Environmental Science & Technology

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Critical Review

Identifying and Understanding Microbial Methanogenesis in CO_2 Storage

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Cite This: *Environ. Sci. Technol.* 2023, 57, 9459−9473

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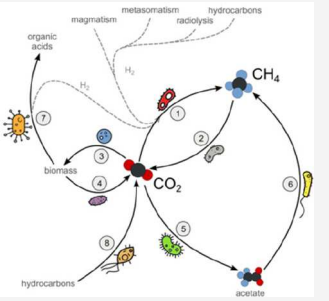
ABSTRACT: Carbon capture and storage (CCS) is an important component in many national net-zero strategies. Ensuring that CO_2 can be safely and economically stored in geological systems is critical. To date, CCS research has focused on the physiochemical behavior of CO_2, yet there has been little consideration of the subsurface microbial impact on CO_2 storage. However, recent discoveries have shown that microbial processes (e.g., methanogenesis) can be significant. Importantly, methanogenesis may modify the fluid composition and the fluid dynamics within the storage reservoir. Such changes may subsequently reduce the volume of CO_2 that can be stored and change the mobility and future trapping systematics of the evolved supercritical fluid. Here, we review the current knowledge of how microbial methanogenesis could impact CO_2 storage, including the potential scale of methanogenesis and the range of geologic settings under which this process operates. We find that methanogenesis is possible in all storage target types; however, the kinetics and energetics of methanogenesis will likely be limited by H_2 generation. We expect that the bioavailability of H_2 (and thus potential of microbial methanogenesis) will be greatest in depleted hydrocarbon fields and least within saline aquifers. We propose that additional integrated monitoring requirements are needed for CO_2 storage to trace any biogeochemical processes including baseline, temporal, and spatial studies. Finally, we suggest areas where further research should be targeted in order to fully understand microbial methanogenesis in CO_2 storage sites and its potential impact.



KEYWORDS: *CO_2 storage, microbial methanogenesis, biogeochemical tracing, clumped isotopes, stable isotopes, noble gases, microbial sequencing*

1. INTRODUCTION

Fossil fuels have been society's primary source of energy for over 150 years and currently account for approximately 80% of global energy production.[1] Anthropogenic emissions have resulted in a 50% increase in atmospheric carbon dioxide (CO_2) concentration and an associated decrease in atmospheric $\delta^{13}C$-CO_2 relative to preindustrial levels. The resulting climate change, global warming, and ocean acidification are not sustainable. Carbon capture and storage (CCS) of anthropogenic CO_2 within geological storage targets is an essential component of many national net-zero strategies (i.e., where the same amount of CO_2 is removed from the atmosphere as is emitted).

Therefore, it is critical to scientifically and systematically demonstrate that captured CO_2 can be safely and cost-effectively stored in geological systems. Geological targets include saline aquifers, depleted hydrocarbon fields, basalts, and coal beds.[2] To date, fundamental scientific research has focused on CO_2 phase behavior, solubility, and fluid migration, the physical characteristics of the geological environment, and the chemical reactions within the fluids and rocks that might modify such a system. Together these factors dictate how both proximal and distal crustal systems respond to injected CO_2 on various time scales.[3] Notably, there has been substantially less research focused on the potential impact of subsurface biological activity on the CO_2 storage system and how this will vary between geological targets.

At temperatures below ∼122 °C, almost all rock environments contain microbes in the water-filled pore spaces and fractures.[4] For perspective, while the biomass of Earth's continental subsurface ecosystem is poorly known, recent estimates suggest that it contains 20−100 Pg of carbon (1 Pg = 10^{15} g).[5,6] This is comparable in size to the total prokaryotic (bacteria and archaea) biomass on Earth, which is estimated at 350−550 Pg of carbon.[7] Our knowledge of the biodiversity and activity of prokaryotes in the deep crust is not well developed, since the majority of microbial biomass comes from deep lineages with no cultured relatives.[8] Many of them are known to form symbiotic ecosystems to exploit limited energy and nutrient sources.[9,10] Many are autotrophic[11,12] and capable of drawing down CO_2 and sequestering it as biomass.[13,14]

Received: November 17, 2022
Revised: May 18, 2023
Accepted: June 1, 2023
Published: June 16, 2023

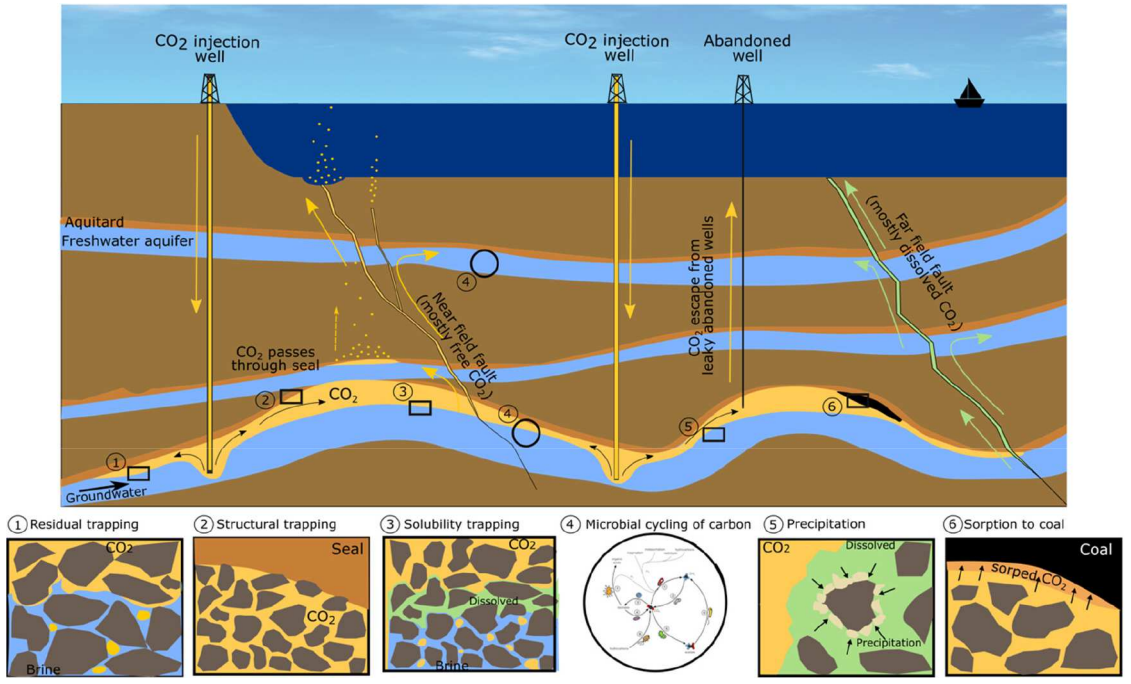


Figure 1. Schematic overview of CO_2 storage. Potential CO_2 storage mechanisms are shown in boxes 1−6, and potential near- and far-field leakage mechanisms are also shown.

Since they use chemical energy, rather than sunlight to power CO_2 consumption, they are called chemolithoautotrophs.

Methanogenic archaea generate methane from a variety of substrates but are dominated by acetoclastic (acetate-consuming) and hydrogenotrophic (H_2/CO_2-consuming) methanogens.[15] Their diversity and activity in marine sediments and wetlands is well-studied, and they have been identified in deep sedimentary fluids.[16,17] The activity of hydrogenotrophic methanogens and the magnitude of the conversion of CO_2 to CH_4 has been shown to be significant within depleted oil fields (e.g., refs 18−20). However, a broader understanding of the potential scale of methanogenesis, the range of geologic settings under which this process operates, and the potential implications of this process—in the context of CCS—are poorly understood. It is therefore critical to understand what happens to such a geological ecosystem when it is perturbed on decadal time scales by injecting CO_2.[20] This has the potential to impact how carbon is being stored in the system and what the monitoring requirements are. Similarly, this will be important when considering how to identify and understand the impact of CO_2 leakage from deep/high-temperature to shallow/low-temperature systems, if methanogenesis occurs during migration and/or in subsequent shallower accumulations. The latter is important for developing a monitoring strategy in both deep and shallow CCS targets that enables quantification of CO_2 trapped via various mechanisms, including the amount of CO_2 that has been converted to methane, and any potential CO_2 loss from the target reservoir interval.

