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Relevance of Deep-Subsurface Microbiology for Underground Gas Storage and Geothermal Energy Production

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Abstract This chapter gives the reader an introduction into the microbiology of deep geological systems with a special focus on potential geobiotechnological applications and respective risk assessments. It has been known for decades that microbial activity is responsible for the degradation or conversion of hydrocarbons in oil, gas, and coal reservoirs. These processes occur in the absence of oxygen, a typical characteristic of such deep ecosystems. The understanding of the responsible microbial processes and their environmental regulation is not only of great scientific interest. It also has substantial economic and social relevance, inasmuch as these processes directly or indirectly affect the quantity and quality of the stored

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oil or gas. As outlined in the following chapter, in addition to the conventional hydrocarbons, new interest in such deep subsurface systems is rising for different technological developments. These are introduced together with related geomicrobiological topics. The capture and long-term storage of large amounts of carbon dioxide, carbon capture and storage (CCS), for example, in depleted oil and gas reservoirs, is considered to be an important option to mitigate greenhouse gas emissions and global warming. On the other hand, the increasing contribution of energy from natural and renewable sources, such as wind, solar, geothermal energy, or biogas production leads to an increasing interest in underground storage of renewable energies. Energy carriers, that is, biogas, methane, or hydrogen, are often produced in a nonconstant manner and renewable energy may be produced at some distance from the place where it is needed. Therefore, storing the energy after its conversion to methane or hydrogen in porous reservoirs or salt caverns is extensively discussed. All these developments create new research fields and challenges for microbiologists and geobiotechnologists. As a basis for respective future work, we introduce the three major topics, that is, CCS, underground storage of gases from renewable energy production, and the production of geothermal energy, and summarize the current state of knowledge about related geomicrobiological and geobiotechnological aspects in this chapter. Finally, recommendations are made for future research.

Keywords CCS · Deep biosphere · Geothermal energy · Hydrocarbon reservoir · Renewable energy · Underground gas storage

Abbreviations

16S rRNA	Ribosomal RNA of a sedimentation rate of 16 Svedberg
AOM	Anaerobic oxidation of methane
bbl	Barrel (oil)
CARD-FISH	Catalyzed reporter deposition-Fluorescence in situ hybridisation
CCS	Carbon capture and storage
cDNA	Complementary DNA
CLEAN	CO_2 large-scale enhanced gas recovery in the Altmark Natural Gas Field
CO_2CRC	Cooperative Research Centre for Greenhouse Gas Technologies
COE	Cost of electricity
CO_2-EGR	EGR using CO_2
CO_2-EOR	EOR using CO_2
CO_2MAN	CO_2-reservoir management
CO_2SINK	CO_2 Storage by injection into a saline aquifer at Ketzin
DAPI	4′,6-Diamidin-2-phenylindol
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
*dsr*AB	Dissimilatory (bi)sulfite reductase gene

EDTA	Ethylenediaminetetraacetic acid
ECBM	Enhanced coal bed mining
EGR	Enhanced gas recovery
EOR	Enhanced oil recovery
EPS	Extracellular polymeric substances
FISH	Fluorescence in situ hybridization
HFC	Hydrofluorocarbons
IEAGHG	International Energy Agency Greenhouse Gas
mcr	Methyl coenzyme M reductase gene
MEOR	Microbial enhanced oil recovery
MIC	Microbially influenced corrosion
MPN	Most probable number
mRNA	Messenger RNA
OIP	Oil in place
P	Pressure
PAH	Polycyclic aromatic hydrocarbons
PFC	Perfluorocarbons
PCR	Polymerase chain reaction
PDS	Bottom-hole positive displacement sampler
PLFA	Phospholipid-derived fatty acids
qPCR	Quantitative polymerase chain reaction
RECOBIO	Recycling of sequestrated CO_2 by deep subsurface microbial-biogeochemical transformation, RECOBIO-1 and RECOBIO-2 are two successive projects
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription-quantitative PCR
SAC	Surface active compound
SC-CO_2	Supercritical carbon dioxide
SIP	Stable isotope probing
SSCP	Single-strand conformation polymorphism
TOC	Total organic carbon
T-RFLP	Terminal restriction fragment length polymorphism
UGS	Underground gas storage
V	Volume

Contents

1 Carbon Capture and Storage (CCS)

1.1 Introduction to Carbon Capture and Storage

Mitigation of greenhouse gas emissions without interference with economic growth is the main concern of climate-change initiatives to prevent global warming. Unfortunately, observations of a 100-year period between 1906 and 2005 already show an increase of the global temperature of 0.74 ± 0.18 °C. Changes in climate are noticeable and include extreme weather such as droughts, heavy precipitation, heat waves, and intensity of tropical cyclones [45].

CO_2 is the principal component of the greenhouse gases in addition to CH_4, N_2O, hydrofluorocarbons (HFC), perfluorocarbons (PFC), and SF_6 (Kyoto Protocol, 1998). Power generation using fossil fuels or biomass, cement production, and other CO_2-emitting industries are the main sources of CO_2. This gas accounts for 64 % of the enhanced "greenhouse effect" [15, 44]. Therefore, removing CO_2 from flue gases would help to maintain the global temperature rise to a maximum of 2 °C.

In this respect, carbon capture and storage (CCS) can be a promising and fast approach to reduce CO_2 emission to the atmosphere. But this approach is limited by availability and capacity of CO_2 storage sites. Despite this limitation, CCS can be a bridging technology that provides a gain in time until an energy supply with renewable energies is secured. Moreover, storage of CO_2 in deep geological formations probably results in natural gas restoration in geological timescales provided that CO_2 is transformed microbiologically to CH_4.

In the special report of the Intergovernmental Panel on Climate Change on carbon dioxide capture and storage [44], CCS is defined as "[…] a process consisting of the separation of CO_2 from industrial and energy-related sources, transport to a storage location and long-term isolation from the atmosphere." A detailed description of the CCS technology is given in this special report. In brief, there are three main strategies to capture CO_2 from flue gases: (i) postcombustion, (ii) precombustion, and (iii) oxy–fuel combustion [44]. In the postcombustion process, chemical sorbents are used to recover up to 95 % CO_2 from the flue gas, which contains mainly N_2 and 3–15 vol % CO_2. In the precombustion process, the fuel is first burned with oxygen, air, and/or steam to generate CO and H_2. Then CO is converted to CO_2 by the addition of steam and finally CO_2 is captured using absorption–desorption methods. In the oxy–fuel combustion process, the combustion of the fuel is carried out by using oxygen, either pure or mixed with a CO_2-rich recycled flue gas, and results in flue gas of up to 98 % CO_2. After CO_2 is enriched from the original flue gas by using one of these capturing strategies, the gas is pressurized for the transport to CO_2 storage sites via pipelines or trucks. The CO_2 capture process accounts for an increase of 20–90 % cost of electricity (COE) depending on the type of power plant [44]. Further technological developments may reduce the extra costs in the future. The sequestration of the original flue gas would cause much higher costs.

