



Review on the impact of water temperature changes due to geothermal activities on groundwater microbiology and associated geochemical processes

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Content

l Motivation	3
2 Introduction	4
B Effects of temperature on water chemical composition	5
3.1. Oxic versus anoxic aquifers	5
3.2. Chemical changes during drilling and construction	5
3.3. Expected chemical changes in the main groundwater types in Switzer	land 6
Fifects of temperature on microbial community composition	7
4.1. Aerobic conditions	7
4.2. Anaerobic conditions	8
4.3. Conditions during construction and operations	9
5 Effects of temperature on microbial activity and biochemical processes	10
Bio-corrosion of the geothermal infrastructure	11
Potential contamination and 'Geothermal Bio-remediation'	11
3 Synthesis and recommendations	12
Remaining open questions	15
9.1. Open questions for Swiss aquifers	16
References	17

1 Motivation

Swiss Parliament has adopted the motion 22.3702 Jauslin, which instructs the Federal Council and all federal offices concerned "to change the [legal] regulations in such a way as to enable the geothermal potential of the subsoil to be optimally exploited including the seasonal storage of heat". More specifically, the motion expresses "the need to increase optimally the limit for groundwater temperature change through heat use or storage", currently set to a maximum of 3 °C at 100 m downward of the facility (Water Protection Ordinance). In doing so, the protection of groundwater used as drinking water as well as the protection of habitats dependent on groundwater must not be affected. With regard to the 3 °C rule, the Water Protection Ordinance does not distinguish between groundwater suitable to be used as drinking water and groundwater that is not suitable. However, primarily groundwater that is not suitable for drinking water use should be able to be used for heat storage, especially deep groundwater if its chemical composition makes drinking water use impossible. Comparison with other European countries is difficult, as it is usually not the permissible temperature change at a certain distance from use that is regulated, but rather the maximum permissible difference between the withdrawal and return temperatures or the minimum and maximum permissible return temperatures. In France, the maximum allowable temperature change for geothermal uses of minor importance is 4 °C at a distance of 200 m from the use, while the maximum allowable return temperature is 32 °C. Austria and Germany do not have any national regulation. The responsible "Bundesländer" require a maximum temperature spread of 6 °C between withdrawal and return temperature and / or a minimum and maximum return temperature between 4 - 6 °C and between 18 - 20 °C, respectively. Denmark allows a minimum and maximum return temperature of 2 °C and 20 °C, respectively, but groundwater in drinking water wells must not be warmed by more than 0.5 °C. The Netherlands only rules the maximum return temperature at 25 °C. None of these countries has a fixed regulation of the permissible temperature change for deep groundwater.

Therefore, an assessment based on a sound literature study of the aquifers' reaction and potential impacts on the microbiome and microbial activity by the geothermal use of (mainly) shallow aquifers is urgently needed before proceeding to extensively exploit the potential of the subsoil.

2 Introduction

Aquifer Thermal Energy Storage (ATES) systems are open-loop systems that extract water from one well, heat/cool buildings, and then inject the heated or cooled water back into the aquifer through the same or another well. Low temperature (LT) ATES are operated below 30 °C and are usually located in shallow aquifers, medium temperature (MT) ATES refer to a temperature range between 30 °C and 50 °C and high temperature (HT) ATES are operated at 50 °C and higher (Lee and Lee, 2013; Ueckert and Baumann, 2019; Collington et al., 2020). Other standard geothermal systems are Borehole Thermal Energy Storage (BTES) systems, closed-loop systems that use a ground heat exchanger array that stores and recovers heat depending on the season, using the earth like a thermal battery. Their temperature can range from 25 °C to 90 °C (for > 50 °C, also called high-temperature HT-BTES).

What to expect? Two main effects can be expected to happen when hotter water is reinjected into a "colder" aquifer or heat is stored in the aquifer: (a) precipitation of minerals with retrograde solubilities such as carbonates and (b) enhanced activities and growth of thermophilic or thermotolerant bacteria. The latter could either result in biofilm formation or in changes within a bacterial community, which can lead to changes in the ecosystem function and result in common microbially-mediated problems such as clogging, biofouling, and corrosion (Dinkel et al., 2020). Such phenomena may also result in increased numbers of pathogenic bacteria in biofilms and increased risks to human health if the same aquifer is used for drinking water supply. Other effects such as gas exsolution with an increase in temperature have not been reported in the literature for shallow geothermics, because gas solubility is more dependent on pressure than on temperature. By contrast, when reinjection of colder water occurs in a "hotter" aquifer, it is expected: (a) an increase of carbonate minerals dissolution, and (b) a lower microbial activity, reducing the risk of bioclogging and corrosion.

