Synthesis of the risks and actions to correct these risks depending on the environment

Dissemination level: PU - Public

Hystories deliverable D 3.4

Date: 16 October 2023

The Project is co-founded by European Union

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Revision History

Revision	Revision date	Summary of changes
0	16 October 2023	Initial version

Checked by:

Name	Institute	Date
Martin WAGNER	MicroPro GmbH	16.10.2023

Approved by:

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Ngoc Dieu HUYNH WP3 Leader	MicroPro GmbH	16.10.2023
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1. Introduction

Underground hydrogen storage in porous structures is a promising technology that enables to balance the energy demand. However, our experience for hydrogen storage in geological formations as well as knowledge of geo-microbial populations and a potential stimulation during hydrogen storage is very limited.

Numerous microbiological and molecular biological investigations over the past decades have shown that geological structures, reservoirs and storages are by no means to be regarded as sterile spaces, but are rather populated by a wide variety of highly adapted anaerobic microorganisms. The degree of colonization of this deep biosphere by bacteria and archaea depends on numerous chemical and physical factors such as availability of electron donors and acceptors, mineral composition, salinity, depth (temperature), and many others.

The microbiological investigations of formation water samples from various European reservoirs within the Hystories-project, Deliverable 3.1 and 3.2 have again confirmed this and clearly demonstrated that porous geological structures are very often habitats for various complex microbial populations, including hydrogen-using microorganisms. Microorganisms are able to adapt even to extreme geochemical and physical conditions of underground storages and develop there. Nevertheless, microbial growth is only possible within certain limits. Beyond these extreme values, their activities can be temporarily or permanently inhibited. This opens up technical possibilities to control microorganisms in geological structures under defined circumstances. For the assessment of existing or even future risks from microbial activities during underground hydrogen storage, it is important to know the nutrient requirements and the effects of the underground storage environment, such as temperature, salinity, pH, pressure and toxicity, on microbial metabolic processes.

This Deliverable 3.4 discusses the conditions affecting the metabolism of microorganisms in the subsurface relevant to hydrogen storage, based on literature studies as well as practical experience from microbiological studies in the Hystories project and other projects conducted at MicroPro. This work package comprises the assessment of microbial risks for underground hydrogen storage with focus on the three most important metabolic pathways based on hydrogen: sulfate reduction, methanogenesis and acetogenesis. In addition, strategies for reducing negative microbial activities in underground hydrogen storage facilities will be discussed.

2. Environmental constraints on subsurface microbial hydrogen consumption.

2.1. Availability of nutrients and trace elements

Although the physico-chemical conditions in underground reservoirs often allow for microbial growth and especially the turnover of hydrogen, there is a high dependency on the availability of nutrients. In general, the electron acceptors or energy sources available to microorganisms in subsurface environments are very limited. However, hydrogen storage provides a quasi-unlimited energy source that can be utilized by numerous microorganisms under the anaerobic conditions of the deep biosphere. It is therefore necessary to look in more detail at the availability of inorganic nutrients and electron acceptors as regulating factors for microbial activity in underground storage systems.

Microorganisms basically require macro elements (C, N, H, P, Ca, Mg, S, and Fe) and trace elements (Co, Mn, Ni, Mo, Cu, Zn, W, and Se) for their growth. Different vitamins can also be required for optimal microbial growth. Despite being transported by reservoir water movements or diffusion nitrogen, in the form of ammonium, is often limited. This is especially the case in depleted oil and gas fields. Phosphorus, derived organically by the lysis of dead cells or inorganically by the dissolution of phosphate-containing silicates (Bennett et al, 2001) is, similarly to nitrogen, often limited. Consequently, phosphorous and nitrogen are considered as limiting nutrients (Head et al., 2003; Herbert et al., 1985; Hagar et al., 2022). Nevertheless, due to the low biomass yields of anaerobes, the requirement for N and P can be low. Therefore, less N and P are required for microbial hydrogen consumption in porous underground storages. Moreover, additional nutrients for microbes can be introduced into reservoirs during storage operations such as water flooding, injection and extraction of gases and fluids (Ivanova et al., 2007).

Dissolution of carbonates such as calcite ($CaCO_3$) or dolomite $CaMg(CO_3)_2$ from the host rock by the reservoir water results in readily available carbon in the form of dissolved HCO_3^-. The aqueous dissolution of calcium sulfur mineral phases such as gypsum ($CaSO_4[2H_2O]$) or anhydrite ($CaSO_4$) can provide sulfate for sulfate-reducing microorganisms. Due to the presence of carbon sources, electron donors (H_2, CH_4, Fe^{2+}, reduced inorganic sulfur or nitrogen compounds, Mn^{2+}, organic carbon) and electron acceptors (CO_2, NO_3^-, SO_4^{2-}, Fe^{3+}, Mn^{4+}) in the rock layers, the subsurface environments allow at least a limited microbial growth.

Hydrogenotrophic microorganisms require a carbon source in addition to hydrogen as an energy source. These can be inorganic (CO_2, HCO_3^-) as well as organic carbon compounds (e.g. acetate). However, organic carbon compounds can also be used by other anaerobes such as methanogenic or sulfate-reducing microorganisms. There is still little known about the metabolic pathways as well as the interaction of hydrogen-consuming microbial groups in the presence of different carbon sources under unlimited hydrogen conditions.

We investigated the effect of different carbon sources on hydrogen consumption by mixed hydrogenotrophic cultures enriched from formation water which were dominated by methanogenic or sulfate-reducing microorganisms. Carbon sources for microbial growth were acetate, lactate, methanol, CO_2 and HCO_3^-. The cultures were incubated at 50 °C in a specific enrichment medium with a salt content of 0.1 % NaCl. The experiments clearly showed that the carbon source has a significant influence on hydrogen turnover (Figure 1).