There are research and industrial projects worldwide that investigate CCS on laboratory and field scales (pilot/demonstration plants) and perform EGR (enhanced gas recovery), EOR (enhanced oil recovery), or ECBM (enhanced coal bed mining) connected to CO_2 storage. The IEAGHG [43] operates a database that lists all research, development, and demonstration (RD&D) projects concerning CCS. Among them, there are projects that store CO_2 in saline aquifers, for example, the Frio Brine Pilot Test (USA) and CO_2SINK (Ketzin, Germany) projects, which store CO_2 in gas fields, for example, the In Salah Gas project (Algeria) and CO_2CRC Otway Basin project (Australia), and EOR projects, for example, Weyburn CO_2-EOR (Canada). In addition, a CO_2-EGR approach was planned for the gas field Altmark (CLEAN project, Germany). The almost depleted gas field Altmark is the second-largest onshore gas field in Europe and would be of great importance for CO_2 storage if CCS is accepted by the German government and society. In addition to research and development in CCS technology, industry and scientists have to include good public relations in their field of duty. In particular in populated regions where CCS in deep geological formations is possible, residents must regularly be informed about the process, the safety, and the risk management of the CO_2 storage site.

Disposal of CO_2 in the ocean and usage of CO_2 for chemical processes are also approaches to reduce emissions of CO_2. But the most promising approach is the injection of supercritical CO_2 (SC-CO_2) into deep geological formations, that is, depleted oil and gas reservoirs, saline aquifers, or into unminable coal beds. In general, geological formations have to fulfill two main requirements to be suitable for long-term CO_2 storage. First, the storage reservoir has to consist of a porous and permeable rock, often sandstone, into which the CO_2 can be injected. Second, there has to be an impermeable cap rock and a succession of further seals up to the surface (multibarrier system). Typical cap rocks and seals consist of mudstone, siltstone, or salts (e.g., anhydrite). In particular, natural gas reservoirs have been demonstrated to be gas tight at least concerning CH_4 for geological timescales. Therefore, CO_2 storage in depleted gas fields is favored. These storage sites can be operated up to a site-specific pressure level, which should remain below the initial pressure level of the reservoir.

The worldwide storage potential has been estimated to be at least 200 Gt CO_2, and might even reach 2,000 Gt CO_2 in sedimentary basins (e.g., oil and gas reservoirs) [44]. For Germany, a summary of the distribution and the storage potential of sedimentary basins has been provided by May et al. [63]. There are three main geological structures, which represent potential CO_2 storage reservoirs in Germany. These reservoirs comprise sandstones rich in (i) feldspar, carbonate, and clay; (ii) iron minerals; or (iii) organic material. The formation waters are often highly saline (up to 300 g/l) and consist of high ammonia content (up to 3,000 ppm). With depth, the brines are increasingly reductive, their content of dissolved metal ions (e.g., iron) increases, whereas the content of sulfate decreases. Aside from these chemical conditions, the deep biosphere, which is likely to be present in such geological formations, has to be adapted to high temperatures, high pressure, and a low supply of electron acceptors, electron donors, or other nutrients.

1.2 Geochemical Effects and Risks of CO_2 in Storage Sites

The CO_2 gas designated for storage can be accompanied by impurities such as SO_x, NO_x, CO, H_2S, NH_3, O_2, condensable water, and hydrocarbons [87]. Therefore, the potential impact of the impurities on the storage site and storage process has to be considered. According to Knauss et al. [51], co-contaminant H_2S showed only minor effects on water–rock interaction, but SO_2 leads to a drastic drop of pH, which will lower the formation of carbonates. However, sequestration of CO_2–SO_2 mixtures into storage sites that contain hematite (Fe_2O_3, red beds) has been reported to result in dissolution of hematite and the release of ferrous iron induced by SO_2 [77]. This iron release will promote the formation of siderite ($FeCO_3$), which can cause an increase in the storage capacity of the reservoir, but can also provoke a negative effect on the storage unit by lowering its permeability:

$$Fe_2O_3 + 2\,CO_2(g) + SO_2(g) + H_2O \rightarrow 2\,FeCO_3 + H_2SO_4 \tag{1}$$

Once CO_2 is injected into the storage reservoir, the gas can be trapped by four mechanisms [38, 77, 78]:

(i) Hydrodynamic trapping: SC–CO_2 is trapped below a cap rock of a depleted gas or oil field. This can be connected to enhanced gas or oil recovery (EGR, EOR), respectively.
(ii) Residual trapping: CO_2 is trapped by capillary forces in the pores of the reservoir rocks.
(iii) Solubility trapping/solution trapping: CO_2 is dissolved in formation water as H_2CO_3, HCO_3^-, and other aqueous species.
(iv) Mineral trapping: CO_2 is trapped as carbonate mineral (calcite, magnesite, siderite, and dawsonite) in deep saline formations. In this respect, silicate minerals are essential because their alteration enhances these mineral trapping processes due to the supply of cations.