While CO_2 storage technologies are under development, there is a need to understand how "safe" a geological storage target is or how to mitigate possible undesirable responses. This requires understanding the microbial responses to injection which have previously been largely overlooked. The objective of this review is to describe the conditions under which the microbial conversion of CO_2 to CH_4 occurs, how this might impact the evolution and fate of CO_2 in a storage site, and the integrated techniques required to identify and quantify the extent of microbial methanogenesis in CO_2 injection wells.

2. CARBON STORAGE: WHERE AND HOW?

CCS is an essential tool for combating climate change, and CCS projects globally are rapidly expanding. From 2021 to 2022, there was a 44% increase in the capacity of projects.[21] With the goal of limiting global warming to 2 °C, estimates suggest that CCS capacity needs to increase from current capabilities of approximately 40 Mt/yr to over 5600 Mt/y by 2050,[1] assuming a 40−70% reduction in greenhouse gas emissions by 2050.[22] Deep saline aquifers likely have the largest long-term storage potential, with depleted (onshore and offshore) hydrocarbon reservoirs having the second largest potential. Other storage targets include basalts and coal beds. Active CCS facilities benefit from geological data collected during oil and gas exploration; however, this will not always be the case for all future potential sites.

Storage of CO_2 is strongly dependent on local geologic conditions, lithology, crustal structure, and reservoir formation conditions (i.e., fluid composition, reservoir pressure, and temperature). The four most common means of CO_2 trapping and storage in geological environments are (1) physical, (2) residual, (3) dissolution, and (4) mineralization (Figure 1).

Physical trapping utilizes the same geological structures or stratigraphic configurations that form oil and gas accumulations. This occurs when buoyant, supercritical CO_2 is trapped below a low permeability caprock (e.g., anhydrite, shale, salt). If the geologic setting is suitably stable, the storage can effectively be considered permanent. Natural magmatic CO_2 can be trapped in geological structures on time scales of millions of years (e.g., ref 23), similar to hydrocarbons, which provide examples of the effectiveness of physical trapping in isolating CO_2 in the subsurface.

https://doi.org/10.1021/acs.est.2c08652
Environ. Sci. Technol. 2023, 57, 9459−9473

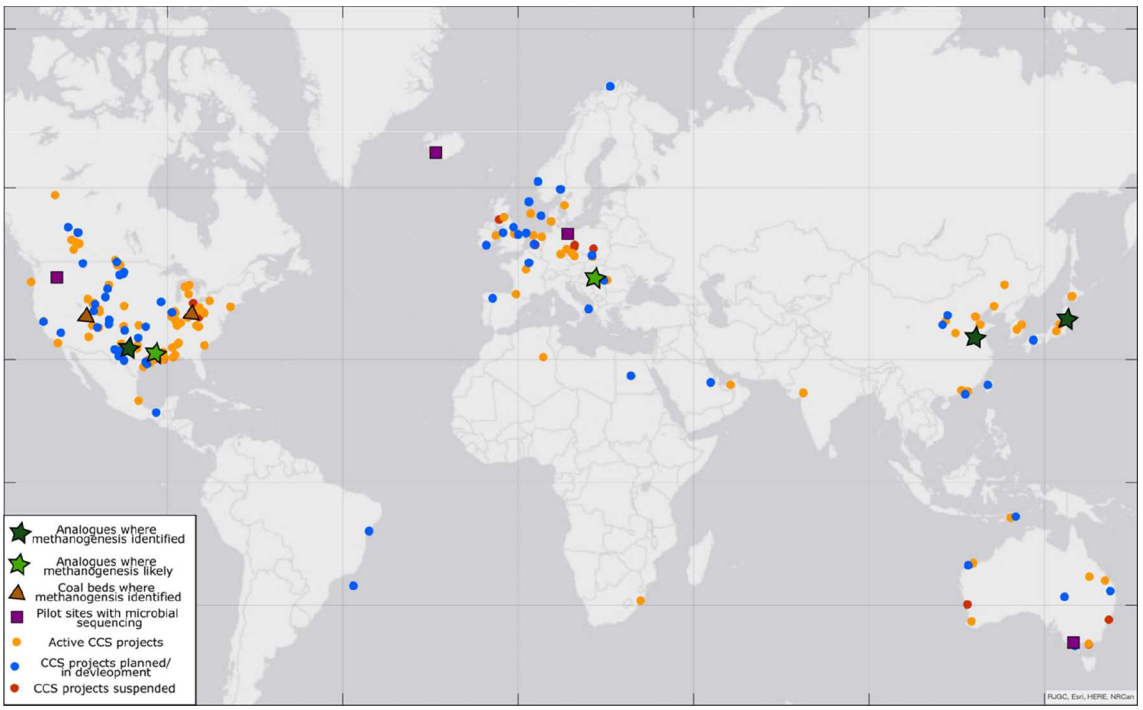


Figure 2. Map showing the global distribution of pilot carbon capture and storage (CCS) sites, sites where methanogenesis has been identified, is likely and CCS pilot sides where microbial sequencing has been completed. Active, in development/planned and suspended operations are also identified using the National Energy Technology Laboratory carbon capture and storage database.[30]

As CO_2 moves through a given reservoir, a portion of the CO_2 is trapped in the pore space by capillary forces. This process, *residual trapping*, is controlled by the connectivity between pores, reservoir lithology, wetability, and pre-existing pore fluid chemistry. Although residual trapping occurs at the microscale, the volume of CO_2 trapped by this mechanism is significant when the process occurs in large reservoirs.[3]

Dissolution trapping occurs when CO_2 interacts with a gas-undersaturated fluid (i.e., briny water) and the CO_2 dissolves into the fluid. The extent of dissolution is controlled by formation water salinity, reservoir temperature, and pressure conditions. Large-scale disposal of CO_2 in deep saline aquifers dates back to 1996 at the Sleipner project, offshore Norway.[24,25] Natural analogues show that dissolution can account for >90% of the injected CO_2 within some geological systems.[26]

CO_2 can also chemically react with the host rocks to form a stable mineral phase, typically in carbonate minerals (e.g., calcite and/or dolomite) in both carbonate or basalts systems. The efficacy of *mineralization* depends on the host rock mineralogy and groundwater chemistry as well as the system pressure and temperature. Primary reservoirs targeted for CO_2 storage (e.g., deep saline aquifers, depleted hydrocarbon fields) have a range of reactivities, with analogues suggesting mineralization is much less important than solubility trapping in naturally CO_2 rich sedimentary systems[23,26] and systems which have been CO_2 injected for enhanced oil recovery.[20] In contrast, injection of CO_2 into basalts can result in mineralization, as demonstrated by the CarbFix project[27], during the lifetime of the storage operation.

The dominant trapping mechanism of injected CO_2 in geologic storage sites is likely to evolve over time from predominantly physical trapping immediately following injection, to increasingly dominated by solubility or mineral trapping mechanisms after ~1000 years (e.g., ref 28). However, this will be limited by the availability of cations or undersaturated groundwater.[23,26] Processes that have the potential to change the chemical state of injected carbon in the subsurface that impact these key trapping mechanisms then have the potential to strongly impact the long-term fate of CO_2 in these sites. Understanding the magnitude and time scales of processes such as the biologically mediated conversion of CO_2 to CH_4 and other byproducts remains a primary focus of fundamental scientific research.