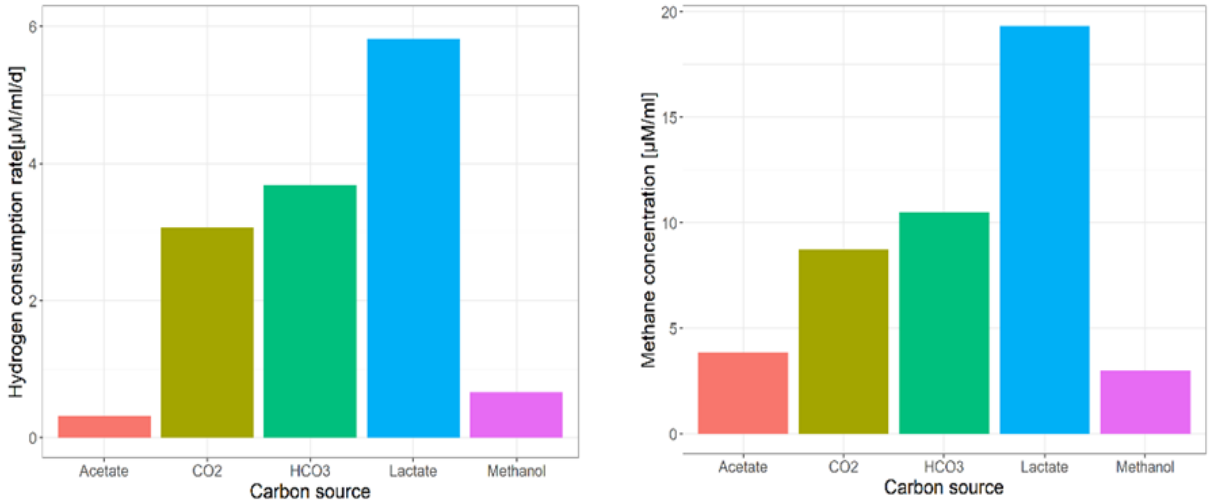


Figure 1: Effect of different carbon source on microbial hydrogen consumption and methane formation

Both the maximum hydrogen consumption rate as well as highest methane formation was in the assays containing lactate, followed by HCO_3^- and CO_2. Noticeably, sulfate reduction only occurred in the cultures with CO_2, methanol and lactate. In addition, acetate was not found to be consumed by neither methanogens nor sulfate reducers during the experiment (data not shown).

Our knowledge about the general nutrient requirements as well as its variation for specific microbial groups is still very limited. Studies have shown that sulfate reduction can occur at

sulfate concentrations as low as 5 – 77 µM (Thaysen et al., 2021; Vroblesky et al., 1996; Havig et al., 2017). A sulfate concentration above 30 µM has been considered to promote sulfate reduction in freshwater sediments (Lovley and Klug, 1986). Furthermore, it was observed that a sulfate concentration of 10 mM (960 mg/l) resulted in maximum hydrogen consumption, while hydrogen consumption almost stopped at 0.2 mM (19.2 mg/l) (MicroPro internal data).

Sulfate reducers compete with methanogens for available substrates and can overgrow the latter depending on environmental conditions (Omil et al. 1997; Muyzer and Stams, 2008; O'Flaherty et al., 1998). However, in a study on hydrogen consumption with a mixed culture dominated by methanogens and sulfate reducers, we observed that, with unlimited hydrogen supply, only methanogenesis was detectable despite the presence of sulfate (Figure 2). Different sulfate concentrations were included in the experiment, ranging from 5 mg/l to 2 g/l. However, although sulfate reducers were present in the culture, no sulfate reduction was observed (data not shown here). Furthermore, only HCO_3^- was consumed during the test periods, although lactate was also present.

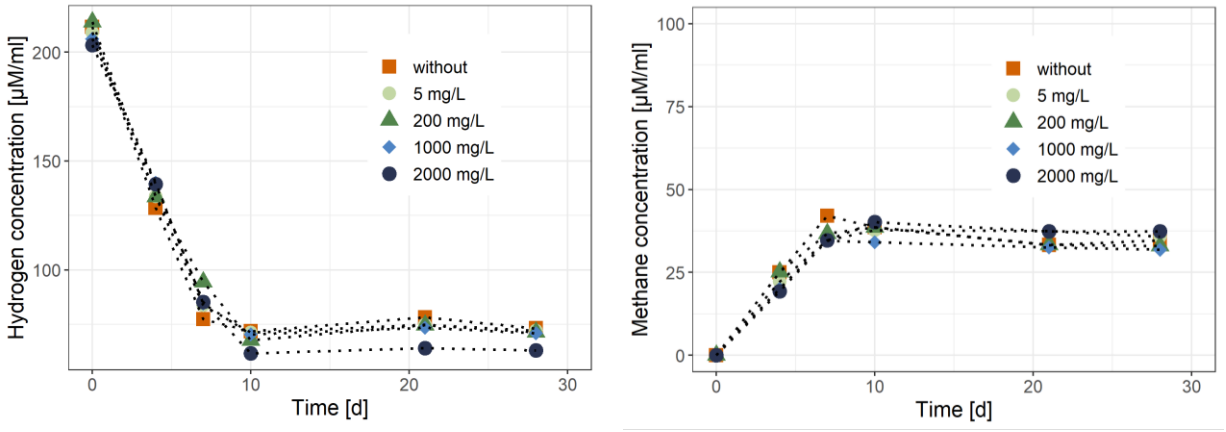


Figure 2: Hydrogen consumption in the presence of different sulfate concentrations (0, 5 mg/l, 200 mg/l, 1 g/l, and 2 g/l)

2.2. Temperature

Microorganisms are able to grow within a wide range of temperatures and can be classified based on their preferred growth temperature (Figure 3). They range from mesophilic to thermophilic to hyperthermophilic bacteria and archaea, which occur in high-temperature environments up to 122 °C, representing the upper temperature limit of life (Takai et al., 2008, Holden, 2009).