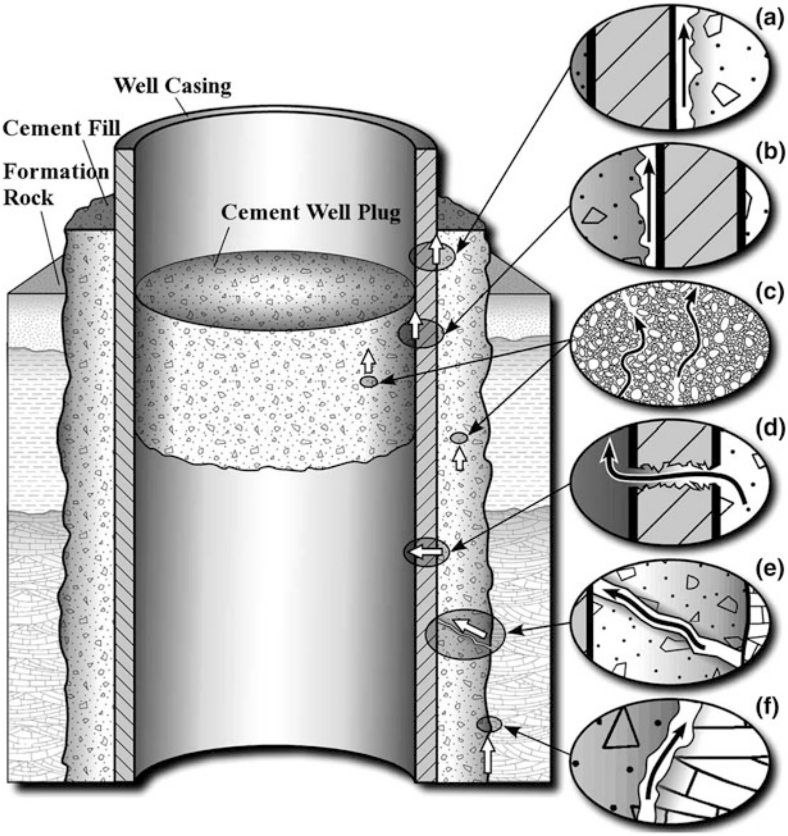

Fig. 1 Schematic illustration of possible leakage pathways through an abandoned well. **a** Between casing and cement; **b** between cement plug and casing; **c** through the cement porespace as a result of cement degradation; **d** through casing as a result of corrosion; **e** through fractures in cement; and **f** between cement and rock (from [33], with kind permission from Springer Science and Business Media: [33], Fig. 1, Copyright Springer-Verlag 2004)

Another trapping mechanism can be the absorption of CO_2 by coal, which could lead to a release of methane and is used in enhanced coal bed methane recovery.

It has to be considered that CO_2 differs from other gases with respect to its solubility, penetration, and reaction behavior. The permeability and penetration behavior of the cap rock is also a crucial aspect for the safety and integrity of the CO_2 storage site. One possible risk could be leakage of CO_2 via undetected fractures and faults and via abandoned wells or failure during the injection process [44]. However, CO_2 exhibits a very good solubility (in contrast to CH_4 and especially to H_2) and will be trapped in any overlying formation water if it leaks vertically through one sealing unit. Leakage through anthropogenic artificial barriers (cement, casing) may occur because of fatigue or alteration of the well bore material due to chemical attack of highly corrosive SC–CO_2 or high pressure (Fig. 1 [33]).

Another risk, especially for CO_2 storage in saline aquifers, could be contamination of overlying groundwater with brines. The saline formation water could be displaced upward due to a spacious pressure build-up. In this case, the pressure of the storage formation would drop, could be detected with monitoring equipment, and an emergency plan could be applied. In general, monitoring strategies have to be operated before, during, and after CO_2 sequestration to assess the baseline conditions, to follow the storage process and detect process failure, and to control long-term reactions and failure, respectively. The migration of CO_2 in the storage formation and the composition of the overlying groundwater and surface soils of the storage site have to be controlled. In addition to geochemical reactions, also biogeochemical reactions, that is, mineral–brine–CO_2–microbe interactions have to be considered.

1.3 Microbial Populations in Potential CO_2 Storage Sites

Geological formations known to be suitable for CCS comprise a deep subsurface biosphere, which is dominated by sulfate-reducing, iron-reducing, acetogenic, and methanogenic microorganisms [60]. Microbial corrosion of tubing and cement of well bores and souring of gas due to H_2S production by sulfate reducers are well-known problems of gas and oil industry [21, 34, 50]. In addition, clogging of well bores and porespace of the geological formation can arise when H_2S precipitates in the presence of ferrous iron first to FeS and then to FeS_2. These technological problems clarify the need to consider biogeochemical reactions in addition to geochemical reactions, although microbiologically mediated processes in the deep subsurface are rather slow compared to microbial activities at the surface [17, 60].

Microbial reactions can have favorable and unfavorable effects on the capacity, integrity, and safety of CO_2 storage sites. Therefore, baseline monitoring of each CCS operation should include the detection of the initial microbial community to deduce possible microbial reactions in advance. In particular, a microbial assemblage as biofilm on mineral surfaces can either inhibit or enhance mineral dissolution [64]. Dissolution of minerals can decrease the storage capacity of the reservoir and could additionally lead to exposure of fractures, which possibly form connections to higher layers of the formation and affect the storage integrity. But dissolution of minerals can also provide microorganisms with, for example, electron acceptors. Biofilms can serve as protective coating of minerals decreasing mineral dissolution and presenting nucleation sites to catalyze carbonate precipitation [23]. On the surface of silica-based minerals, for example, the formation of amorphous silica gels, whose crosslinking is facilitated in the presence of biomolecules, can lead to a self-sealing effect of microfissures and porespace of disturbed claystone and cements [36, 49]. Hence, self-sealing and enhanced carbonate formation may contribute significantly to integrity and safety of the storage site and additionally stabilize the injected CO_2 into solid carbonates [64]. Another indirect way to favor carbonation can be the adjustment of physicochemical conditions (e.g., increase of pH) due to metabolic activity in the deep subsurface.

The injection of CO_2 into potential storage formations causes changes in reservoir temperature and pressure, and also leads to considerably higher CO_2 concentrations. All variations in the physicochemical conditions will stress the indigenous biosphere of the storage formation. Beyond that, a sterilization can take place at the center of the CO_2 injection well. However, Mitchell et al. [66, 67] have demonstrated that the resilience of biofilms to SC–CO_2 is higher than that of planktonic microorganisms.

Microbial monitoring before and during CO_2 injection into a saline aquifer near Ketzin (Germany) has revealed that the microorganisms adapted within five months to higher CO_2 concentrations and were even more metabolically active [70]. Furthermore, during the propagation of CO_2 in the storage reservoir, CO_2 will form a plume that develops a gradient of CO_2 concentrations. Thus, regions with lower CO_2 content can directly provide autotrophic microorganisms with their carbon source and an electron acceptor. Heterotrophic microorganisms probably metabolize organic compounds (e.g., organic acids, methylalkanes) that were mobilized by SC–CO_2 from the sandstone of the storage formation [85]. Hence, CCS can even stimulate microbial growth.

The consumption of CO_2 due to microbial activity has reproducibly been shown to be connected to a considerable increase in the formation of TOC (total organic carbon) in experiments with a bioreactor and a sterile control reactor under elevated H_2 and CO_2 partial pressure [26]. The experiments have been performed with milled material of a drilling core, which originated from the gas field Schneeren-Husum (Germany), and formation water collected at well heads of well bores of this gasfield. Therefore, microbial transformation of CO_2 into biomass and organic compounds can additionally contribute to the storage capacity of a reservoir.

One problem that may result from the stimulation of, in particular, sulfate-reducing microorganisms is the increase in H_2S production, which in turn can affect the integrity of well bores and storage equipment via biocorrosion.

Methanogenic microorganisms form another microbial group to be considered, which would transform injected CO_2 either directly (autotrophically) or indirectly (acetoclastically) to CH_4. Although CH_4 represents a far more potent greenhouse gas than CO_2 if it would leak from the reservoir, CH_4 can possibly be used as an energy source in geological timescales.