3. CURRENT STATUS OF CCS PROJECTS

CCS projects are now operational or in development in 25 countries, with the United States and Europe accounting for 75% of the projects in development[29,30] (Figure 2). Basins which have so far been developed for CCS are generally near emission-intensive regions, where storage has been in depleted hydrocarbon reservoirs. Typically, only countries with a history of hydrocarbon production have adequately assessed their sedimentary basins for geological storage.

CCS projects are expanding rapidly, with more than 100 new CCS facilities being announced in 2021. However, even with spiking interest and investment in CCS, only 3 Mt/yr of CO_2 capturing ability has been added worldwide each year since 2010,[31] with annual capture capacity of approximately 40 $MtCO_2$. When compared with the global CO_2 output of approximately 43 Bt/yr, there is clearly a huge shortfall. In fact, estimates suggest that capacity would need to increase to 1.6 Bt/yr by 2030 to align with a pathway to net zero by 2050.[30,32]

4. MICROBIAL METHANOGENESIS

Microbial methanogenesis produces ~1.2 Gt of methane per year and occurs naturally in a wide range of (mostly anoxic) environments.[3,15] The main substrates for methanogenesis,

https://doi.org/10.1021/acs.est.2c08652
Environ. Sci. Technol. 2023, 57, 9459−9473

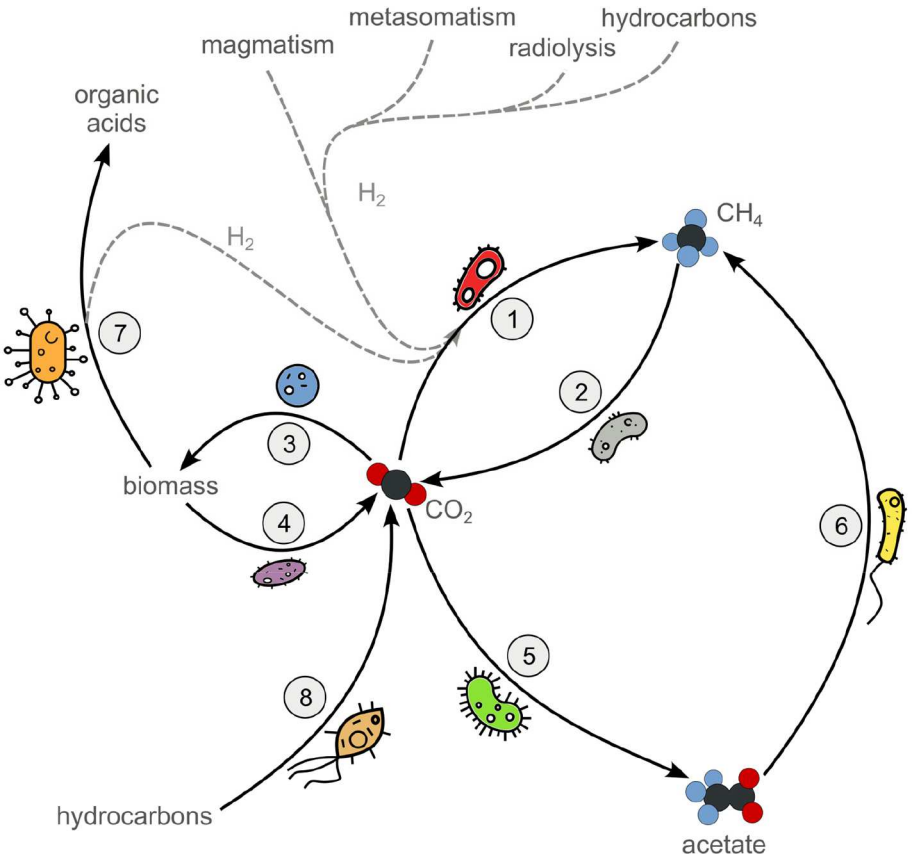


Figure 3. Schematic diagram showing the competing microbial processes involved in CO_2 cycling in CCS storage sites. The relative contributions of the different processes varies depending on the CCS storage site and availability of substrates beside CO_2. (1) Hydrogenotrophic methanogens. The source of hydrogen can be either from the biological fermentation of organic carbon sources and biomass ((7) in the figure) or from diverse abiotic sources (see main text for discussion). (2) Methanotrophy. (3) Chemolithoautotrophy, associated with diverse electron acceptors and donors that can vary in different CCS storage sites. (4) Heterotrophy. (5) Acetogenesis. (6) Aceticlastic methanogens. (7) Heterofermenters. (8) Hydrocarbon degraders. The diverse microbial cartoons used are abstract representations of different microorganisms and do not represent actual microbes.

H_2/CO_2, acetate, formate, methylated organic compounds, or hydrocarbons, can come from the natural fermentation of organic matter.[15] Methanogenesis from H_2/CO_2, called hydrogenotrophic methanogenesis, may be the most important process for CCS, since CCS adds CO_2 into the environment. Hydrogenotrophic methanogenesis consumes H_2 and CO_2 in a 4:1 molar ratio.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

hydrogenotrophic methanogenesis

Therefore, hydrogen has a large control on the kinetics and energetics of methanogenesis, especially in CCS systems where CO_2 is not limiting.[34] While water reacts with mafic rocks in the continental crust to produce hydrogen (a processes called serpentinization), and natural radioactivity can generate hydrogen through water radiolysis, the rates of natural in situ abiotic hydrogen production in the continental crust are on average low[35] (e.g., Figure 3) and insignificant on a CCS engineering or storage time scale for most geological systems. Within ultramafic rock types (e.g., ophiolites) or engineered systems (injection into basalts) faster reaction rates occur.[27,36]

Faster water−rock reactions also result in increased alkalinity, which can limit the bioavailability of CO_2 since most of it will be heavily deprotonated to carbonate.[37] While some methanogens have carbonic anhydrase,[38] which might allow them to convert bicarbonate to CO_2, none have been found to use carbonate. In such environments, the additional CO_2 from well injections may decrease the high pH of serpentinizing environments, pushing the carbonate equilibrium toward CO_2. This may alleviate the CO_2 limitation of methanogens, which can then use the H_2 from serpentinization to produce methane. Hydrogen sources also include the natural fermentation of organic matter (sedimentary organic matter can be found in saline aquifers and depleted oil fields) or the dehydrogenation of hydrocarbons. Methane production therefore can occur in a wide range of CCS systems, but at rates which will vary greatly depending on the availability of electron donors from local sources.

While the locations of biogenic CH_4 production are widespread, including ocean sediments, coal deposits, oil reservoirs, landfills, rice paddies, insects and animal guts, wetlands, and a wide variety of soils,[33] the number of organisms capable of methane production is comparably quite limited. Until recently it was thought that the entirety of CH_4 producing Archaea was contained within one single phylum, Euryarchaeota. New genomic evidence (e.g., refs 39−41) has recently expanded methanogenic phyla to include alkane oxidizing methanogens found in the Archaeoglobi, Hadesarchaeota, and various clades within the TACK superphylum including Nezhaarchaeota, Korarchaeota, and Verstraetearchaeota.[42] Even with this recent expansion and

https://doi.org/10.1021/acs.est.2c08652
Environ. Sci. Technol. 2023, 57, 9459−9473

with the wide range of methanogenic substrates used, the core of the methanogenic pathway remains genetically similar, with the methyl coenzyme M reductase (MCR) being the highly conserved canonical signifier of microbial methane metabolism.[15] MCR can therefore be used as a means to detect the presence of methanogens in the environment, but an additional method would be required to determine whether methanogenic taxonomic groups are present.