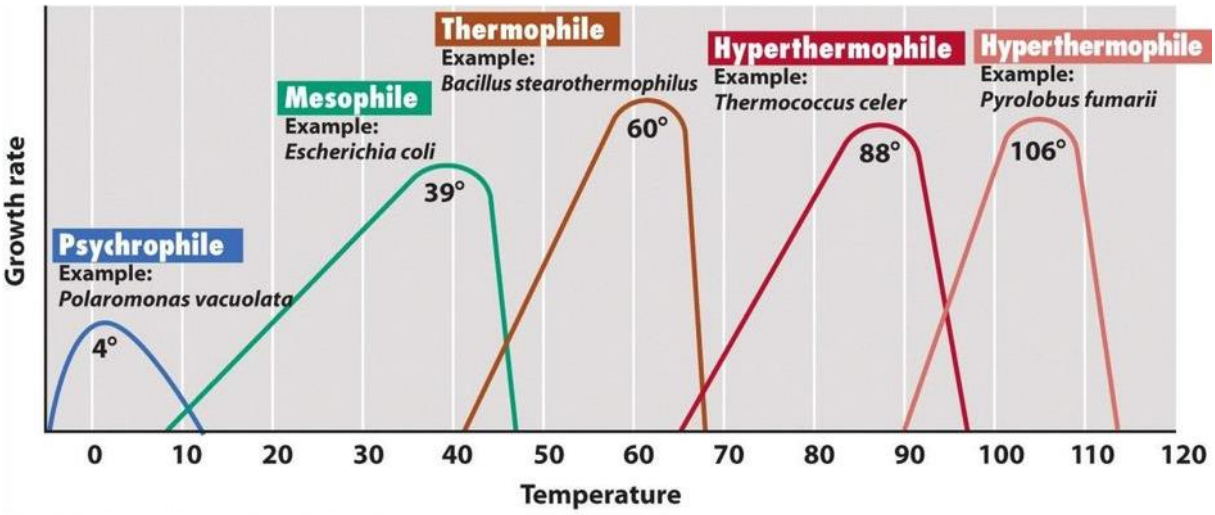


Figure 3: Classification of microorganisms on the basis of their preferred growth temperature (in °C)

Underground gas reservoirs typically have a temperature range of 20 to 110 °C or higher. This temperature range allows for the development of microorganisms from mesophilic to hyperthermophilic. Studies have shown that optimal growth temperatures range from 15 to 98 °C for methanogens, 20 to 70°C for sulfate reducers and 20 to 30 °C for acetogens (Thaysen et al., 2021). Microbiological investigations by MicroPro also detected various groups of microorganisms, especially in the underground storage tanks and reservoirs, at temperatures of 30 to 70 °C (Hystories, Deliverable 3.1). Consequently, high microbial activity can certainly be expected when temperatures in a reservoir are between 20 and 70 °C.

Some methanogens or sulfate reducers have been detected at a temperature above 100 °C. However, thermophilic and especially hyperthermophilic hydrogen-oxidising microorganisms are rare and the rate of metabolic reaction decreases abruptly when the temperature rises above the optimal temperature. The critical temperature for methanogens, sulfate reducers and acetogens is 121 °C, 113 °C and 70 °C, respectively (Thaysen et al., 2021). Although the presence of hyperthermophiles is evident, *in-situ* observations show that microbial activity in oligotrophic reservoirs is limited to temperatures below 80-90 °C (Wilhelms et al., 2001). High temperatures can have a detrimental effect on cells, e.g. through DNA damage, reduction of protein stability and alteration of the fluidity of biological membranes (Holden, 2009; Jaenicke and Sterner, 2006). High temperatures also have a strong effect on cellular maintenance energy, which increases more than three thousand-fold when the temperature rises from 0 to 100 °C (Hoehler et al., 2010). As a result, it is likely that microbial activity will be low in milieus at temperatures above 80 °C.

In addition, temperature, together with pH and pressure, can also strongly influence the solubility of gases and the solution equilibrium of ions, which affects the availability of nutrients to microorganisms. Thus, temperature is a very important criterion for the occurrence of microbial hydrogen consumption in underground storage.

2.3. Salinity

Due to osmotic stress and toxic ions, increased salt concentration commonly results in decreased microbial activity and even causes cell death. However, there are microorganisms that are very well adapted to high salinity and can grow even in saturated brine. Microorganisms can be classified according to their optimum salt concentration for microbial growth (Imhoff, 2001)

Table 1: Classification of microorganisms according to their salt optima (modified from Imhoff., 2001)

Type of natural habitat	Classification of microorganisms	Optimum salt concentration (% NaCl)
Fresh water	Freshwater (non-halophilic)	< 0.5
Brackish water	Brackish water (slight halophilic)	0.5 - 2
Seawater	Marine (moderately halophilic)	2 - 7
Hypersaline water	Extremely halophilic	7 -15
Highly saline water	Extremely halophilic	> 15

Microorganisms that live in high salinity environments must be able to tolerate high osmotic pressure and ion concentration. Different groups of microorganisms use different strategies to maintain osmotic balance. There are two main mechanisms to counteract the effect of osmotic efflux of water in high salinity environments: Accumulation of ions (K^+, Cl^-) to high intracellular concentrations and accumulation of organic compounds known as compatible solutes (Oren, 2002).

Halophilic hydrogenotrophic microorganisms, especially methanogenic and sulfate-reducing microorganisms, are often found in the formation water of reservoirs. For example, sulfate-reducing microorganisms obtained from water in reservoirs in northern Germany could be cultivated at salinities of up to 270 g/l. Sulfate reducers isolated from reservoirs in Lausitz, Kirchheilingen and Langensalza grew in saturated brine at 320 g/l (Wagner and Ballerstedt, 2013). Although most microbial processes that occur at low salinity can also take place at high

salinities up to NaCl saturation, some dissimilatory processes are inhibited at high salt concentrations.