In open-loop (ATES) systems, heat is stored in the aquifer in summer, while cool (i.e., groundwater temperature) is simultaneously pumped, increasing the average temperature of the aquifer. In winter, the stored heat is pumped and cooler water is reinjected into the aquifer, but in the latter case, many times at a higher temperature than the one of the aquifers (i.e., heat rejection). For close-loop (BTES) systems, in most cases, heat is stored in summer and recovered in winter, with the possibility of also being used for cooling in summer. Hence the most expected reaction in the aquifer is an increase in temperature. A decrease of aquifer temperature below its background temperature is less probable. It only occurs in deep aquifers (> 1000 m). Further, it has been demonstrated the resilience of the aquifer to almost recover the initial hydrogeochemical and microbial state with the cessation of geothermal activities. Therefore, in this review, we mainly focus on the effects of a temperature increase on water chemical composition, microbial community composition, and microbial activity, and also during the recovery of aquifer background temperature.

3 Effects of temperature on water chemical composition

3.1. Oxic versus anoxic aquifers

In general, a temperature difference of +1 °C in groundwater is associated with a 4% decline in oxygen saturation and a pH drop of 0.02 because of the accumulation of CO₂ (Brons et al., 1991; Riedel, 2019). In unconfined aquifers, small temperature differences ($\Delta T \leq 10$ °C) at which most open systems operate do not significantly influence the concentrations of the main chemical constituents (Possemiers et al., 2014). However, for confined aquifers, a small increase of concentrations in components such as B, Si, Li, dissolved organic carbon, NH₄, Na, and K, and a decrease in Mg have been reported for similar temperature differences (Saito et al., 2016).

If the temperature increases (e.g., 7 °C, from 17 °C to 24 °C) in **shallow-oxic aquifers**, although this can enhance the precipitation of carbonate minerals and dissolution of silicate minerals, a weak correlation between temperature and the concentration of trace elements (including heavy metals) has been observed. In general, there is a positive correlation between trace element (Ni, Cd, B, Ba) concentrations with Alkalinity, pH, and redox (Garcia-Gil et al., 2016a). Further, the oxidation of minerals such as pyrite and the oxidation of organic matter by oxygen and nitrate proceed significantly faster as temperature increases.

In the case of **shallow-anoxic sediments** (e.g., in The Netherlands), barely present in Switzerland, an increase in aquifer temperature up to 25 °C can induce a significant increase in As concentrations. For temperatures > 60 °C, the dissolution of silicate minerals (e.g., K-feldspar) is enhanced, increasing the concentration of K and Si, but also F, P, B, Mo, and V, and Cr if it is present. Desorption from and potentially reductive dissolution of iron oxides is believed to be responsible for increasing concentrations of As, B, Mo, V, and P. However, it is also possible that some of the reductively dissolved Fe²⁺ reacts with HS⁻ formed during the sulfate reduction at lower temperatures to precipitate as an iron sulfide mineral, which could sequester part of the released As. An increase in dissolved organic carbon has also been reported, and explained by a combination of i) desorption of organic acids from iron oxides and ii) microbial respiration (mineralization) of solid organic matter (Bonte et al., 2013a).

3.2. Chemical changes during drilling and construction

The drilling and construction establishment of a new geothermal facility inevitably causes disturbance to an aquifer. The use of drilling fluids and the possible mixing of waters from different depths can cause temporary changes to the groundwater composition. However, it has been demonstrated that the groundwater chemistry stabilizes after some (three) months period (Dinkel et al., 2020). The mixing of waters (i.e., the existence of chemical gradients) or stratified water (Hartog et al., 2013) with

different redox, salinity, pH, or alkalinity, is another risk (Bonte et al., 2011; Burté et al., 2019). If oxygen- or nitrate-rich water (usually shallow) comes in contact with iron-containing water (usually deep), then the formation of gases (N₂, CO₂) and clogging (precipitation of oxides) can occur. Therefore, it is crucial in the case of open systems, that screen filters are at the same depth (Houben, 2006; Possemiers et al., 2016).