5. METHANOTROPHY

Even if methane is produced by microbes at CCS storage sites, its accumulation can be limited by microbial consumption through methanotrophy. This process requires the presence of an oxidant, such as O_2, NO_3, NO_2, SO_4, Mn^{2+}, or Fe^{2+}, and can be performed by a single organism, like in the case of aerobic methanotrophy and some special cases of anaerobic methanotrophy (like nitrite-dependent anaerobic methane oxidation) or within a microbial consortia. For the other oxidants besides oxygen, methanotrophy often occurs within a consortium of two types of microbes, the first which transfers reducing equivalents, in the form of diffusible molecules or directly transferred electrons, to another organism that respires the oxidants.[43] The end result is that CH_4 is oxidized back to CO_2. However, if these oxidants are present in high enough concentrations, they can inhibit methanogenesis by decreasing concentrations of methanogenic substrates.[44] Methanotrophy, both aerobic and anaerobic, is as globally important and widespread as methanogenesis.[33]

Aerobic methanotrophy occurs mostly in bacteria of the Alphaproteobacteria, Gammaproteobacteria, and Verrucomicrobia phyla and can sometimes be enhanced by a syntrophy with methylotrophic bacteria that prevent the buildup of intermediate molecules.[45] Anaerobic methanotrophy using sulfate as an electron acceptor often requires consortia of different organisms, and members of the Euryarchaeota, where anaerobic methane oxidation is best studied. These are called ANME-1 and ANME-2, and they are closely related to methanogens. Further, they may be able to reverse back and forth between methane production and consumption, depending on the hydrogen concentrations.[46,47] Anaerobic methanotrophy using oxidized nitrogen compounds occur nonsyntrophically by bacteria such as *Methylomirabilis* sp.[48] and archaea such as *Methanoperedens* sp.[49] that can also use oxidized iron.[50] Molecular markers for aerobic methanogenesis are particulate and soluble methane monooxygenases (PMO and MMO).[51] Most anaerobic methanotrophy occurs with the same enzyme as methanogenesis, MCR, since this process uses methanogenesis in reverse.[52]

6. GEOCHEMICAL TRACERS FOR METHANOGENESIS + AOM

The use of geochemical and stable isotope fingerprinting to determine the provenance and history of carbon compounds in the shallow to deep subsurface is well established. Stable isotope geochemistry of carbon ($\delta^{13}C$) compounds, coupled with concentration measurements, is routinely used to identify the processes responsible for CO_2 sequestration in the subsurface associated with different trapping or conversion processes.

In the context of CCS, there is a requirement to characterize (i) the initial injected fluid composition, (ii) the baseline composition of dissolved gases and ions within formation water in the storage reservoir target, and (iii) the compositional evolution of both reactants and products associated with in-reservoir processes such as hydrogenotrophic methanogenesis or carbonate mineral precipitation. The injected supercritical fluid is likely to be near pure CO_2, while the initial $\delta^{13}C_{CO_2}$ will depend on the feedstock and the capture mechanism. A detailed discussion of the variability of $\delta^{13}C_{CO2}$ values of CO_2 feedstock compositions used to supply active CCS projects can be found in Flude et al.[53] The baseline composition of dissolved gases or ions within formation water can be quite variable depending on the origin of the given compounds. Dissolved inorganic carbon, which is typically present as HCO_3^- at the pH of most formation waters, is likely to have a $\delta^{13}C$ value that varies between approximately 0 and −25‰, depending on wheter the CO_2 is associated with carbonate minerals (e.g., more positive in cement production) or derives from the oxidation of organic matter (e.g., more negative in hydrocarbon burning). These baseline compositions will quickly be modified during CO_2 injection to reflect the relative mass or volumes of baseline fluids to that of the injected fluids. As the system evolves toward a fluid composition that is dominated by the injected fluid, the dissolved carbonate species will begin to equilibrate with the injected supercritical CO_2, giving rise to a new subsurface composition which is indistinguishable from that of the injected fluid plus or minus any intramolecular equilibrium isotope exchange reactions between CO_2 and other dissolved carbonate species. Any subsequent evolution in this composition is then likely to be associated with specific trapping mechanisms or alteration processes.

At the pH of most natural waters, CO_2 can be trapped during carbonate mineral precipitation, and results in the $\delta^{13}C_{CO_2(g)}$ evolving to more negative values, with a magnitude of less than 2‰ following removal of 20% of the injected CO_2 to secondary carbonate minerals.[26] Similarly, the same authors showed that the dissolution of CO_2 into formation water (solubility trapping) also gave rise to an evolution in $\delta^{13}C_{CO_2(g)}$ to more negative values (in the pH of most natural waters) but of a smaller magnitude than precipitation. In contrast, the consumption of CO_2 during hydrogenotrophic methanogenesis results in a shift to more positive values of $\delta^{13}C_{CO_2(g)}$, with a magnitude approximately 5 times greater (10‰) for 20% conversion of CO_2 to CH_4.[20] In addition to the evolution of the reactant CO_2, it is also possible to monitor the evolution in the abundance and isotopic signature of the product methane. As with CO_2, the stable isotopes of carbon and hydrogen (δD) are used as the primary methodology to identify the products of CO_2 consumption during hydrogenotrophic methanogenesis and differentiate this from microbial methane produced through acetoclastic fermentation or methylotrophic methanogenesis.[54−57] This differentiation is generally based on genetic fields of composition space,[55,57,58] which characterizes microbial methane by strongly negative values of $\delta^{13}C$ between −50 and −110‰, while δD displays a large range with values between −150 and −450‰.[55] Within this broad genetic field, methane generated by methylotrophic methanogenesis is typically found to have more negative δD values, while hydrogenotrophic methanogenesis can generate methane with more negative $\delta^{13}C$ compositions. The isotopic separation between these pathways was proposed to occur at approximately $\delta^{13}C_{CH_4} = -60$‰ and $\delta D_{CH_4} = -250$‰. This

https://doi.org/10.1021/acs.est.2c08652
Environ. Sci. Technol. 2023, 57, 9459−9473

difference in the carbon and hydrogen isotopic composition of methane between these two pathways arises as a result of a combination of differences in the isotopic composition of the precursor compounds (i.e., CO_2, acetate, and water) and a difference in the kinetic isotope effect associated with the two pathways. The pathway associated with hydrogenotrophic methanogenesis imparts a kinetic isotope effect for $\delta^{13}C$ that is typically more than 25‰ and up to 60‰ at low temperature and high pressure under substrate limitation,[59] greater than that of the acetoclastic or methylotrophic methanogenesis pathway.[55] As a result, these ranges have been shown to be broadly indicative of the origin of microbial methane in the subsurface when comparing fluids over a global scale.[55] However, there is considerable overlap in the composition space of the respective pathways that can become significant at a local scale. This can arise from site-specific conditions, such as variability in the isotopic composition of precursor organic compounds, formation water composition, availability of reactants (e.g., sulfate or H_2), and rates of methanogenesis, that can yield methane from one pathway that has a composition more characteristic of the other (e.g., refs 55−58).

Recent developments in clumped isotope geochemistry now provide further constraints on the origin of microbial methane in the subsurface. For isotopically equilibrated methane, the doubly substituted isotopologues of methane, $^{13}CH_3D$ and $^{12}CH_2D_2$, have been shown to yield temperatures associated with methane generation, which aid in the identification of microbial methane (e.g., refs 60−67). Furthermore, simultaneous measurement of both doubly substituted isotopologues has also been shown to provide new constraints on the microbial methane generation pathway. This is because methane produced via hydrogenotrophic methanogenesis in combination with methanotrophy has been shown to produce methane at or close to equilibrium for $^{13}CH_3D$, with only minor departures observed for $^{12}CH_2D_2$. In contrast, methane produced by methylotrophic methanogenesis has been shown to yield significant deviations from equilibrium in both isotopologues.[63,68−74] However, as with all isotopic approaches, clumped isotopic signatures can also yield ambiguous results due to (i) combinatorial effects, that represent a statistical artifact associated with the addition of hydrogen from precursors with very different isotopic signatures (e.g., refs 65, 75, and 76), (ii) chemical and/or biological processing of product methane, such as via anaerobic oxidation of methane,[68,77] or (iii) speed of metabolic processes.[78] In this context, it is important to consider the independent constraints provided by the inert noble gases that are able to trace carbon addition and loss from the system alongside the process understanding provided by stable isotope geochemistry.