For example, methanogenesis from reduction of CO_2 with H_2 or from acetate has never been detected in natural environments at the salinity above 100 g/l (Head et al., 2010; Oren, 2002). The upper salinity limit for microbial processes therefore depends on the specific pathway utilized, with tolerance increasing with increasing substrate energy yield. Oxidation of acetate by sulfate-reducing microorganisms does not occur at salt concentration exceeding 100 - 150 g/l whereas growth of sulfate reducers that use lactate, propionate, or H_2+CO_2 was found between 30 to 230 g/l salt with an optimum at 80 - 100 g/l. Formation of acetate from H_2 + CO_2 could occur at salinity up to 250 g/L (Oren, 2002).

Life at high salt concentrations is energetically expensive. ATP requirement for osmoregulation per cell increases by an order of magnitude between 0 and 400 g/L salt concentration (Head et al., 2014). Moreover, HEAD et al. (2014) also showed that the maximum salt tolerance for methanogenesis is lower at higher temperature, indicating an interaction between salinity and temperature. However, the relationship between salinity, temperature, and microbial processes in reservoirs is currently unknown and still require more studies. Generally, high salinity could reduce microbial activity because a certain part of available energy must contribute to stress adaptation. Therefore, storage with reduced microbial activity could be expected in storages with high salt concentrations.

2.4. pH-Value

The pH value also has a significant influence on microbial growth. It regulates the ionisation state of the components (e.g. cell enzymes) in a system (Dixon and Webb, 1979). According to the optimal pH for growth, microorganisms can be classified as acidophilic (pH < 5.0), neutrophilic (pH 6.5 - 7.5) and alkaliphilic (pH 8.5 – 11.0).

Although some hydrogen-consuming microorganisms have been found in both acidic and alkaline environments (Hoehler et al., 2018), they generally prefer a neutral pH for optimal growth. Outside the pH range of below 4.5, or above 7.5, most methanogens and sulfate reducers cannot grow (Thaysen et al., 2021). The metabolism of methanogens and sulfate-reducing prokaryotes is severely restricted by low pH (Goodwin et al., 1988). A pH value below 3.6 and above 10.0 - 10.7 is considered critical for acetogens (Thaysen et al., 2021).

In the Hystories project, the effects of pH on hydrogen consumption were investigated with an enrichment culture of methanogens and sulfate reducers. At 50 °C and a salinity of 0.1 % hydrogen consumption was measured at different pH values between 6.0 and 10.0. The

experiment clearly showed that the pH has a significant effect on microbial activity, with pH values above 7.0 causing a significant decrease in hydrogen consumption (Figure 4).

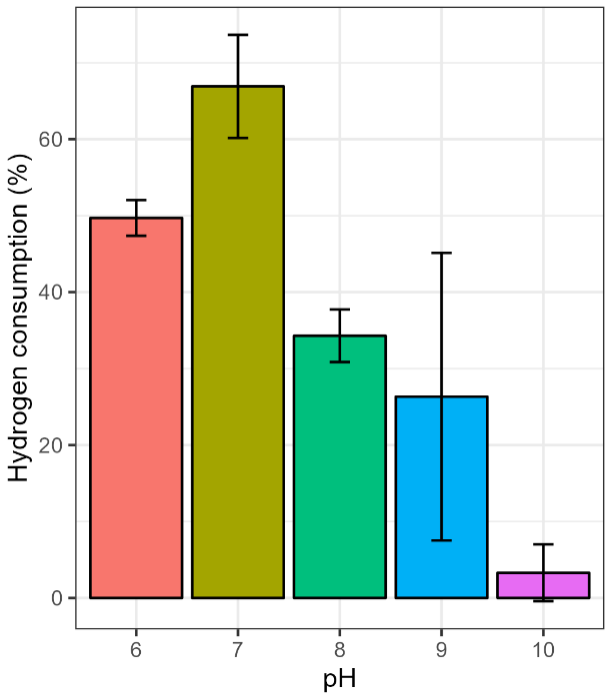


Figure 4: Effect of pH on hydrogen consumption by a mixed methanogens and sulfate reducers dominated cultures enriched from reservoir formation water

The formation waters of most of the porous reservoir rocks studied have pH values ranging from slightly acidic to moderately alkaline (Hystories-Deliverable 3.1; Wagner and Ballerstedt, 2012). In the context of hydrogen utilization, carbon dioxide plays a crucial role both as a carbon source and in terms of a pH regulating agent. Carbon dioxide dissolved in formation water forms carbonic acid and consequently causes a lowering of the pH value. (1)

$$CO_{2(g)} \rightarrow CO_{2(aq)} + H_2O \rightarrow H_2CO_3 \quad (1)$$

The carbonic acid thus formed is an important agent for the dissolution of carbonates e.g. calcite. The solubility of carbonate increases with decreasing temperature, increasing pressure and increasing carbon dioxide partial pressure. This leads to an increase of the pH value in natural systems (2)

$$CaCO_3 + H_2CO_3 \rightarrow Ca^{2+} + 2\ HCO_3^- \quad (2)$$

Depending on the physico-chemical parameters, the pH of the formation water can therefore remain at an almost neutral level. This allows the development of microbes in porous

underground storage systems. Under these conditions, pH alone may not be the limiting factor for the occurrence of microorganisms in underground storage systems.