So far, the extent of the microbial impact on CCS on short-term and long-term scales remains to be clarified. Even if no viable microorganism survives the CO_2 injection, there would be biological residues such as endospores, organic clusters, enzymes, or lysed cells that can have an influence on the CO_2 storage performance [62, 64]. There are many biogeochemical processes in the deep subsurface that are not yet understood or have even been subjected to investigation. In this respect, one challenge is to obtain reliable samples of the deep subsurface biosphere. Then, other challenging aspects are the very low doubling times of these microorganisms and the creation of their physicochemical requirements for cultivation. Despite these aspects, only a small number of CCS projects to date consider biogeochemical processes [17].

In some projects, which store CO_2 in hydrocarbon reservoirs, microbial monitoring of surface soil (total cell counts, In Salah Gas project; Algeria [47]) or microbial mats above the storage reservoir (microbial community compositon and total cell counts, Sleipner project (Norway [98]) has been performed to survey possible CO_2 leakage. In contrast, formation waters of the Paaratte formation have been sampled in situ at 1,400 m depth (60 °C, 13.8 MPa) for 16S rRNA gene analyses in the framework of the CO_2CRC Otway Basin project (Australia). Bacterial sequences of the reservoir community have been related to the genera *Thermincola*, *Acinetobacter*, *Sphingobium*, and *Dechloromonas* [72]. Microorganisms, stained with the DNA-specific dye DAPI, have been reported to be microscopically visible mainly as filamentous cells of 5–45 μm length. The injection of a gas mixture of 75.4 mol % CO_2 and 20.5 mol % CH_4 to the Paaratte formation started in 2008 [13].

Detailed microbial analyses have been performed for formation fluids of the almost depleted natural gas reservoir Altmark (Permian—Upper Rotliegend, Germany). This gas reservoir comprises extreme environmental conditions, for example, in situ temperatures of 110 °C up to 130 °C and high salinity brines of >300 g salts per liter.

The hydraulic isolated subfield block "Altensalzwedel" has been considered for EGR and storage of 100,000 t CO_2 in 3,000 m depth (Fig. 2). Although injection of CO_2 in the Altmark gasfield was not possible due to political obstacles and public opposition, a comprehensive reservoir monitoring, which includes 16S rRNA gene analyses and cell quantification of the deep subsurface biosphere, has been performed during the CLEAN project [56].

Formation fluids of three different well bores of the subfield block "Altensalzwedel" (S10, S13, S17) have been sampled in situ using a double-ball lining sampler. Analyses of bacterial 16S rRNA genes of these fluids have revealed that the microorganisms at the site are related to hydrogenotrophic bacteria of *Hydrogenophaga* sp., *Acidovorax* sp., *Ralstonia* sp., and *Pseudomonas* sp. and to representatives from saline, hot, anoxic, and deep environments [69]. In addition, relatives of *Diaphorobacter* sp., a thiosulfate-oxidizing bacterium, were present in the formation fluid of one well bore (S17), and an uncultured biocorrosive thermophilic bacterium has been detected in fluids of two well bores (S13, S17). The formation fluids of one well bore (S10) have also been sampled with a bottom-hole positive displacement sampler (PDS). This sampling device can be inserted sterile and closed into the well bore, can be opened in the depth to collect the formation water in situ, and closed again to be moved out. In contrast, the double-ball lining is a more open system for in situ sampling. However, the two different sampling procedures have principally revealed the same microbial community structure in the formation water of well bore S10. In addition to the microorganisms, which

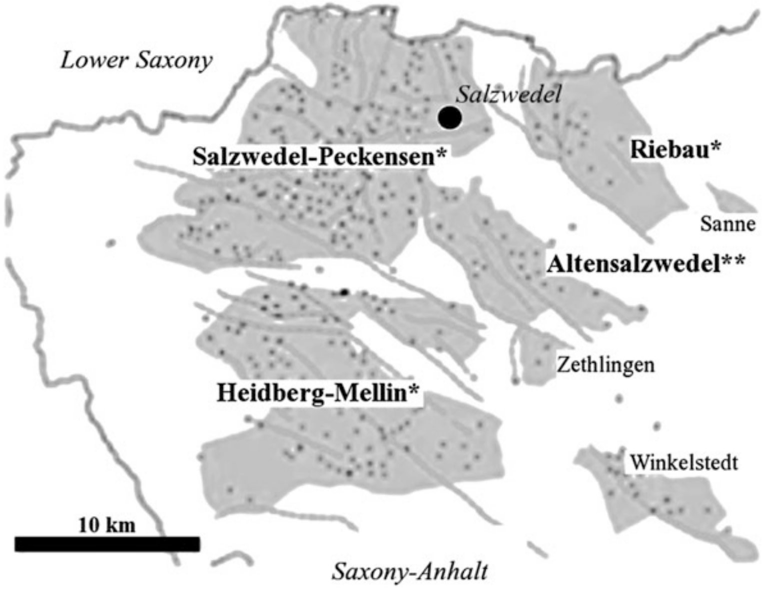

Fig. 2 Subfield blocks of the Altmark gasfield as indicated by *grey* shades (modified after [31]). *Three subfield blocks, which have been the focus of the RECOBIO-2 project. **One subfield block, which has been the focus of the CLEAN project

have also been found in the formation water sampled with the double-ball lining sampler, additional 16S rRNA gene sequences similar to dissimilatory metal-reducing bacteria (*Pantoea* sp. described by Francis et al. [32]), aromatic-degrading and metal-corroding bacteria of deep-sediment origin (*Sphingomonas* sp. [3, 99]), and extremophilic Fe(III)- and Mn(IV)-reducing bacteria (*Bacillus* sp. [12]) have been found in formation water sampled with the PDS [56]. Cell quantification using cell counting of SYBR Green-stained cells (mainly particle-associated cells) and quantitative PCR analyses have shown only very low cell numbers [69].

In the RECOBIO-2 project, the deep biosphere of three subfield blocks of the Altmark natural gasfield, surrounding the "Altensalzwedel" subfield block, has been investigated (Fig. 2) [42]. The formation waters of CLEAN and RECOBIO-2 sampling sites mainly differed in their concentration of sulfate, which was between 400–1,800 mg/l and almost no detectable sulfate, respectively.

Microscopic analyses using CARD-FISH and DAPI-stained cells also showed rather low cell numbers of at most 10^5 cells/ml in the formation water samples. There were only minor differences in the bacterial community composition in the formation water, which had been sampled at the well head (produced) and in situ (double-ball lining) of a well bore of the "Heidberg-Mellin" subfield block [35, 41]. The 16S rRNA gene sequences were similar to representatives of sulfate-reducing *Desulfotomaculum* sp., thiosulfate-reducing *Thermoanaerobacterium* sp., elemental sulfur-reducing and fermenting *Petrotoga* sp., and to uncultured bacteria found in, for example, geothermal water or petroleum reservoirs. A first 16S rRNA gene sequence analysis of the archaeal community of the formation water sample, which had been collected in situ, indicated the occurrence of members of hydrogenotrophic *Methanomicrobiales*.