Noble gases (He, Ne, Ar, Kr, Xe) are powerful tracers for CCS applications due to their inert nature and diagnostic isotopic characteristics. They are naturally occurring and ubiquitous on Earth; however, different terrestrial sources have markedly different noble-gas characteristics. For example, surface-derived noble gases are distinct from radiogenic gases in Earth's crust and/or primordial gases, derived from Earth's mantle.[79−81] Notably, noble gases are also environmentally friendly—due to their nonreactive nature—and thus have the potential to be extremely useful in tracing migration of CO_2. They are found in trace amounts in captured CO_2 (e.g., ref 53) and vary as a function of the source from which CO_2 was captured.[82] Thus, noble-gas analysis of CO_2 from CCS plants allows the source of CO_2 in pipelines or tankers to be fingerprinted. If subsurface leaks occur at CCS sites, they too can be readily identified using noble-gas isotopes.[83] In previous studies, noble gases have been used to explain the fate of CO_2 in the subsurface, to quantify the extent of groundwater interaction, and to understand CO_2 behavior after injection into oil fields for enhanced oil recovery.[20,23,26,84−86] Further, they have been used for monitoring of subsurface CO_2 migration and leakage in CO_2-rich soils, CO_2-rich springs, and groundwaters.[87] In many of these applications the CO_2 to noble-gas ratio (e.g., $CO_2/^3He$) is used in combination with noble gas and stable isotopes (e.g., carbon) to determine both gas sources, the dominant subsurface processes and their rates (e.g., Figure 4).

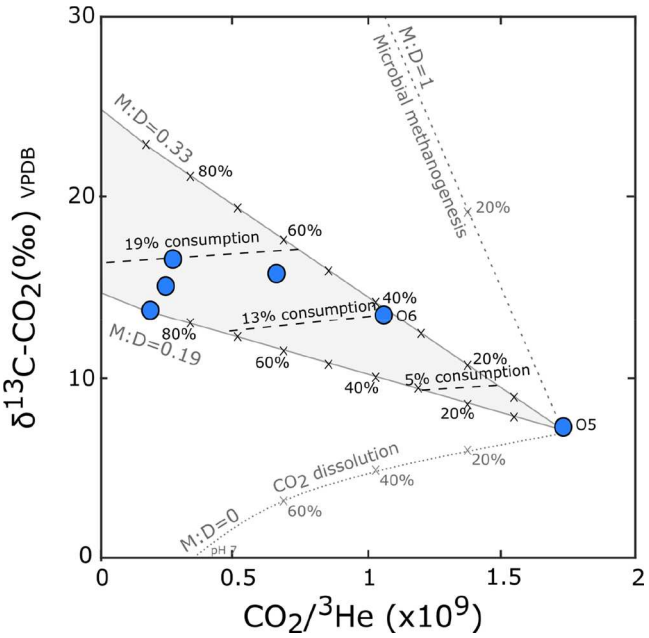


Figure 4. Carbon isotopic composition of CO_2 ($\delta^{13}C$) as a function of $CO_2/^3He$ in the Olla oil field. The dashed lines show the fractionation trajectories for the endmember processes (methanogenesis and dissolution (pH 7)). The tick marks represent the total amount of CO_2 trapping within the Olla system, relative to the most pristine sample (O5). The shaded region represents trapping by the combination of both microbial methanogenesis and dissolution. The upper and lower methanogenesis:dissolution ratios (M:D) are 0.33 and 0.19, respectively, showing that dissolution accounts for approximately 3 times more CO_2 removal (M:D = 0.26) than microbial methanogenesis. The lines labeled "consumption" show the portion of original injected CO_2 that has been removed by net microbial methanogenesis. Reprinted with permission from ref 20. Copyright 2021 Nature.

7. RECENT IDENTIFICATION OF METHANOGENESIS IN CCS

Reservoirs where natural or anthropogenic CO_2 is or was previously present are excellent targets for investigating the behavior of CO_2 during subsurface storage. For example, a recent study described the biogeochemical behavior of CO_2 in a CO_2 injected Olla oil field in Louisiana[20] (Figure 4). The Olla field was CO_2-injected (with natural magmatic CO_2) for enhanced oil recovery in the mid 1980s, leaving behind approximately one-third of the CO_2.[88] This CO_2 subsequently

https://doi.org/10.1021/acs.est.2c08652
Environ. Sci. Technol. 2023, 57, 9459−9473

migrated into all the producing formations. By using an integrated biogeochemical (gene sequencing, stable, clumped, and noble gas isotopes) approach, it was determined that between 13 and 19% of the remaining injected CO_2 was, within 30 years, converted into CH_4 by in situ microbial methanogenesis[20] (Figure 3). This conclusion was reached based on the following evidence.

First, a combined noble-gas and stable isotope approach was used to provide insights into the processes affecting the CO_2 at depth (e.g., refs 20, 23, and 26). Primordial ^3He is inert, with no significant sources or sinks within the crust, and thus changes in $CO_2/^3He$ can be interpreted to result from the addition/loss of CO_2. A decrease in $CO_2/^3He$ from the injection value was observed, suggesting that CO_2 was removed from the system. Elevated $\delta^{13}C$ of CO_2 (13.6 ± 3.2‰) is inconsistent with a mantle origin (−5‰) and common CO_2 trapping mechanisms (dissolution and precipitation) which would result in a decrease in $\delta^{13}C$-CO_2.[20] The $\delta^{13}C$ values of CO_2 and CH_4 are also observed to be in internal thermal isotopic equilibrium, yet reservoir temperatures are too low to drive thermodynamic equilibration over such time scales. This shows that equilibrium must be biologically controlled through a combination of microbial methanogenesis and anaerobic oxidation of methane (AOM). Net microbial methanogenesis would result in an increase in $\delta^{13}C$ values of CO_2 and CH_4, accounting for the observations.[18,20] Clumped methane isotopologues are consistent with a component of microbial methane in the system. They also appear to be trending toward thermal equilibrium under current reservoir temperatures, again consistent with AOM occurring within the system.[20,68,69] Further evidence of low-temperature biological activity was found in the molecular geochemistry and elevated $\delta^{13}C$ of propane. Finally, 16S rRNA gene sequencing of microbial communities in the reservoir brines identified hydrogenotrophic methanogens, methanol/methylotrophic methanogens, and anaerobic methanotrophs, again supporting microbial methanogenesis and methane oxidation.[19,20] By modeling the impact of these microbial processes on $\delta^{13}C$-CO_2 and $CO_2/^3He$, the amount and rate of microbial methanogenesis was found to be significant (up to 19%[20]).

Microbial methanogenesis has also been identified in systems that previously had high naturally occurring CO_2 concentrations such as in the Pannonian Basin (e.g., Figure 2). $C/^3He$ in the basin is 7.9×10^9, consistent with the European subcontinental lithospheric mantle, despite most of the C now being in CH_4,[89] and this CH_4 is postulated to be primarily sourced from CO_2 but until recently there was no mechanism for this conversion.[89] The physiochemical conditions within the basin are conducive for microbial methanogenesis and H_2 availability from thermogenic hydrocarbons.[90] Recent work has shown that similar to Olla, $CO_2/^3He$ and $\delta^{13}C$-CO_2 are consistent with a combination of microbial methanogenesis and dissolution.[20]

Methane is less reactive and less soluble and has different wetting properties than CO_2, impacting three of the four main CCS trapping mechanisms/strategies. This identification and quantification of microbial methanogenesis warrants careful consideration of reservoir processes at potential CCS sites worldwide.