Nevertheless, changes in pH could affect microbial metabolic pathways and *vice versa*. WORMALD et al. (2020) showed that acetoclastic methanogenesis does not occur at alkaline pH values (pH > 9.0). DOPFFEL et al. (2023) described a significant pH increase in sulfate-reducing cultures incubated with hydrogen, where the pH exceeds growth limits (pH > 9.0) and hydrogen was not completely consumed. Furthermore, studies have shown the influence of pH variations on metabolic reactions in anoxic environments containing iron-containing (oxyhydroxide) minerals (Postma and Jakobsen, 1996; Marquart et al., 2019). Electron donor oxidation was 85 % lower for iron reduction and 61 % higher for methanogenesis at pH 7.0 compared to pH 6.0 (Marquart et al., 2019). Similarly, as pH increases, sulfate reduction becomes more energetically favourable than iron reduction (Postma and Jakobsen, 1996).

2.5. Pressure

Increased pressure is a major characteristic of subsurface structures. Microorganisms of the deep biosphere have evolved different mechanisms and strategies to adapt to high pressure conditions. Studies have shown that barotolerant microbes can survive at pressures of up to 500 bar. However, the optimal range is between 1 and 200 bar. This range is relevant to most underground reservoirs and storages. Extremely barophilic bacteria isolated at a depth of 11,000 m required a pressure of over 500 bar for growth and grew optimally at 700 bar (Kato et al., 1998). MILLER et al. (1988) recorded methanogen activity at a pressure of 750 bar.

The correlation of temperature and pressure for microbial growth has also been reported (Horikoshi, 1998). High pressure is required to maintain a fluid environment for microorganisms at a temperature above 100 °C. Hyperthermophilic microorganisms generally prefer high pressure, while mesophilic microorganisms can be inhibited by pressure above 300 – 500 bar (Abe et al., 1999; Holden, 2009). Mesophilic sulfate reducers have been reported to grow optimally at a pressure of 100 – 500 bar and thermophilic sulfate reducers have been reported to grow optimally at a pressure of 300 – 420 bar (Steinsbu et al., 2010).

In our study, microorganisms from reservoirs were enriched at a pressure between 100 and 200 bar (Hystories, Deliverable 3.1). It was found that the activity of microorganisms such as acetogens is increased at a hydrogen pressure of 45 bar compared to a pressure of 1 bar (Hystories, Deliverable 3.2). SILVA AND WEBER (1993) demonstrated that a pressure of more than 1,000 bar is required for a significant change in biochemical components, such as pressure

denaturation of proteins, whereas pressures for hydrogen storage are usually between 10 and 200 bar (Matos et al., 2019; Shi et al., 2020). Therefore, high pressure in the reservoir is not expected to have a negative impact on the growth and activity of microorganisms. On the contrary, since microorganisms can only use gases such as hydrogen and carbon dioxide when they are dissolved, increased pressure improves the solubility of the gases and thus their availability for microbial processes. In addition, pressure, in conjunction with temperature and pH, determines the dissolution of rock materials, which could, for example, increase the concentration of HCO_3^- from calcite. (Lexikon der Wissenschaft) The effect of pressure on microorganisms in underground gas reservoirs is therefore more likely to be due to its effects on substrate transport.

2.6. Thermodynamic drivers and inhibitors

In addition to physical and chemical environmental conditions that control the activity of hydrogen-consuming microorganisms, parallel microbial processes as well as resulting metabolic products of the hydrogen utilization process can also have an impact on the hydrogenotrophic microorganisms in underground storage systems. Due to their high affinity for hydrogen, sulfate-reducing microorganisms usually displace methanogens and acetogens under conditions with limited hydrogen concentrations. One study describes that methanogenesis is inhibited at sulfate concentrations as low as 0.2 mM due to competition with sulfate reducers (Winfrey et al., 1977). However, in hydrogen storage systems where the hydrogen concentration is generally not limited, the interaction between the different microbial groups is very difficult to predict (Hystories, Deliverable 3.2). Sulfate reducers, methanogens and homoacetogens can be inhibited by hydrogen sulfide at concentrations above 3.8 mM (Hulshoff Pol et al., 1998; Ntagia et al., 2020; Dopffel et al., 2023). Inhibition of homoacetogenesis and sulfate reduction by nitrate and nitrite has also been described (Fröstl et al. 1996; Carlson and Hubert 2019). Wang et al. (2015) showed that ammonia-rich substrates exert inhibition on hydrogenotrophic methanogens. In addition, sulfate reduction can be inhibited by elevated concentrations of volatile organic compounds, especially at low pH values (James et al., 1998, Reis et al., 1990; Voskuhl et al., 2022). According to Omil et al. (1997), Isa et al. (1986) and others, the ratio of organic carbon and sulfate is also decisive for the competition between methanogenic and sulfate reducers: if the proportion of organic carbon outweighs sulfate, the methanogenic ones dominate. According to Lens and Hulsholl Pol (2000), if sulfate is not limiting, the hydrogen is completely converted by sulfate reducing bacteria (SRB), since the sulfate reducers achieve a higher energy yield in the conversion of hydrogen than the methanogens. According to Isa et al. (1986) and AIVasidis

(1990), however, autotrophic SRB are favoured over methanogenic ones only at correspondingly low hydrogen partial pressures (< 50 mol H_2). At higher concentrations, methanogens are favoured by H_2- concentrations.

2.7. Ranking of microbial risks for underground hydrogen gas storages

As a result of this study, the parameters temperature and salinity of the storage systems were identified as essential environmental factors for the control of hydrogenotrophic microorganisms. As described above, upper limits can be specified for temperature and salinity, at which microbial activity can be excluded or is very strongly reduced. However, if both stress factors act simultaneously, the respective limits decrease considerably, so that microbial colonisation of reservoirs with temperatures above 55 °C and salinities above 1.7 M is very unlikely and these therefore appear to be well suited as hydrogen reservoirs (Thaysen et al., 2021, 2023).