The in situ-sampled formation water of a well bore of the “Salzwedel-Peckensen” subfield block was more diverse and comprised 16S rRNA gene sequences, which were assigned predominantly to uncultured bacteria detected in, for example, volcanic deposits, petroleum reservoirs, geothermal water, or hydrothermal vents. In addition, sequences have been affiliated with *Desulfotomaculum* sp., *Thermoanaerobacterium* sp., *Petrotoga* sp., and to *Delftia* sp. found in PAH-contaminated soils. Interestingly, *Desulfotomaculum* sp. has also been detected in the 16S rRNA sequence analysis of the living bacterial community of the same formation water sample [41].

Projects storing CO_2 in deep saline aquifers are, for example, Frio Brine Pilot Test (USA), CO_2SINK and CO_2MAN (Ketzin, Germany), Sleipner (Norway), and Nagaoka project (Japan). However, the deep subsurface biosphere has been considered only in CO_2SINK and CO_2MAN, two projects on the small-scale pilot CCS test site in Ketzin, Germany.

Since 2008, CO_2 has been injected (~ 60,000 t of mainly food-grade CO_2) into a saline aquifer, which is located in the “Roskow-Ketzin”double anticline, at a depth of 630–650 m below surface [53]. The CO_2 plume reached the first of the two observation wells two weeks after the start of CO_2 injection. The drill mud was removed from the injection well and the two observation wells using a N_2 lift at each well.

For microbial analyses, formation water of the first observation well has been collected in situ using either a flow-through sampler or a double-ball lining before and after CO_2 injection and at the well head during the N_2 lift. The microbial community has been analyzed using 16S rRNA gene fingerprinting methods (PCR-SSCP, DGGE) and cell counting with FISH and DAPI staining [70, 71]. Predominant microorganisms could be detected independently of the sampling procedure, which indicates negligible contamination effects during sampling. The microbial community was dominated by anaerobic halophilic fermentative bacteria (*Halanaerobium* sp., *Halobacteroidaceae*) and sulfate-reducing bacteria (*Desulfohalobium* sp., *Desulfotomaculum* sp.). Other members of the bacterial community were affiliated with phenanthrene-degrading *Comamonas* sp., to *Empedobacter* sp. from petroleum-oil contaminated soil and to oil-degrading bacteria of *Bacteroidetes*. After CO_2 arrival at the observation well, chemolithotrophic microorganisms temporarily outcompeted chemoorganotrophic microorganisms.

Microscopic analyses revealed total cell numbers of $2–6 \times 10^6$ and $2–4 \times 10^6$ cells/ml of living microorganisms before N_2 lift and CO_2 injection in formation water of the first observation well [70]. After N_2 lift, there were hardly any microorganisms detectable, but after CO_2 injection, total cell numbers were again determined to be 10^5 cells/ml. Moreover, after five months of CO_2 injection, total cell numbers again reached 2×10^6 cells/ml and comprised almost exclusively living microorganisms. Representatives of *Alpha*-, *Beta*- and *Gammaproteobacteria*, sulfate-reducing bacteria (*Desulfovibrionales*, *Desulfotomaculum* cluster I, and other *Firmicutes*, *Desulfobacteraceae*), and methanogenic archaea were detected using specific probes via FISH analyses.

Sulfate-reducing bacteria were detected in formation water of the injection well and were shown to be responsible for a decrease in the sulfate concentration and an increase in iron sulfide formation, which caused a decrease in permeability of the injection well and could be removed by a N_2 lift [102].

In addition, samples of drilling cores were investigated in long-term laboratory experiments with synthetic brine (172.8 g/l NaCl, 0.62 g/l KCl, 8.0 g/l $MgCl_2$ * $6H_2O$, 4.9 g/l $CaCl_2$ * $2H_2O$) under in situ conditions (5.5 MPa and 40 °C) and high CO_2 partial pressure to detect indigenous microorganisms and to quantify microbial activity [97]. The microbial community of the sandstone has been affiliated with members of *Alphaproteobacteria* (*Rhizobium* sp., *Agrobacterium* sp.), *Betaproteobacteria* (*Burkholderia* sp., *Hydrogenophaga* sp.), and *Actinobacteria* (*Propionibacterium* sp.). Except for *Agrobacterium* sp. and *Hydrogenophaga* sp., all other bacteria survived the exposure to CO_2. Sulfate-reducing bacteria and archaea were not detected in sandstone material. Mineral dissolution due to CO_2 exposure caused an increase in porosities during long-term experiments [96]. However, after 24 months, porosities again decreased due to precipitation [30].

1.4 Conclusion and Perspectives

Carbon capture and storage can be a fast-acting approach to mitigate CO_2 emissions and can provide a gain in time for the development of energy-efficient renewables. Hence, if all safety precautions were considered and a reasonable handling secured, CCS could contribute considerably to prevent climate change.

Enhanced gas and oil recovery using CO_2 or storage of natural gas are known, long-performed, and CCS-analogue approaches. Therefore, findings from these approaches can help to deduce geochemical and biogeochemical reactions in a CCS operation. Nevertheless, research on CCS depends on pilot and demonstration tests to gain detailed process knowledge.

An interdisciplinary approach combining geophysical, geochemical, and biogeochemical monitoring of the whole CCS operation (baseline, injection, long-term storage) will be required to understand complex processes in the storage site and to be able to react properly if any problems in operation occur. In this respect, determination of the baseline conditions, the original microbial community composition, and knowledge of process behavior are essential to predict and then prevent any failure in advance. For example, a decrease of injectivity has occurred as an immediate consequence of microbial activity and has been recovered with a N_2 lift at the CCS pilot plant near Ketzin (Germany). During the Frio Brine Pilot Test (USA), dissolved organic carbon (DOC) has increased by a factor of 100 from 1–5 to 500–600 mg/l after 20 days of CO_2 injection [48]. The organic carbon, mainly formate, acetate, and toluene, can probably be extracted by SC–CO_2 from the rock of the geological formation, but can also be a result of microbiological metabolism generating biomass and organic compounds.

During the RECOBIO-1 project 2005–2008, Ehinger et al. [26] already showed that microbial activity can have an impact on the performance of CCS in depleted natural gas fields. However, only four CCS pilot plant projects considered the deep subsurface biosphere in their monitoring concept to date. These are CO_2CRC Otway Basin project (Australia), CLEAN project (Altmark, Germany), and CO_2SINK and CO_2MAN projects (Ketzin, Germany). In the CO_2SINK project, recovery of microbial cell numbers and microbial activity was shown after CO_2 injection into the subsurface saline aquifer near Ketzin.