3.3. Expected chemical changes in the main groundwater types in Switzerland

Among the different types of groundwater identified in Switzerland (Wanner et al., 2023), three of them stand out for their representativeness. The following is a summary of how geothermal activities can alter the chemical composition of each of them:

In Ca-(Mg)-HCO₃ water types, the most common shallow groundwater type in Switzerland (carbonate aquifers) (Wanner et al., 2023), an increase in temperature can precipitate minerals such as calcite and dolomite (Garcia-Gil et al., 2016b), but also undersaturated water for silicate minerals, therefore, increasing the potential of dissolving them too in case they are present (Blasco et al., 2019; Choi et al., 2020). Note that in the case of organic-rich groundwater, microbiology can contribute to silicate minerals weathering (Hiebert and Bennet, 1992). Further, metals such as Cr (from naturally occurring Cr-spinels) can be remobilized because of an increase in temperature. The reinjection of oxygen-rich water in geological formations containing sulfide minerals (e.g., pyrite, Figure 1) eventually mobilizes other metals such as aluminum (Regenspurg et al., 2020).

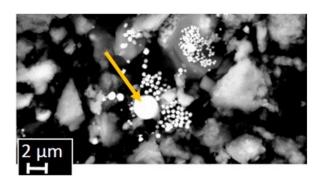


Figure 1. Energy-dispersive X-ray spectroscopy reveals the presence of pyrite (bright white spots, the largest indicated by a yellow arrow) in sand and lignite-containing, brownish sediment (Tertiary "Rupelbasissand", Berlin, Germany). Modified from Regenspurg et al. (2020).

Ca-HCO₃-SO₄ is also a common shallow groundwater type in Switzerland (evaporite-bearing aquifers) (Wanner et al., 2023). Geothermal activities have been observed to be responsible for the incrustations of iron oxides in low-saline shallow aquifers, a reduction in calcium and magnesium ion concentrations in water (Ueckert and Baumann, 2019), and the formation of iron sulfides in more saline deeper ones (Stenvik et al., 2022). The injection of water into these aquifers can trigger an important evaporitic rock dissolution (Garrido Schneider et al., 2016).

Ca-HCO₃-SO₄ or Ca-SO₄-HCO₃ is the water type in crystalline rock aquifers in Switzerland, which are located in the basement below the Molasse Basin in the North

of the country, and much shallower in the South (Stober and Bucher, 1999; Sonney and Vuataz, 2008). An increase in temperature will dissolve silicate minerals. Further, the presence of metalloids such as As (from As-bearing minerals) can be remobilized because of an increase in temperature. The increase in temperature can also promote the proliferation of microbiology, contributing to the minerals' weathering (Hiebert and Bennet, 1992).

The possible changes in the chemical composition of near-surface groundwater in Switzerland resulting from geothermal activities can be found in more detail in Wanner et al. (2023).

4 Effects of temperature on microbial community composition

Several impacts are described on microbial community composition because of an increase or decrease in groundwater temperature. Further, functional microbial diversity explains groundwater chemistry (Flynn et al., 2013). Microorganisms can be classified in terms of their optimal growing temperature (Parker et al., 2016):

- psychrophiles with a temperature preference range of 0 °C to 20 °C
- mesophiles with a range of 15 °C to 45 °C
- thermophiles with a range of 45 °C to 80 °C
- hyperthermophiles that thrive above 80 °C.

Previous investigations have reported changes in bacterial community compositions as a result of thermal impacts (Sowers, et al., 2006; Brielmann et al., 2009; Würdemann et al., 2014; Griebler et al., 2016; Metze et al., 2021; Bin Hudari et al., 2022), e.g., an increase in total microbial number (York et al., 1998; Keller et al., 2021) or even the highest diversity at 70 °C (Westphal et al., 2017). But other studies did not detect any or significant impacts (Schippers and Reichling, 2006; Dinkel et al., 2020; Keller et al., 2021), especially for temperature changes of up to 6 °C. This invites further investigations and suggests that results obtained from different geothermal energy systems cannot be readily transferred from one system to another (Casasso and Sethi, 2019).