8. MICROBIAL RESPONSE TO DIFFERENT PHASE CO_2 INJECTION

The phase of CO_2 (i.e., supercritical, gaseous, dissolved) that is injected depends on the temperature and pressure (and therefore depth) of the targeted reservoir (Figure 5). In most

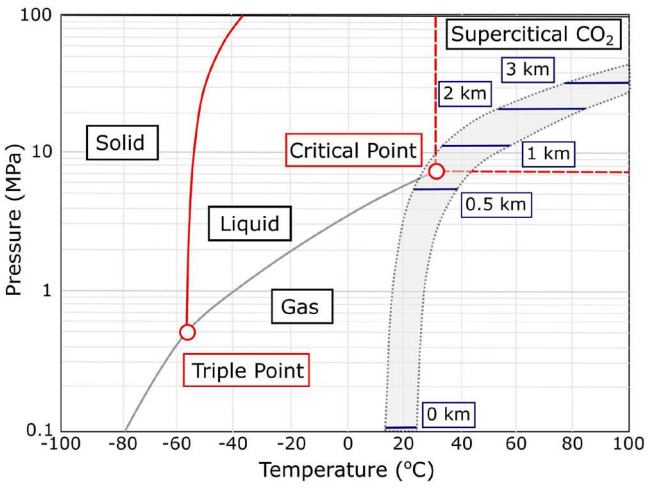


Figure 5. Phase stability diagram for CO_2. Typically, $scCO_2$ becomes stable below 800 m when pressures are >7 MPa and temperature ≳35 °C. Adapted with permission from ref 92. Copyright 2013 Mineralogical Society America.

scenarios CO_2 will be injected in a supercritical phase ($scCO_2$), as it can be stored most efficiently in the subsurface. CO_2 can also be injected in the gas phase (< 800 m deep, common in CO_2-EOR) or dissolved in water (e.g., CarbFix[91]).

The injection of $scCO_2$ was previously regarded to have harmful effects on the microbial communities, resulting in osmotic stress and cytoplasmic acidification for example, and it was thus considered a sterilizing agent.[93−95] However, recent laboratory, biogeochemical modeling, and field experiments have shown microbial activity at the interface of the $scCO_2$ and aqueous phase and shifts in the microbial communities present in the subsurface (e.g., refs 96−100). Initial $scCO_2$ exposure causes a decrease in cell numbers, due to the more acidic conditions immediately following injection.[98,99] However, in the subsequent months, injection ceasing cell numbers can rebound to preinjection values. Therefore, the injection of $scCO_2$ does not preclude methanogenesis from occurring and could even stimulate the process.

For CO_2 to be bioavailable, it must first be dissolved. It is expected that the injection of dissolved CO_2 will result in CO_2 being readily available for methanogens. In contrast to $scCO_2$ injection, experiments that have injected dissolved diluted CO_2 into shallow potable aquifers found an initial increase in total cell counts immediately after injection, despite similar metal mobilization and pH responses.[101] While these methods do not distinguish methanogens from other microbes, this demonstrates the principle that the CO_2 is bioavailable and does not create toxic conditions. For example, initial injection of dissolved CO_2 at CarbFix resulted in a biomass bloom in the months following the maximum DIC concentrations.[102] Where CO_2 is injected in the supercritical or gaseous phase, CO_2 must first dissolve to become bioavailable. Natural and anthropogenically injected CO_2 analogues have shown that CO_2 dissolution is the dominant loss mechanism from the gas phase

https://doi.org/10.1021/acs.est.2c08652
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(e.g., refs 20, 23, and 26) and a large proportion of this can happen on relatively short (i.e., decadal) time scales.[20] Microbial methanogenesis has previously been observed in reservoirs where CO_2 injection had been in a gaseous phase (e.g., Olla field, Louisiana[20]). Likewise, chemolithoautotrophic subsurface microbial communities have previously been shown to be CO_2-limited.[13] Combined with the associated decrease in pH (from the dissolved CO_2), it is likely that if the physicochemical and environmental conditions are conducive for microbial methanogenesis, this process will be promoted after the injection of CO_2, with increasing amounts of methanogenesis occurring once the CO_2 has dissolved and becomes bioavailable. Thus, it is likely that the amount of methanogenesis will be dependent on the dominant trapping mechanism and the rate of dissolution of CO_2 into formation water. Beside hydrogenotrophic methanogenesis, injected CO_2 might be additionally transformed by the subsurface microbial communities directly into biomass (through chemolithoautotrophy), as already observed during the biomass bloom of the CarbFix experiment,[102] and potentially cycled through a variety of other substrates which include acetate and other organic acids (Figure 4). These alternative pathways of microbial CO_2 utilization might overtime support acetoclastic and methylotrophic methanogenesis, supporting CO_2 conversion to methane even in CCS sites where H_2 might be limiting.

9. METHANOGENESIS POTENTIAL IN CCS ENVIRONMENTS

Reservoir type and geology could also be important parameters in reservoirs where the environmental conditions for methanogenesis are met. The targets with the greatest capacity for carbon storage are deep saline aquifers, but other targets include (but are not exclusive to) depleted hydrocarbon reservoirs, coal beds, and basalts.[2] It is therefore important to consider the possibility and probability of microbial methanogenesis occurring within these different storage reservoirs.

9.1. CO_2 Injection into Saline Aquifers. The environmental conditions within a saline aquifer are likely to be within the environmental window for microbial methanogenesis. Pilot CO_2 storage projects for example in the Ketzin Aquifer in Germany (Figure 2) have shown that, when within the environmental window for microbial activity, the acidic conditions associated with CO_2 injection result in a rapid temporal shift in the microbial community[101] toward chemolithoautotrophic (e.g., methanogens) organisms,[98] due to increased dissolved CO_2 concentrations and hydrogenotrophic methanogens ability to survive at lower pH (>3.8).[103,104] The shift to higher rates of methanogenesis are also correlated with elevated H_2 and CH_4 concentrations.[98] In the months after injection ceased, cell numbers rebounded to preinjection values and the microbial community shifted back to being dominated by chemoorganotrophic organisms;[98,99] however, methanogenesis was likely still be occurring. The presence of methanogens has also been detected post CO_2 injection in other test sites, for example in the Otway basin.[97] The rate of methanogenesis in the saline aquifers is also likely dependent on the rock types present (e.g., sandstone vs carbonate, if shales are present), as this will likely affect nutrient/H_2 availability and the capacity of the reservoir lithology to buffer changes in pH.

9.2. CO_2 Injection into Depleted Hydrocarbon Reservoirs. Microbial methane and methanogens have been found in oil fields with suitable physiochemical conditions worldwide.[105,106] Numerous studies have identified both acetoclastic and hydrogenotrophic methanogenesis following oil biodegradation ("methanogenic biodegradation") and the methane produced is referred to as "secondary microbial gas" (e.g., refs 107−109). A review focused on secondary microbial methane found it to be produced in 40 possible basins worldwide.[108] Many of these basins (e.g., Carnarvon, Gippsland, and Otway basins, Australia,[110] West Sak Field/North Slope basin, USA,[111] and North Sea[112]) are possible CO_2-EOR or CO_2 storage sites.