In addition to the CLEAN project, the deep subsurface biosphere in formation waters of well bores around the subfield block, which was formerly considered for CO_2 injection in the natural gasfield Altmark, was investigated in the RECOBIO-2 project 2008–2011.

In general, besides sulfate-reducing, metal-reducing, fermenting, and biocorrosive bacteria, many uncultured microorganisms have been detected by molecular genetic analyses. Cultivation of microorganisms of the deep subsurface is challenging due to low cell numbers, low microbial activity after sampling and extreme physicochemical requirements. However, cultivation approaches are required, because successful enrichment, isolation, and description of so far unknown microorganisms will further improve knowledge of biogeochemical processes.

Carbon capture and storage provides not only a possible measure to promote climate protection, but also valuable insights into subsurface environments.

2 Underground Gas Storage (Methane, Hydrogen) for Energy Generation

2.1 Introduction to Underground Gas Storage

The underground storage of natural gas has its origin in the beginning of the twentieth century when gas companies searched for a solution to balance out the seasonal fluctuation in the demand for gas used for space heating of buildings [16]. Currently, around 630 underground gas storage (UGS) facilities are in operation worldwide [29]. New interest in large-scale underground storage of energy has been sparked by the expanding renewable energy production worldwide. The increasing utilization of solar or wind sources [91, 92] leads to a high fluctuation in energy production, which can be adapted to the actual demand by using the electrical energy to form hydrogen or methane and subsequent storage of the gases. Large volumes of storage capacities are required for this issue, which most likely can be solved by underground storage [94].

Although underground gas storage has been standard for engineering for decades, the impact of microbial processes on underground gas storage has hardly been explored. An early example of the impact of microbial processes on underground gas storage is provided by an underground town-gas reservoir near Lobodice, Czech Republic, where conspicuous changes in the gas volume and composition have been observed during a seven-month period of gas storage in the 1980's. The gas volume decreased by 10–20 % in conjunction with an approximately 1.5-fold increase in the methane content and significant losses of hydrogen, carbon dioxide, and carbon monoxide. Cultivation of microbial communities present in the reservoir water and rocks revealed methanogenic archaea as drivers of the changes in stored town gas. Changes in the carbon-isotope signature of methane in the stored town gas supported the result [88]. As exemplified in this study, microorganisms living in the deep subsurface can have profound effects on underground gas storage with respect to gas loss and alteration of gas composition. The consequences of this gas alteration are discussed below in more detail.

2.2 Microbiology of Gas Storage Sites

Underground gas storage is performed in depleted gas or oil reservoirs, aquifers, and salt caverns. These reservoirs are characterized by temperatures above 35 °C with a temperature increase of ~3 °C per 100 m depth, high pressure (>7 MPa) [29], absence of oxygen, and high salinity. Microbial life is widespread in the crust of the earth and numerous mechanisms to deal with different environmental factors have evolved [79, 82]. Microorganisms have been isolated that withstand hydrostatic pressure of 100 MPa [90], salt concentrations of up to 300 g/l [76], or temperatures of 113 °C [10]. Therefore, UGS facilities cannot be considered simply as a geological formation with unique physicochemical characteristics, but need to be seen also as a microorganism habitat. Indeed, between 10^3–10^6 microorganisms per ml reservoir water have been recorded in porous rock reservoirs [29, 46, 88] and microbial life has also been proven in salt formations [95]. Here the questions arise how microorganisms live in such habitats, which factors control the microbial activities, and how the microbial processes affect the underground gas storage.

Free water is vital for microbial life so that the residual reservoir water serves as habitat for microorganisms. Microbial life in deep geological storage systems, such as oil and gas reservoirs, is controlled by the reservoir temperature, salinity, abundance of essential inorganic nutrients, and appropriate energy resources [60]. The temperature is generally seen as the limiting factor for the presence of living microorganisms, while the other factors control the size and activity of the microbial populations [40, 61]. Despite the documented growth at 113 °C of the Archaeon *Pyrolobus fumarii* [10], in situ observations indicate that microbial activity in oligotrophic reservoirs is restricted to temperatures below 80–90 °C [100]. Microorganisms gain their energy from complex electron transfer processes involving the oxidation of organic and inorganic compounds and subsequent reduction of a

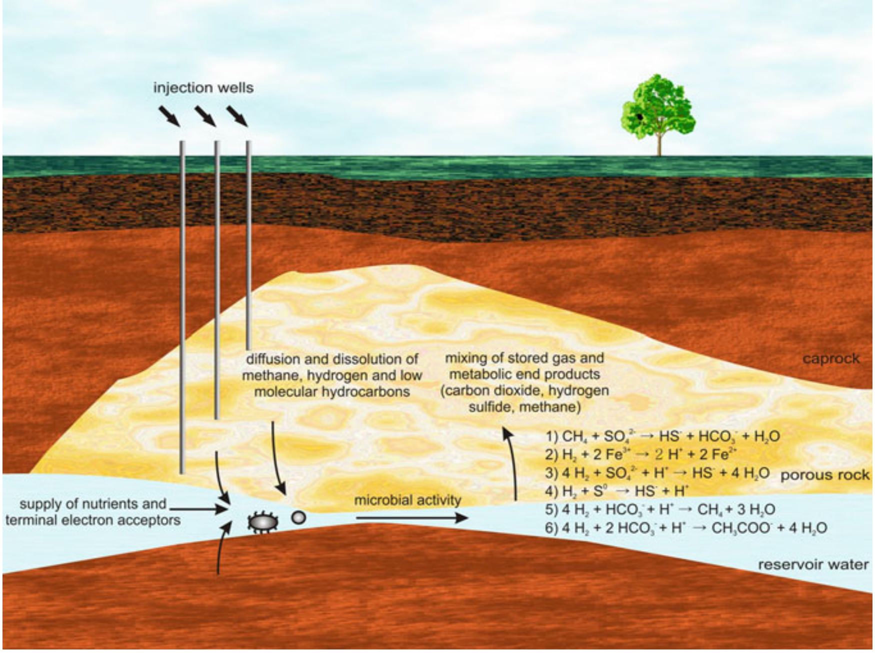

Fig. 3 Scheme of possible microbially mediated processes in underground gas reservoirs. Stored gas diffuses in the reservoir and dissolves in residual water, where gas components such as methane and hydrogen can be cosumed by microorganisms. Microorganisms derive nutrients such as phosphorous and nitrogen from mineral dissolution reactions, hydrolysis of dead cells, or from the nitrogen gas stored. Terminal electron acceptors are provided from marine evaporites, the mineral matrix, coal and shale layers, or also, in the case of carbon dioxide, from the stored gas itself. Volatile metabolic end products of microbial processes mix with the stored gas resulting in a change of the gas