4.1. Aerobic conditions

Microbial community structure can be significantly altered by the increase or decrease in temperature. This community shift is recognized in the increase of several species' metabolic activity and growth rate (e.g., forming biofilms, Figure 2a). For a temperature range of 8 °C to 18 °C, with slight changes in geochemical composition, weak but significant impacts on bacterial abundance and bacterial productivity have been observed. However, there is no improved survival or growth of coliforms (e.g., *Escherichia coli*) with a temperature increase (Brielmann et al., 2009). The presence of

native microorganisms seems to negatively impact *E. coli* survival (John et al., 2005). Viruses also show a temperature dependency with greater inactivation at greater temperatures; however, this occurs largely at temperatures greater than 20 °C (Gordon and Toze, 2003; John et al., 2005). Therefore, it poses no likely threat to ecosystem functioning and drinking water. An increase in temperature to 40 °C can be followed by an increase in the concentration of total organic carbon and by a strong increase in bacterial abundance, especially sulfate-reducing bacteria in shallow aerobic aquifers (Lienen et al. 2017). When the temperature goes 60 °C above the aguifer temperature, the inhibition of growth can occur (Regenspurg et al., 2020) but also thermophilic bacteria can become dominant (Lienen et al. 2017). A shift again towards the initial community structure is expected during the cooled-down phase. However, if this phase is short, this shift will not be observed and the variation of cell numbers not correlate with the temperature decrease (Regenspurg et al., 2020). These effects on a functional level have also been observed after the abandonment of a high-temperature ATES site, where the lowered temperature resulted in a population similar (but not equal) to the original population, performing the same functions (Hartog et al., 2013). This also occurs downstream with the mixing of the heated water with the non-affected groundwater, showing the resilience of the groundwater microbiome, with no significant differences in the total bacterial cell counts for an aguifer with an average temperature of 10 °C after 28 days at 13 °C and after 7 days at 35 °C (Keller et al., 2021).

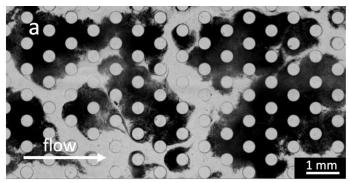
4.2. Anaerobic conditions

In reduced aquifers, no changes in the microbial community composition have been observed for moderate temperature changes as long as no oxygen enters the system. However, continuous operation can result in changes in bacterial composition (Dinkel et al., 2020; Bin Hudari et al., 2022).

A temperature increase from 11 °C (aquifer temperature) to 25 °C caused a shift from iron-reducing to sulfate-reducing and methanogenic microbial community. A further temperature increase to more than 45 °C can result in the emergence of a thermophilic microbial community specialized in fermentation and sulfate reduction. However, organisms adapted to temperatures between the typical temperature range of mesophiles and thermophiles can also be resilient to temporary heat changes, e.g., between 6 and 60 days for an indigenous aquifer microbial community at 45 °C that is changed to 12, 25, and 60 °C (Bin Hudari et al., 2022).

In open systems, the entry of oxygen into the system and the subsequent recovery of anoxic conditions are reflected in the microbial community. After the introduction of oxygen, $Fe(OH)_3$ can precipitate (Ni et al., 2018), and the abundance of the psychrotolerant, facultative anaerobic, iron- and manganese-reducing bacterium R. ferrireducens declines, and others like aerobic, iron-oxidizers such as Gallionella increases. The subsequent reduction of $Fe(OH)_3$ is accompanied by an increase in

Rhodoferax and Geothrix and the disappearance of *Gallionella*. The shutdown of the operation favors the enrichment of sulfate reducers and iron oxidizers bacteria in the proximity of the wells (Figure 2b, Würdemann et al., 2014). This interaction of sulfate reducers and iron oxidizers bacteria might have further enhanced corrosion.



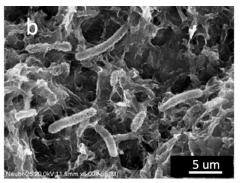


Figure 2. a. Bright-field image of biofilm formation (*Bacillus subtilis*) under imposed flow in a micromodel containing circular obstacles (modified from Kurz et al., 2022). b. Scanning electron microscopy image of a biofilm with microorganisms covered with minerals (modified from Würdemann et al., 2014).