Microbial methanogenesis has also been identified in reservoirs hosting hydrocarbons where native CO_2 has been converted into CH_4 (e.g., Pannonian basin, Hungary, Nebo-Hemphill oil field, Louisiana,[20] and Minami-Kanto gas field[113]) and methanogenesis has been postulated in other basins (e.g., Subei, JM Brown Basset[20]). As described above, it has also been identified from (bio)geochemical signatures in systems which have undergone CO_2-EOR, for example, the Olla oil field (see section 7) and Lost Hills oil field.[20]

9.3. CO_2 Injection into Coal Beds. Microbial methanogenesis is a commonly identified process within coal beds (e.g., refs 114−118) and is sustained by the degradation of coal. The type of methanogenesis is determined by the degradation products and can be acetoclastic (e.g., Cook inlet[117]), hydrogenotrophic (e.g., Illinois basin[117]), or a mix of both (e.g., San Juan basin[114]) (Figure 2). The degree of methanogenesis appears to be related to groundwater residence time with older water having undergone greater extents of microbial methanogenesis.[118] AOM has also been identified in coal beds, suggesting the microbial cycling of carbon.[114] The rate of methanogenesis is likely limited by the methanogen biomass rather than degradation of coal constituents, and therefore a change in conditions following CO_2 injection, which favors methanogens over chemoorganotrophic organisms, could significantly increase the rate of methanogenesis in such environments.

9.4. CO_2 Injection into Basalts. Methanogens have been identified in multiple basalt formations, where they sometimes even dominate these ecosystem (e.g., refs 100 and 119−121). For example, high methane concentrations within the Columbia River basalts are thought to be a result of methanogenesis.[119] Hydrogen is hypothesized to be derived from the reduction of H_2O, driven by Fe in ferromagnesian silicates or from weathering of the basalts, and is limited by the reacting surface exposure to groundwater, as well as the abundance of microbes that make use of the H_2.[119] Methanotrophs and the microbial cycling of carbon between CO_2 and CH_4 have also been identified within basaltic formation waters.[100,120−122] It has been hypothesized that CO_2 injection, in particular $scCO_2$, will decrease the pH, increase rock weathering, and increase dissolved products (e.g., cations of Fe, Al, Mg, Ca). The initial pH drop may likely thermodynamically favor Fe(III) reducing microbes, depending on the mineralogy of the source material, limiting access of methanogens to available electron donors. Evidence for this comes from modeling[100] and bioreactor[123] studies. Over the long term, the liberated Fe will likely increase the production of H_2 and increase pH as iron reduction progresses, resulting in increased methanogenesis.[100] The length of time that increased methanogenesis will occur over, as a result of CO_2 injection, will likely depend on how mobile the CO_2 within the reservoir is and how reactive the CO_2 remains. Additionally, the increase

https://doi.org/10.1021/acs.est.2c08652
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in CH_4 within the system will likely lead to a stimulation of methanotrophs and the cycling of a portion of injected CO_2, depending on the availability of suitable electron acceptors (e.g. ref 100).

9.5. Relative Methanogenic Potential in Different Storage Environments. Physicochemical conditions will likely be some of the key parameters controlling whether microbial methanogenesis will occur. For microbial methanogenesis to occur, the reservoir must be below ~122 °C, which is thought to be the upper temperature limit for methanogens.[4] Study bias of storage analogues may also be a critical component, since temperature is not the only reason that microbial activity might be reduced.[114] Multiplicative effects of stressors such as pressure, pH, metal toxicity, or starvation conditions may reduce the temperatures at which microbial activity is curtailed.[124,125,126] In addition, complex microbial ecosystems in the subsurface make the microbial response to CO_2 additions more complicated. Other organisms, such as acetogens, could outcompete methanogens for the available CO_2, limiting the amount of methane produced. Alternatively, acetogens could partner with acetoclastic methanogens, increasing the amount of methane produced (Figure 4). Other redox-active compounds such as sulfate, nitrate, nitrite, iron, and manganese can support populations of microbes that compete effectively against methanogens.[124,127] Therefore, subsurface temperature profiles alone may be insufficient for predicting methanogenic activity. Combined ecological, geochemical, and physiological assessments are important for each proposed storage setting.

Hydrogen availability is another key parameter controlling the kinetics and energetics of methanogenesis (e.g., ref 34) and therefore the relative potential for methanogenesis during the lifespan of a CCS project. However, where and how H_2 becomes available in these systems, and whether it is from biotic or abiotic sources (Figure 4), is not well understood. We consider the absolute abundance potential of hydrogen to reflect the sum of the rates and yield of hydrogen production relative to those of hydrogen consumption and the potential hydrogen availability (relative to injected CO_2 volume) to be a function of the residence time of hydrogen within the reservoir target. While it is exceedingly difficult to quantify the differences in H_2 availability between the different geologic settings due to the paucity of calibration data for H_2 in these settings, we have attempted here to provide a qualitative framework based on a more conceptual understanding of the major differences between these sites. We suggest that hydrogen abundance and availability is likely to be greatest in depleted hydrocarbon fields and coal beds. We base this assumption on the fact that methanogens are frequently observed in reservoirs hosting oil and gas (e.g., refs 105, 106, and 109), and despite there being no known microbial oil biodegradation or other symbiotic processes that generate H_2, we previously demonstrated significant consumption of CO_2 in a depleted oil field associated with hydrogenotrophic methanogenesis.[20] It is also possible that H_2 could derive from abiotic dehydrogenation pathways associated with radical decomposition of hydrogen transfer reactions during hydrocarbon decomposition or equilibration.[60,128,129] Regardless of the mechanism responsible for hydrogen liberation, we consider it unlikely that a fraction of the hydrogen is not made available to other biogeochemical processes. In environments where serpentinization can occur, such as in basaltic host rocks, H_2 is produced via reactions of water with ferrous iron rich minerals and can generate up to 350 mmol H_2/kg of rock[130,131] and can therefore also support relatively high concentrations and availability of H_2, but likely less than in depleted hydrocarbon fields. Hydrogen can also be produced from the natural fermentation of organic matter (most likely H_2 source in saline aquifers). Notably, this source of hydrogen is from lower H_2 density (shales) and thus less labile compared to liquid or gaseous hydrocarbons and hence unlikely to yield H_2 in the same high concentrations or produced at high rates to support strong bioavailability compared to the other target environments.

10. IMPACT OF METHANE ON SUBSURFACE CO_2 STORAGE

The addition of relatively small volumes of non-CO_2 contaminants into a geologic repository can have potentially significant implications for the storage capacity of the repository and the mobility of the injected fluid. The addition of methane during methanogenesis to a pure $scCO_2$ fluid will gradually decrease the density of the fluid stored within the geologic repository, as noted during a recent study of the Greater Captain aquifer in the North Sea.[132] This decrease in density can be further supplemented by processes such as exsolution of methane from formation water, which can occur as CO_2 dissolves into formation waters as a result of its lower solubility and because most brines are close to methane saturation.[133] This decrease in fluid density, while negligible at trace concentrations of CH_4, becomes significant when the concentration of the contaminant fraction increases. Indeed, this process has been shown to reduce the storage capacity of a reservoir by as much as 40% when the methane fraction reaches 15% of the combined fluid under the pressure−temperature conditions typical of potential saline aquifer and depleted oil field repositories.[134] Any reduction in storage capacity has the potential to impact the economics of large-scale CCS projects. Furthermore, increases in methane concentrations have the potential to increase the fluid buoyancy and rising velocity, which reduces the interaction time of the bulk fluid with formation water. This is important because it has the net effect of reducing the relative amount of CO_2 that can be trapped by solubility trapping in formation water, increasing the reliance on other trapping mechanisms for long-term sequestration. Finally, the viscosity of methane under typical subsurface conditions means that methane will likely form a bank at the front of the plume boundary and migrate as a distinct fluid ahead of the CO_2 plume.[133] Methane cannot be trapped in minerals and is less likely than $scCO_2$ to dissolve into formation water downstream of the injection site. It thus has much greater mobility in the subsurface environment and potentially has a greater dependence on structural or stratigraphic trapping over greater time scales than CO_2. It is therefore important to understand how much CH_4 could be generated over the range of conditions that are likely to prevail at CCS sites and how this could impact fluid storage and retention in these systems.