terminal electron acceptor. The energy obtained is used for maintenance of microbial metabolism and growth [60]. Currently, methane and hydrogen are both considered as high-performance carriers of renewable energy, either directly produced in biogas plants or from the conversion of solar or wind energy. Both carriers will increasingly replace natural gas, which is a hydrocarbon mixture consisting primarily of methane and to a small extent of other low molecular hydrocarbons, carbon dioxide, nitrogen, and hydrogen sulfide [74] in both pipeline and storage systems. Hydrogen and methane as well as other low molecular hydrocarbons can serve as electron donors for microorganisms [40], so that the gas stored in a respective deep geological storage system provides sufficient energy sources for microbial activity (Fig. 3). Therefore, the depletion in essential inorganic nutrients, mainly phosphorous and nitrogen, and the availability of electron acceptors are considered as regulating factors for microbial activity. Suitable electron acceptors are ferric iron, manganese, sulfate, elemental sulfur, and carbon dioxide. Nitrate and nitrite are generally only present in low amounts [46, 60]. The electron acceptors are provided from the embedded or overlying marine evaporates, the mineral matrix (e.g., ferric iron containing siderite), coal, and shale layers or in case of carbon dioxide also from the stored gas itself. Nitrogen is present as ammonium ions in the water, which can be transported by reservoir water movements or diffusion or it can be assimilated from the nitrogen gas by nitrogen-assimilating microorganisms. Phosphor is considered as the much more likely limiting nutrient [40], which is present organically or inorganically bound and is mobilized by hydrolysis of dead cells or microbial-induced weathering of minerals such as phosphate-containing silicates [8, 83].

Methane, and with regard to future storage concepts, also hydrogen can be regarded as the dominant energy sources for microorganisms affecting the long-term fate of these stored gases. Although the solubility of both gases in water decreases with increasing temperatures and salinity [20, 101], the elevated pressure has a far greater impact on the solubility resulting in high dissolved gas concentrations in the water phase [5]. At elevated gas partial pressure, an increase in microbial activities has been recorded, which is attributable to the high availability of gaseous substrates in the water phase [22, 24, 55, 75]. In principle, anaerobic oxidation of methane (AOM) can proceed with sulfate as the terminal electron acceptor. The process is believed to be mediated by a syntrophic consortium of methanotrophic archaea and sulfate-reducing bacteria ([52] and references therein) or by methanotrophic archaea alone [65]. Furthermore, there are indications that methane oxidation is coupled with the reduction of manganese and ferric iron [6]. Thus far there is no single study addressing the role of AOM in gas reservoirs so that we can only speculate about its role.

Hydrogen plays a central role as an energy source in subsurface anoxic environments and can be utilized by a wide range of bacteria and archaea ([68, 89] and references therein). Hydrogen oxidation in such environments can be coupled to the reduction of ferric iron, sulfate, elemental sulfur, or carbon dioxide [19, 89]. Ferric iron reduction results in iron mobilization because the highly water-insoluble Fe(III) is reduced to the much more soluble Fe(II) [59]. Reduction of sulfate or elemental sulfur is highly undesirable in UGS because the formed hydrogen sulfide creates a serious problem for the industry due to its toxicity to humans [81], deterioration in quality, odor, souring, and corrosion of steel material of the well-tubing [7, 73, 93]. Moreover, hydrogen sulfide reacts with ferrous iron to form iron sulfide, which precipitates and can cause clogging of the operation equipment. Microbial mediated formation of hydrogen sulfide has been found repeatedly in gas and oil reservoirs [34, 80] pointing to the impact of sulfate reduction in UGS facilities. When all other electron acceptors are depleted methanogenesis and/or homoacetogenesis will appear, which are less favorable processes from a thermodynamic point of view [18]. In the course of methanogenesis, hydrogen oxidation is coupled to the reduction of carbon dioxide under formation of methane, a process which is exclusively mediated by archaea. Alternatively, homoacetogenic bacteria catabolize hydrogen and carbon dioxide to acetate. Both processes are widespread in the deep subsurface [54] and have been observed in gas reservoirs [46, 88]. In addition to the particular importance of methane and hydrogen as energy sources, microbial growth might also be stimulated by drilling fluids providing additional energy sources and nutrients as modeled by Baker [4]. This involves the risk of clogging of technical equipment by microbial biofilms or damage by microbial corrosion [11].

2.3 Implications and Future Perspectives

Overall, microbial activities lead to a loss of the stored gas, especially of hydrogen. Little is currently known about the extent. For example, in the course of methanogenesis, 4 mol hydrogen and 1 mol carbon dioxide are required to produce 1 mol methane and 3 mol water, which, for the operator of a UGS facility, means a substantial loss in the stored gas. Although the heating value of methane is with 35.9 MJ/m^3 higher than that of hydrogen (10.7 MJ/m^3), methanogenesis also means a loss in calorific power.

One may speculate that the highest microbial activity occurs near the gas–water contact where a plentiful supply of electron donors is given, but high microbial activity also occurs at the mineral–water contact. From shallow aquifers, it is known that sessile bacteria contribute over 90 % of the total bacterial community and only less than 10 % exist in the planktonic lifestyle [2, 37, 39]. The first cultivation experiments with samples from an underground town gas reservoir showed a much higher activity of methanogenic archaea using water and rocks from the reservoir compared to the sole use of water [88].

To summarize, the major microbial-induced risks associated with underground gas storage are (i) loss of the gas and thereby calorific loss; (ii) damage of technical equipment by biocorrosion and clogging through precipitates and biomass; and (iii) risk to operational safety and deterioration in quality by hydrogen sulfide formation. Therefore, the understanding of microbial activities in the deep underground is crucial for an economically successful operation of UGS. Microbiological studies are required to shed light on the identity of indigenous bacteria, their metabolism and activity, and factors controlling the type of microbial processes. This should be done in close cooperation with UGS operators, hydrogeologists, geologists, and chemists ensuring a comprehensive understanding of the complex processes in the deep subsurface.

3 Geothermal Energy Production

3.1 Geothermal Energy

Geothermal energy is the heat generated in the Earth. In geothermal plants, this energy is used as a source for heat supply ($T > 60$ °C) or to drive geothermal power plants ($T > 120$ °C).

The use of geothermal energy is generally subdivided into the operation in shallow depth (down to 400 m) and the operation in great depth (2,000–4,000 m). Shallow geothermal energy can be exploited principally worldwide and is often installed in private households for autonomous heat supply. Deep geothermal energy, on the other hand, is most efficient in regions where large temperature reservoirs exist ($T > 100$ °C), which are sufficient for electricity production. Worldwide, five countries use geothermal energy to produce around 20 % of their electricity (Costa Rica, El Salvador, Iceland, Kenya, and the Philippines) [9]. However, those are not the countries with the highest geothermal capacities. Even higher capacities are found in the United States of America (Table 1).