Increased use of geothermal energy may also systematically reduce aquifer water temperatures (Ratchawang et al., 2022) and thereby affect microbial populations (Brielmann et al., 2009). For example, in deep (1'250 m) saline and anoxic aquifers (Lern et al., 2013) but also in shallow (60 m) oxic aquifers (Lern et al., 2011), a higher abundance of bacteria and sulfate-reducing bacteria, in particular, in cold fluids compared with warm fluids has been observed.

The salinity's apparent effect on incrustation type could also be indirect, by its influence on the microbial community. The common iron-oxidizing bacteria *Gallionella* and *Leptothrix* have been observed to prefer lower salinities. However, high iron concentrations are favorable for *Gallionella* (Eggerichs et al., 2014; Stenvik et al., 2022).

4.3. Conditions during construction and operations

Drilling mud has been demonstrated to have a strong impact on the microbial community with a significant change in the cell numbers over time and the variation in bacteria species (Regenspurg et al., 2018).

The aquifer layers' communication with different chemistry either during construction or operations can induce changes in the microbial community composition. For example, the dissolved organic carbon that enters a reduced aquifer can be subjected to fermentative degradation releasing CO₂, which can then be used as a carbon source for autotrophic or heterotrophic organisms with the proliferation of *Gallionella* or sulfate-reducing bacteria (Dinkel et al., 2020).

Open systems can introduce fecal bacteria into the groundwater and stimulate the growth of heterotrophic microorganisms, increasing the risk of proliferation of pathogenic microorganisms (e.g., *Legionella*, *Mycobacterium*, *Pseudomonas aeruginosa*) (Bonte et al., 2011).

5 Effects of temperature on microbial activity and biochemical processes

While the microbial community can have similar metabolic capabilities, although the species differ depending on the temperature (Westphal et al., 2017), an increase or decrease in temperature in the aquifer has the potential to change microbial activity, i.e., it has a potential impact on biogeochemical processes. A shift from aerobic to iron-reducing, sulfate-reducing, methanogenic, and fermentation processes in fluids due to temperature increase has been reported (Westphal et al., 2017). While for temperature changes < 6 °C, changes in microbial activity can be moderated, i.e., a small increase in enzyme activity and therefore in microbial growth, for a 10 °C increase, a higher microbial growth rate is expected and therefore a potential exists for biological clogging, especially if oxygen enters into the system (Douglas et al., 2015). Higher changes in groundwater temperature had a greater influence on the activity of thermophilic bacteria (e.g., *Anoxybacillus tepidamans*), responsible for bioclogging, than on the psychrophilic bacteria among autochthonous bacteria (Kim and Lee, 2019).

Seasonal fluctuations of groundwater levels, and therefore changes in the water saturation degree (fraction of the pore volume occupied by water) of the unsaturated zone, have been demonstrated to have an effect on the effectiveness of borehole heat exchangers, with an increase of effectiveness as water saturation degree increases (Smith et al., 2018). This is expected to have also an impact on microbial respiration in the unsaturated zone (Pietikäinen et al., 2005; Liu et al., 2018), and therefore in the production of greenhouse gases. Carbon dioxide is an indicator of microbial respiration, heterotrophic respiration in particular, which is maximized at intermediate water saturation degrees (where substrate diffusion and oxygen diffusion counterbalance) and that is higher as temperature increases (Hendry et al., 1999; Or et al., 2007).

An increase in temperature can promote the growth of bacterial groups such as *Rhodoferax sp.*, *Geothrix fermentas*, sulfate-reducing bacteria, and *Gallionella sp.* (e.g., *Gallionella ferruiginea*). These bacterial groups are known to promote Fe²⁺ and Mg²⁺ oxidation and SO₄²⁻ reduction under anoxic conditions inducing precipitation of oxides that clog the system, reducing its efficiency, and bio-corrosion. Note that thermophilic (45 °C to 80 °C) sulfate reduction has a higher activation energy (\sim 130 kJ/mol) than mesophilic (15 °C to 45 °C) sulfate reduction (\sim 45 kJ/mol). This has been explained by the enzyme's stability and activity. Therefore, microbial sulfate

reduction can withstand a substantial rise in temperature, while other biochemical processes are more temperature-sensitive (Bonte et al., 2013b). The interplay of sulfate-reducing and sulfate-oxidizing bacteria at high temperatures facilitates closed sulfur cycling and diminishes harmful sulfur species (Lienen et al., 2017). In recent works, it has been observed that phylotypes affiliated to the order *Spirochaetales* and to endospore-forming sulfate reducers of the order *Clostridiales* mineralize acetate at 45 °C, which is inhibited at temperatures < 25 °C and > 60 °C (Metze et al., 2021). Microbially mediated reductive dissolution of the precipitated oxides can occur at high temperatures (> 70 °C) (Jesußek et al., 2013a).