The investigations within the scope of this study revealed that microorganisms enriched from the reservoir samples were still very active at a temperature of over 60 °C. In agreement with previous isolates from similar sites, it can be stated that microbial activity is still present in the temperature range between 55 °C and 70 °C. A salinity of above 1.7 M generally leads to an inhibition of numerous microorganisms. Above this salinity, the activity of hydrogenotrophic methanogens drops drastically. However, at salinities of 1.7 M - 3.4 M, effects of the sulfate reduction process have been shown to still occur (Oren, 2011). Therefore, especially the risk related to sulfate reduction, should not be excluded for repositories with a salinity of 1.7 M - 3.4 M (corresponding to 10 - 20%). At a salinity of more than 3.4 M, the activity of hydrogen utilization by sulfate-reducing microorganisms is significantly reduced, as the energy yield of dissimilatory sulfate reduction is low and the energy costs of maintaining osmotic equilibrium are high (Research Report: Twenty20 - HYPOS 2022; Oren, 1999, 2006; McGenity and Oren, 2012).

In the Hystories project, almost 500 traps, storages and hydrocarbon reservoirs were analysed in terms of salinity and temperature and shown in Figure 5. Based on the critical limits for salinity and temperature, a range of 192 traps can be identified with a correspondingly low microbial risk. Accordingly, 136 structures have a high-risk potential and 164 a medium risk. On this basis, we can already make a very basic risk classification for potential storage sites at a very early planning stage. The structures with medium or high risk must be evaluated with regard to further parameters in order to further limit the risk if necessary.

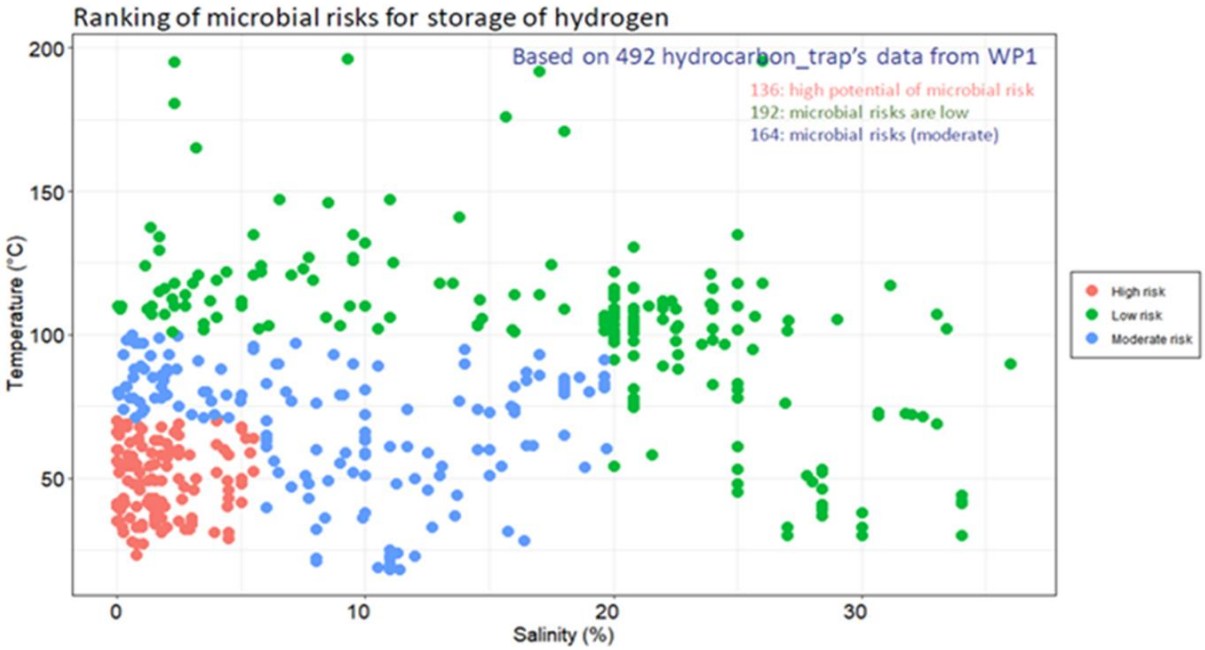


Figure 5: Ranking of microbial risks for storage of hydrogen (Source Hystories, D7.3)

Clearly, in addition to temperature and salinity, other factors such as carbonate availability, sulfate concentration, organic compounds, mineral composition (carbonate and sulfate source for microorganisms), pH and microbial community need to be included in the assessment of microbial risks of hydrogen storage. Table 2 lists important parameters that should be considered in the microbial risk assessment. Each parameter is categorized according to its individual impact on microbial activity: low risks, moderate risks and high risks. A low microbial risk has conditions where microbial growth is almost impossible or microbial activity is extremely limited. In contrast, a storage with high microbial risk has conditions that allow microbial growth and activity to an optimal extent. A moderate-risk repository has conditions that are not in the optimal range or inhibit microbial activity, but there may be development of specific microbial groups in the medium term. Due to the huge volumes of underground storage and the long operating periods, there is some risk to the storage.

