, m^{-1}
ε = turbulent energy dissipation, m^2s^{-3}
κ = absorbance coefficient, m^{-1}
μ = dynamic viscosity, kgf s m^{-2}
μ_{gr} = growth rate, h^{-1}
μ_T = turbulent viscosity
ρ = density, kg m^{-3}
σ_S = scattering coefficient, m^{-1}
τ = turbulent stress
ϕ_χ = continuous phase fraction, -
ϕ_δ = dispersed phase fraction, -
ω = rotational velocity, rad s^{-1}

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