Low molecular weight organic acids and sulfate concentrations seem to be suited to determine microbial activity/impact of geothermal use of shallow aquifers. For deep aquifers, especially with high salinity and sulfate concentration, variations in low molecular weight organic acids and dissolved organic carbon concentration are suggested to be a marker for changes in fluid chemistry and can be an initial indication of fermentative microbial colonization (Vetter et al., 2010).

6 Bio-corrosion of the geothermal infrastructure

Brine environments are more suitable for inducing corrosion in geothermal pipes than freshwater environments (e.g., Soultz brine, Upper Rhine Graben, Germany, Mundhenk et al., 2013; Stenvik et al., 2022; Molasse Basin, South Germany, Alawi et al., 2011). Corrosion can also be microbially induced (Valdez et al., 2000, 2009; Lern et al., 2013). Bacterial attachment to the metal surfaces, e.g., metal sulfides, is among the mechanisms that are involved in microbiologically influenced corrosion. Sulfatereducing bacteria (specialized, lithoautotrophically bacteria, Desulfovibro Desulfuricans DSM 642) are protected from oxygen in a biofilm and form as the end product of their metabolism nitric and sulfuric acid that together with the Fe²⁺ ions can form insoluble iron sulfides like FeS and FeS₂ (Sand et al., 2003; Alawi et al., 2011; Lern et al., 2013; Ura-Bińczyk et al., 2019). G. fermentans (Coates et al., 1999), Rhodoferax ferrireducens (Finneran et al., 2003), and Fe³⁺-reducing bacteria may enhance the release of Fe²⁺ resulting in an increase of dissolved metal concentrations in the aquifer, increasing the risk of clogging. In the case of saline anoxic aquifers, hydrochemical conditions in iron oxide-clogged systems are favorable for the growth of iron-oxidizing bacteria (Stenvik et al., 2022).

7 Potential contamination and 'Geothermal Bio-remediation'

The different organic anti-freeze compounds used especially in closed-loop systems add a risk of groundwater contamination in case of leakage. For example, ethylene

and propylene glycol have been found to be readily biodegradable under both oxic and anoxic conditions. However, while other compounds such as betaine are also expected to be readily biodegradable in oxic and anoxic conditions, they can form by complexation of metal ions and therefore mobilize toxic metals. Further, additives such as corrosion inhibitors or biocides inhibit the biodegradation of organic and antifreezing compounds (Klotzbucher et al., 2007).

"Geothermal Bio-remediation" could leverage the subsurface heating resulting from geothermal systems to accelerate the biodegradation of certain petroleum-based pollutants (Jesußek et al., 2013b; Zeman et al., 2014; Ni et al., 2016, 2018; Meng et al., 2021; Kaur et al., 2021; Roohidehkordiband Krol, 2021) and contaminants of emerging concern (Pujades et al., 2023). It has been shown that thermophile bacteria play a dominant role in biodegradation in comparison to mesophile and psychrophile bacteria. Thermophile bacteria (e.g., Geobacillus thermodenitrificans, Thermos thermophilus HB27, Acidianus brierleyi, Sulfobacillus thermosulfidooxidans, Bacillus licheniformis M2-7, Pseudomonas sp. W6, Bacillus thermoamylovoran, Methanothermobacter thermautotrophicus) can survive under harsh conditions, exhibit higher growth rates, have higher stability, and are capable of degrading recalcitrant pollutants (Sawle and Ghosh, 2011). Biosorption of different metals such as Cd, Cu, Ni, Zn, and Mn by thermophiles (Chatterjee et al., 2010) and textile dyes biotransformation by Bacillus licheniformis M2-7 (Guevara-Luna et al., 2018) have also been reported.

Air surging has been used to unclog geothermal systems and recover efficiency. The bacterial diversity of the groundwater increases after air surging. Nevertheless, the proportion of bacterial genera thought to be related to microbiological clogging decreased (Kim et al., 2017).