Table 2: Important parameters for microbial risks assessment of a hydrogen underground storage sites

Parameter	Sample	Microbial risk assessment		
		Low risk	Moderate risk	High risk
Temperature (°C)	Downhole-measurement	> 100	70 – 100	< 70
Salinity (%)	Formation water	> 20	10 – 20	< 20
Carbon source (carbonate/CO_2, organic compounds)	Formation water Rock/sediment	Not available Extremely limited	Available	Available
Sulfate concentration (SO_4^{2-})	Formation water Rock/sediment	Not available Extremely limited	Available	Available
pH	Formation water	< 4, > 10	4 – 6; 9 – 10	6 – 9
Potential toxic compounds	Formation water Rock/sediment	Available at inhibitory concentrations	Not available Below toxic levels	Not available Below toxic levels
Total cells	Formation water Rock/sediment	Not detected Extremely low	Detected	High cell density
Microbial community	Formation water Rock/sediment	Not detected	Presence of hydrogenotrophic microorganisms	Active hydrogenotrophic microorganisms

From the storage facilities operators' point of view, an initial pre-selection (ranking) of available storage facilities makes sense by considering those parameters for which data are already available. For this purpose, the following parameters from Table 2 can be used for a preliminary evaluation: Temperature, salinity, pH, carbon source and sulfate availability. The classification of the actual storage conditions gives a first indication of the extent to which microbial processes are to be expected. It should be emphasized that the sum of the various factors affecting microbial growth and activity must be taken into account, as microorganisms respond to several factors simultaneously. Unfortunately, research on the effects of combined factors on microorganisms is still very limited, and information on the brine composition of reservoirs, such as presence of various inhibitors, is not always available.

Therefore, we propose a risk assessment diagram (Figure 6) as an initial guide for classifying microbial risks (low risks, moderate risks, and high risks) for underground gas storages based on temperature, salinity, carbon availability, and sulfate concentration. The diagram is structured in such a way that the four most important parameters are considered one after another (from top to bottom) and ultimately results in a microbiological evaluation of the reservoir. A parameter becomes the primary inhibitor when it has reached a threshold for microbial life (low risks and moderate risks).

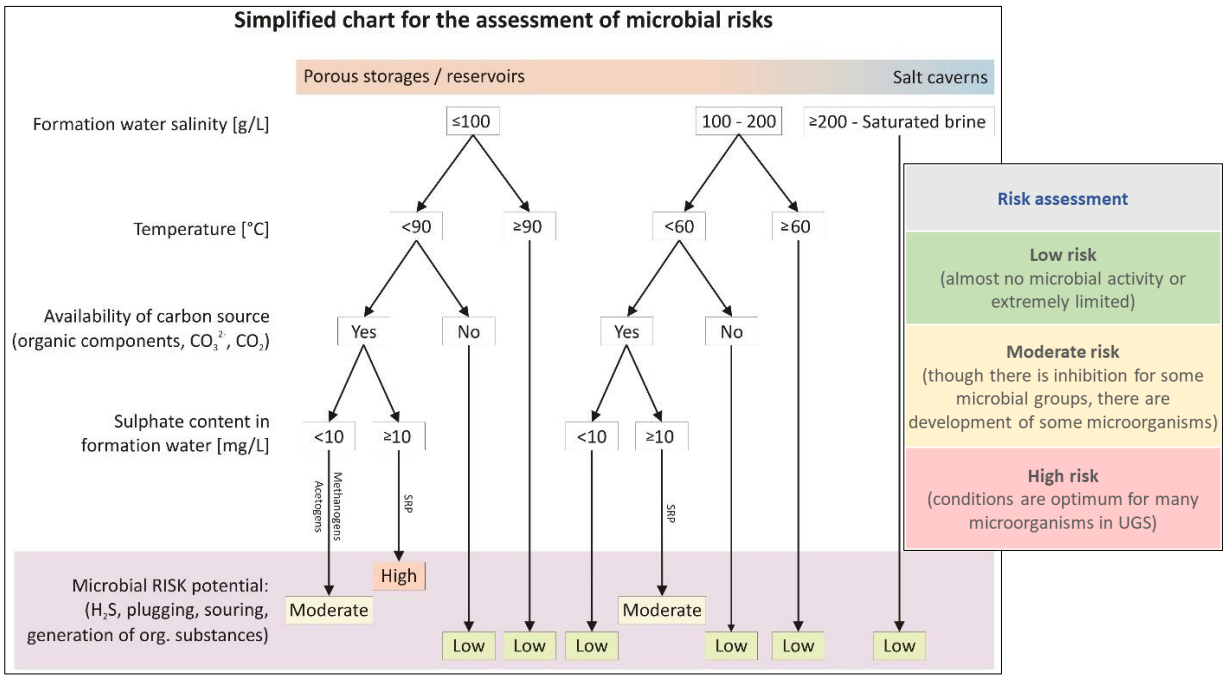


Figure 6: Simplified chart for a risk assessment for UGS based on temperature, salinity, carbon and sulfate availability

As shown in Figure 6, a low microbial risk can be assumed, if a reservoir with a salinity of 150 g/L (2.5 M) and a temperature of over 60 °C is taken as an example. This does not mean that the reservoir is completely free of microorganisms or that no microbial activity is to be expected. However, it is very unlikely that the microbial turnover will reach a critical dimension.

In contrast, for reservoir containing a carbonate host rock with a low salinity, presence of sulfate (e.g. 20 mg/L; 0.3 M) and a temperature of 40 °C, a high risk can be deducted from Figure 6. The formation of H_2S and plugging due to the activity of sulfate-reducing microorganisms as a result of hydrogen storage is very likely. In order to characterise possible substance conversions more precisely, further parameters must be taken into account in each individual case.

It should be taken into account that rock and formation water samples from the reservoir only provide a tiny insight into the often very complex and inhomogeneous geological structure. Therefore, it may only be possible to draw limited conclusions about the entire reservoir from these individual samples. In addition, the risk assessment for hydrogen storage may also change over time, as nutrients, carbon or sulfate sources for microorganisms may have been introduced into the storage system during the previous use of the reservoir or during storage operation. On the other hand, the microbial risk may also decrease to a lower level if inhibitors for microorganisms are present in the reservoir. Therefore, other parameters must of course

be taken into account for a more accurate assessment of the risks. These include, in particular, parameters that influence the microbial metabolism or can be influenced by it. The more data available, the better the risks for hydrogen storage can be assessed.