10.1. Need for Monitoring Methane Formation. The potential for methane formation during CO_2 storage requires a reconsideration of potential monitoring techniques. Many proposed monitoring methods are insensitive to the composition of the fluid that is accumulating and migrating in the subsurface. For example, 4D seismic and electromagnetic surveys of future CCS sites will be able to detect changes between repeated surveys over the same volume of

https://doi.org/10.1021/acs.est.2c08652
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Table 1. Biogeochemical Techniques Useful for Monitoring CO_2 Storage Sites for Methanogenesis

technique	what can it tell us about?	considerations
noble gases	inert tracers can inform on physical processes presence of injected CO_2	difficult if reservoir has a similar composition to injected CO_2 highly sensitive to contamination
$\delta^{13}C$ of CO_2	chemical/biological/physical processes equilibrium processes	considerable overlap in the composition space
$\delta^{13}C$ of CH_4	origin of methane methanogenesis pathway equilibrium processes	considerable overlap in the composition space
δD methane	methanogenesis pathway	complicated by multiple H sources and system openness
clumped methane isotopes	origin of methane biological process	complicated by combinatorial effects mixing, and calibration of microbial pathways
$CO_2/^3He$	-changes in CO_2 in the system combine with other tracers to quantify processes	need to know original composition or most pristine
16S rRNA genes	only depict taxonomic identities of microorganisms	genes may be present, but not in use contamination
Mcr genes	methanogens and anaerobic methanotrophs	genes may be present, but not in use contamination
Pmo and Mmo genes	aerobic methanotrophs	genes may be present, but not in use contamination
rRNA and mRNA transcripts of the above genes	RNA is a short-lived molecule, so if it is present, the functions are likely to be active	genes may have other functions, for instance MCR produces and oxidizes methane.

rock. These changes can confidently be assumed to reflect an evolving fluid effect, given that rock properties are unlikely to be heavily modified by the injection process. Such surveys can provide useful constraints on the extent that the plume has migrated during and following injection, as well as identify any vertical migration into shallower intervals that may provide redundancy to the primary storage target. However, such studies are not well suited to characterizing any evolution in the fluid composition that could occur as a result of methanogenesis. Additional integrated monitoring requirements are needed in order to trace any biogeochemical processes. As microbial community structures and therefore processes as well as trapping mechanisms will evolve with time, both temporal and baseline studies may be required in order to fully understand the behavior of carbon at depth.

Additionally, monitoring for this process is not only required for the injected aquifer but also in any overlying aquifers where leakage could occur. For example, in CO_2 leakage simulation experiments into a shallow aquifer in the Newark basin, there was an observed increase in the number of methanogens in response to CO_2 injection.[101] Any methanogenesis in such systems could lead to an associated oxidation of reactive minerals within the aquifer and mobilization of trace metals.[135] This is therefore an important consideration for any onshore CCS targets where overlying aquifers may be utilized as a potable water source.

Understanding the amount of CO_2 converted using geochemical tracers and the time over which this occurred can inform us about the average rate of microbial methanogenesis within a given system. Once microbial methanogenesis has been identified within the system, the amount of CO_2 converted can be calculated in many ways, for example using the $CO_2/^3He$ combined with $\delta^{13}C$ of CO_2 (e.g., ref 20), the change in CH_4 concentrations (assuming no other CH_4 source to the system), or labeling the C added to the system (for example using ^{14}C). In addition to the amount consumed, the timing of injection, amount of CO_2, and injected CO_2 composition are critical for calculating a rate of methanogenesis. Analyses from microbial communities show a clear microbial succession after CO_2 injection, and thus it is likely this rate will be highly variable depending on the dominant microbial community and any rates will be an average rather than a single time rate.

Integrated multitracer studies investigating the biological and geochemical responses (e.g., those given in Table 1) may prove essential to effectively monitor CO_2 storage and the biogeochemical processes that result as a consequence of it. However, questions remain about exactly what conditions will control or provide limits to methanogenesis in these environments. It is thus essential to characterize baseline conditions in systems prior to injection, and for periodic sample recovery over the project lifespan to monitor these different environments.

11. SUMMARY AND FURTHER WORK REQUIRED

Many national net-zero targets include CCS, and as CO_2 storage facilities are being developed globally it is critical to understand all possible processes that will affect regulations and injection procedures. Microbial methanogenesis has recently been shown to be a significant process in potential

https://doi.org/10.1021/acs.est.2c08652
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CO_2 storage sites over decadal time scales.[20] The conversion of CO_2 to methane could have important implications on how the carbon is trapped within the system. CO_2 will most likely be injected as $scCO_2$, which was previously regarded to be a sterilizing agent (e.g., refs 93−95). However, more recent experiments have provided direct evidence of microbial activity at the interface of the $scCO_2$ and a rebound in cell numbers to preinjection values (e.g., refs 96−100). For this CO_2 to become bioavailable, it must first dissolve. Indeed, dissolution has been shown to be the dominant trapping mechanism for both natural and anthropogenic gaseous CO_2. The rates of dissolution of injected CO_2 will play a key role in determining how quickly the CO_2 becomes bioavailable.

Pilot CO_2 injection studies, natural analogues, and baseline studies of microbial communities in the proposed target environments (saline aquifers, depleted hydrocarbon fields, coal seams, and basalts) show that methanogens can be present in each environment type. In addition, in those where injection has occurred (e.g., refs 97−99 and 101) an increase in methanogens was detected immediately after injection. These studies did not determine whether injection had any long-term (e.g., >6 months) impact on the microbial community structure. Critically, an understanding of the long-term community effects is required as well as the rate and impact of methanogenesis on the reservoir. Key parameters controlling whether methanogenesis will occur and the extent to which it will do so are the physicochemical conditions (e.g., temperature, pH, salinity, metal toxicity, and any combined multiplicative effects these may have[125,126]) and competition among microbial communities. Reservoir type and geology could also be important parameters in reservoirs where the environmental conditions are met, especially within the context of H_2 generation and accumulation, which will likely be limiting to the kinetics and energetics of methanogenesis. In this context, we expect that the bioavailability of H_2 (and thus potential of microbial methanogenesis) will be greatest in depleted hydrocarbon fields and least within saline aquifers.

The extent and rate of methanogenesis are important considerations during CO_2 storage because methane itself is not trapped in minerals and has a lower solubility than CO_2. As such, the conversion of CO_2 to CH_4 has the potential to reduce the total mass of CO_2 trapped in minerals, solubility and residual mechanisms as a function of the increasing extent of conversion of CO_2 to CH_4. and cause a greater reliance on structural and stratigraphic trapping. Therefore, combined ecological, geochemical and physiological assessments are important to conduct for each storage site and careful baseline, temporal and spatial studies are needed that consider both the original and evolved fluid compositions to ensure safe long-term storage and minimize associated time and costs.

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Notes

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ACKNOWLEDGMENTS

R.L.T. acknowledges the Postdoctoral Scholar Program at Woods Hole Oceanographic Institution, with funding provided by the Weston Howland Jr. Postdoctoral Scholarship. P.H.B. and K.G.L. were funded by NSF FRES grant 2121637. K.G.L. also acknowledges funding from the US Dept. of Energy DE-SC0020369. D,G. was partially supported by funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program Grant Agreement No. 948972-COEVOLVE-ERC-2020-STG.

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https://doi.org/10.1021/acs.est.2c08652
Environ. Sci. Technol. 2023, 57, 9459−9473