8 Synthesis and recommendations

From a hydrogeochemical point of view, the remobilization of organic matter and contaminants (e.g., arsenic), the depletion of dissolved oxygen, and the subsequent shift to less oxic or even anoxic conditions are some of the major concerns in the geothermal use of aquifers for large changes of temperature in particular. Anaerobic processes may produce toxic products, corrosive products (e.g., H₂S), and greenhouse gases (e.g., CO₂ and CH₄). From a microbiological point view, moderate changes of temperature are not a major concern, especially if there are not changes in the microbial community. However, large changes of temperature are known to reduce microbial diversity with the proliferation of atypical bacteria prone to induce biogeochemical reactions. The detailed literature review presented above on the impact of water temperature changes due to geothermal activities on groundwater microbiology and associated geochemical processes is synthesized in Table 1.

Table 1. Summary of the impacts of temperature increase in geothermal activities on hydrogeochemistry, microbial communities (see also Westphal et al., 2017) and microbial activity and biochemical processes.

Microorganisms (optimal growth temperature)	Temperature change and absolute temperature	Changes in hydrogeochemistry	Microbial community composition			Microbial activity and biochemical processes	
			Aerobic		Anaerobic		Microbial interactions
					Entry of oxygen	-	meradions
Psychrophiles (0 °C – 20 °C)	Changes of 6-10 °C (absolute temperature 20 °C)	No significant changes in natural water chemistry in groundwater suitable for drinking water (e.g., dissolved organic carbon ~ 1 mg/L and nitrate ≤ 25 mg/L).	Weak impacts on bacterial abundance and bacterial productivity. No improved survival or growth of pathogens.	No significant changes in the microbial community composition. Continuous operation can result in changes in bacterial composition.		< 6 °C: Changes in microbial activity can be moderated. A small increase in enzyme activity and therefore in microbial growth. 10 °C: A higher microbial growth rate and potential for bio-clogging, especially if oxygen enters into the system.	
Mesophiles (15 °C – 45 °C)	Increase of temperature up to 40 °C (absolute)	Significant changes in water chemistry (e.g., dissolved organic carbon or sulfate) in both aquifers suitable and non-suitable (i.e., contaminated by chlorinated hydrocarbons, heavy metals, and/or agrochemicals – mainly nitrate and derivates) as a source of drinking water.	Strong increase in bacterial abundance, especially sulfate-reducing bacteria.	Shift from iron- reducing to sulfate- reducing and methanogenic microbial community	Aerobic bacteria and iron- oxidizers increase. Fe(OH) ₃ can precipitate and the abundance of the psychrotolerant, facultative anaerobic, iron- and manganese-reducing bacterium declines.	Under anoxic conditions, promote the growth of sulfate-reducing and fermentative bacteria. Endospore-forming sulfate reducers mineralize acetate at 45 °C (inhibited at < 25 °C and > 60 °C).	The interplay of sulfate-reducing and sulfate-oxidizing bacteria at high temperatures facilitates closed sulfur cycling and diminishes harmful sulfur species.
Thermophiles (45 °C – 80 °C)	Increase of temperature up to 60 °C (absolute)		Inhibition of bacterial growth can occur. Therrmophilic bacteria can be dominant.	Thermophilic community specialized in fermentation and sulfate reduction.		Greater influence on the activity of thermophilic bacteria responsible for bio-clogging.	
Hyperthermophiles (> 80 °C)	> 80 °C					Microbially mediated reductive dissolution of the precipitated oxides can occur at high temperatures (> 70 °C).	

A number of recommendations can be made regarding building operations of geothermal facilities, the maximum concentrations of chemicals in dissolution and thresholds of physicochemical parameters for their successful operation, especially the open ones (ATES), minimizing the impacts:

- During the works to build the geothermal facility, it is of extreme importance to a well-planned and clean drilling progress and installation, sealing off the overlying groundwater levels and thus avoiding mixing of waters with different chemical compositions (e.g., redox conditions) and different microbiomes.
- To avoid chemical clogging, iron and manganese concentrations should not exceed c(Fetotal) < 0.1 mg/L and c(Mntotal) < 0.05 mg/L, respectively (UMBW, 2009). However, other authors establish some of these thresholds in higher values, e.g., [Fe²⁺] < 11.2 mg/L (Pérez-Paricio, 2000; Douglas et al., 2015). Further, pH should be below 7.5, total dissolved solids < 150 mg/L, and [Cl⁻] < 500 mg/L (Pérez-Paricio, 2000; Douglas et al., 2015).
- To avoid biological clogging, dissolved and total organic carbon, concentrations below 2 mg/L and 10 mg/L, respectively, are recommended. Further, pH should be above 7.2, especially in the presence of iron bacteria, in order to avoid biologically induced clogging, i.e., biomineralization (Pérez-Paricio, 2000; Douglas et al., 2015).