The productivity of a geothermal plant depends on a variety of factors, including the chemical composition of the thermal water, the water temperature, and the water production rate. Another important, yet rarely considered factor is the microbiology. In the subsurface, the majority of the microorganisms live attached to the rocks. However, microorganisms can also become detached and carried off with the produced thermal water and thus enter geothermal power plants. Therefore, the interaction of microbiological processes with geothermal plants should be considered from both sides. First, how do microorganisms influence the use of geothermal energy and second, how does the use of geothermal energy influence the subsurface microbiology (Fig. 4).

3.2 Geothermal Energy and its Effects on Subsurface Microbiology

3.2.1 Shallow Geothermal Energy

For the extraction of shallow geothermal energy, closed loop systems are installed (Fig. 4). The fluid inside the system extracts heat from the underground, which is used in different ways depending on the season: in winter, heat is extracted from the underground and used for heat supply of buildings; in summer, when the ambient temperature is higher than the underground temperature, the cold fluid is used to cool buildings. Subsequently, the warmed water is re-injected into the underground. As a consequence, the aquifer temperature range (of 10–12 °C) decreases and increases, respectively, and microorganisms will have to manage temperature fluctuations of ± 6 °C [14, 86].

Changes in temperature not only affect the metabolic activity of microorganisms, but also the composition of the overall microbial community. In summer, locally increased temperatures (e.g., at injections sites) can promote growth of mesophilic bacteria whereas heat extraction in winter promotes microbial species that grow at lower temperatures (psychrophilic microorganisms). Temperature fluctuations also affect the chemical composition of the groundwater as it changes the solubility of solids, liquids, and gases, including potential organic and

Table 1 Top 15 countries using geothermal energy

Geothermal Electricity Production		**Geothermal Direct Use**	
Country	GWh/yr	Country	GWh/yr
USA	16,603	China	20,932
Philippines	10,311	USA	15,710
Indonesia	9,600	Sweden	12,585
Mexico	7,047	Turkey	10,247
Italy	5,520	Japan	7,139
Iceland	4,597	Norway	7,000
New Zealand	4,055	Iceland	6,768
Japan	3,064	France	3,592
Kenya	1,430	Germany	3,546
El Salvador	1,422	Netherlands	2,972
Costa Rica	1,131	Italy	2,762
Turkey	490	Hungary	2,713
Papua New Guinea	450	New Zealand	2,654
Russia	441	Canada	2,465
Nicaragua	310	Finland	2,325

Source www.iea.org

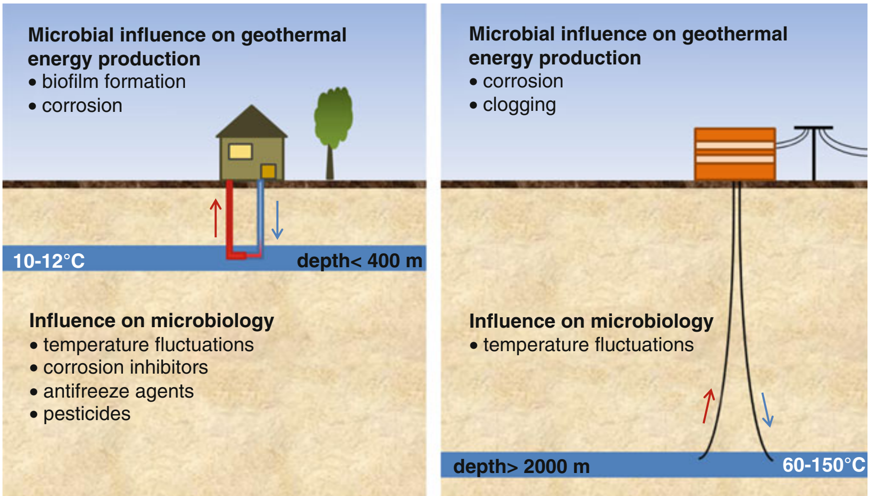

Fig. 4 Mutual influence of microbiology and geothermal energy in both shallow (*close loop*, *left*) and great (*open loop*, *right*) depth, exemplified for heat extraction from shallow and deep aquifers

inorganic substrates. Therefore, different substrate spectra will become available. For example, increased temperature will lower the solubility of oxygen (and other gases) and lead to a limitation of oxygen-dependent metabolic pathways.

3.2.2 Deep Geothermal Energy

Deep geothermal energy plants operate as open loop systems (Fig. 4) where hot water is extracted from the deep subsurface and is re-injected after passing the heat exchanger. The microbial community in greater depths considerably differs from that in shallow depth [57]. Despite the extreme conditions encountered in deep habitats (high temperatures and pressures, high salinity), deep aquifers have been shown to harbor a live and active biosphere [84]. Such ecosystems are often dominated by thermotolerant and thermophilic bacteria and archaea with mainly anaerobic metabolisms (e.g., fermenting, methanogenic, sulphate-reducing microorganisms) [1, 27, 71]. The deep subsurface also harbors populations of spore-forming bacteria, which are able to survive adverse conditions (e.g., heat, drought, substrate limitation) by formation of endospores. Metabolically, spores are largely inactive, but might germinate when temperature and nutrient supply conditions change and thereby influence the quality of geothermal water.

The operation of geothermal plants faces problems that mainly arise from the activity of sulfate-reducing bacteria. Sulfate reducers are capable of oxidizing iron ferrous metals, which results in corrosion of tubings and pipes [25, 28]. Also, formation of sulfidic precipitates (e.g., FeS) can lead to clogging and therefore to reduced water production rates [58]. Both corrosion and clogging can cause serious economic problems based on reduced performance of the geothermal plant.

3.3 Further Research

Temperature is an important factor that influences both microbial viability and metabolic activity. Therefore, research should focus on the effects of geothermal-induced temperature fluctuations on the chemical groundwater composition, nutrient supply, and the microbial community in both shallow and great depths.

Concerning the exploitation of shallow geothermal energy, potential leakage of fluid additives into groundwater raises questions concerning degradability and toxicity of the released substances, and the associated effects on microbial community composition. Also, the preservation of high groundwater quality is important because shallow groundwater is a source of drinking water (in Germany, 75 % is produced from it).

In the deep subsurface, most concerns arise from clogging and corrosion mediated by sulfate-reducing microorganisms. Sulfate reduction rates should be determined in order to estimate the extent of economic damage caused by these processes. Concerning spore-forming microbial populations, investigations are needed that address the effect of temperature fluctuations and/or changes in nutrient supply conditions on both the formation and germination of endospores.

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