Based on this bibliography review on the impacts of geothermal activities on aquifers' microbial community and activity, and for the case of Swiss shallow aguifers, with mean annual temperatures of ~ 14 °C and mainly carbonatic water chemistry, a warming up of groundwater at a certain distance (to be defined) from the geothermal facility of more than 6 °C to 10 °C is not recommended. Ideally, no more than 6 °C, which would avoid a clear transition from psychrophile to mesophile microbial communities, the latter more prone to induce biogeochemical reactions. For the often oxic Swiss shallow aguifers, the main risk is the reduction of oxygen content because of an increase in temperature. A shift to reducing conditions might stimulate sulfate-reducing bacteria and the release to the water of toxic constituents, for example: arsenic in crystalline rock aquifers (locally exceeding the Swiss drinking water limit of 10 µg/L), or chromium (close to the Swiss drinking water limit of 20 μg/L) in the consolidated sandstone aquifers (e.g., Molasse), compromising the quality of water for certain uses. In the case of polluted aguifers, this recommendation (+ 6 °C) should be followed more cautiously depending on the present chemicals/contaminants, i.e., of the initial conditions.

9 Remaining open questions

Geothermal activities can have various impacts on aquifers' microbiology. Even though research efforts have increased, some key questions and topics of interest remain still unanswered:

- Microbial diversity and adaptation: How do microbial communities adapt to higher temperatures and the presence of various chemical compounds in geothermal environments? Understanding the diversity of microorganisms and their adaptive mechanisms is a fundamental question.
- Biogeochemical cycling: What roles do microorganisms play in biogeochemical cycles in geothermal systems, such as sulfur, nitrogen, and carbon cycling? Elucidating these processes can provide insights into the global biogeochemical cycles, including trace elements.
- Microbial interactions: How do different microbial species interact with each other in geothermal systems, and what are the implications for their survival and functions? This includes studying both synergistic and competitive interactions.
- Microbial metabolism and energy sources: How do microorganisms in geothermal systems obtain their energy and nutrients, and what metabolic pathways are involved? Understanding these processes can shed light on the sustainable utilization of aquifers for geothermal purposes.
- Biofilm formation: What is the role of biofilms in geothermal systems, and how
 do they influence heat storage efficiency? While the formation of biofilm in the
 aquifers can be a priori understood as a counterproductive effect, the
 reduction of hydraulic conductivity and the presence of biomass can enhance
 the energy storage (i.e., the loss of heat by water flow or dissipation).
- Conservation and management: How can we protect the unique microbiological diversity in aquifers while still harnessing geothermal energy for sustainable development?

These open questions highlight the complexity of microbial communities and activities in geothermal applications and the need for further research to better understand their roles and adaptations. More studies in this field will contribute to both fundamental microbiological knowledge and practical applications in geothermal energy and environmental management.

9.1. Open questions for Swiss aquifers

When considering the impacts of (shallow) geothermal energy on aquifers in Switzerland, several open questions arise:

- What is the expected oxygen depletion because of increased temperature and microbial activity for the three main types of groundwater identified in Switzerland? The transition from an oxidized to a hypoxic or anoxic state might stimulate sulfate-reducing bacteria activity, which might result in a significant deterioration in water quality and ecological integrity.
- What are the expected changes in chemistry for the three main types of groundwater identified in Switzerland? Besides the ones that can be predicted using non-isothermal geochemical modeling, there is the potential for the release of undesired metals and metalloids, which compromise their uses.
- What is the current status of shallow aquifers in Swiss urban and peri-urban areas? The chemical (e.g., hydrocarbons) and biological (fecal indicators and pathogenic microorganisms) contamination could prevent an increase in the working temperatures.

Addressing these open questions will be crucial for advancing the sustainable development of geothermal energy in Switzerland and maximizing its potential contribution to the country's energy transition.

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