The ranking of microbial risks for the reservoirs investigated in the Hystories project based on available data and laboratory analyses is shown in Table 3. Since no complete information was available on the rock composition of the investigated reservoirs, it was assumed as a worst-case scenario that carbon and sulfate sources are present in the rock.

Table 3: Ranking of microbial risks for storage sites investigated in Hystories project

Storage site	Formation water	Salinity (NaCl %)	Temperature (°C)	Risk ranking 1	pH	Hydrogen-consuming groups detected	Risk ranking 2
1	1	1.5	49	High	6.8	Yes* (SRB, methanogens, acetogens)	High
2	2	4.8	60	High	7.4	±	Moderate
	3	1.7	60	High	5.8	Yes* (SRB, acetogens)	Moderate
3	4	0.1	66	High	6.2	Yes* (SRB, acetogens)	High
4	5	1.4	91	Moderate	10.2	not detected	Low
5	6	0.1	34	High	7.5	Yes* (SRB, methanogens, acetogens)	High
6	7	3.6	41	High	6.5	±	Moderate
	8	3.7	41	High	6.5		Moderate
	9	5.2	48	High	6.4		Moderate
	10	6	48	High	7.0		Moderate
	11	3.6	48	High	6.8		Moderate
7	12	10	64	Moderate	5.9	±	Moderate
	13	0.6	64	High	6		Moderate
8	14	2.8	40	High	6.5	Yes* (SRB, methanogens)	High
9	15	16.3	88.3	Moderate	5.7	±	Moderate

± detected by molecular analysis but not by viable cultivation

∗(): hydrogen-consuming groups successfully enriched at the laboratory

As can be seen, based on temperature and salinity (Table 3 “Risk ranking 1”), there are 12 samples with high microbial risk and 3 samples with moderate microbial risk. However, when other parameters such as pH and microbial community from the laboratory analysis are included, the ranking of microbial risk changes (Table 3 “Risk ranking 2”).

If all parameters of the formation water (Temperature, salinity, pH, and analysis of the microbial community) are taken into account, there are 4 samples with high microbial risk, 1 sample with low microbial risk and 10 samples with moderate microbial risk. The result of risk ranking 2 (Table 3) is also consistent with our simulation tests, where microbial hydrogen consumption activity was measured under conditions close to real storage conditions. Consequently, the detection of microorganisms, especially hydrogen-consuming microbial groups, in the formation water samples, in addition to the temperature, salinity and pH readings, is a direct and reliable indication of the microbial risk of a hydrogen storage facility.

The risks for reservoirs 6 and 7 are reduced from HIGH (Table 3 “Risk ranking 1”, only temperature and salinity were considered) to MODERATE because hydrogen-consuming microorganisms could not be detected in the formation water samples (Table 3 “Risk ranking 2). However, it should be noted that the fact that there is no clear evidence of the presence of microorganisms in an underground storage facility at the time of the investigation does not mean that the microbial risk is low.

As mentioned above, a single sample can only represent a tiny part of the structure and can in no way provide conclusions about the entire reservoir. In addition, during the operation of a reservoir not only nutrient inputs but also contamination with microorganisms can occur, especially during long-term operation. In addition, only formation water samples have been used for microbiological characterization in our study so far, which may underestimate the presence of microorganisms that usually prefer to colonies rock surfaces in the repositories. If other parameters are appropriate for microbial growth, we recommend that reservoirs are considered to be at moderate risk and the risk should not be underestimated. It is important to monitor microbial activity if microbes can potentially develop under the storage conditions.

Again, microbial metabolism and microbial interaction with geological formations can alter geological conditions, which in turn affects the microbial community and its activity in the subsurface system. Therefore, in addition to the preliminary assessment, regular monitoring of microbial activity in the storage system is recommended to minimize microbial risks in underground hydrogen storage.

3. Mitigation of microbial risks for underground hydrogen storages

Porous reservoirs (former crude oil or natural gas reservoirs and more rarely aquifers) are porous rocks (e.g. sandstone, limestone) filled with reservoir/formation water. The pore space, in particular the surfaces of the rock particles, represent the habitat of the microorganisms. Porous structures offer biofilm-forming microorganisms gigantic areas for settlement and intensive contact with the rock matrix. Due to the operation of a porous storage facility, the reservoir water is displaced from the top of the structure during the gas injection and pushed into the edge zones. During gas withdrawal, this process is reversed. This operation mode leads to a cyclical change between water-saturated storage gas and reservoir water in the upper working range of the storage facility. Through the transport processes thus set in motion, the microorganisms are constantly supplied with substrates.

Another critical area with a very intense flow of material is near the bottomhole. Experience has shown that this space (a few meters around the bottom of the borehole) is a particularly critical area where blockages of the pore space due to biofilm formation or chemical precipitation (e.g. FeS) frequently occur. From the edges of the reservoir, fresh formation water can in turn enter the reservoir, which is why porous reservoirs, unlike salt caverns, must be considered as open systems. This is important for any treatment strategy.

The ability to control biological activity in geological structures, particularly in open systems such as porous reservoirs, is very limited. Biocides and other biologically active substances (e.g. nitrate) have been used successfully in oil reservoirs and gas storages (Dieterich and Wagner, 2011). The treatment of local reservoir damage due to bioactivity (e.g. FeS precipitation) can be controlled by acidification or intensive biocide application, especially in spatially limited areas (near-borehole areas). (Schmitz, 2011)

3.1. Parameter limit utilisation for suppression of microorganisms

Based on the risks and growth parameters described above, strategies to modify the ecological conditions of a reservoir can be derived. For this purpose, pH values below 6.0 and above 10.0 were considered in our model experiments with pH value test series (see also page 13), whereby microorganisms are restricted in their metabolic activity and inhibited at extreme pH values. Increasing or decreasing the pH can therefore be considered as a possible treatment strategy, although the possible consequences (solubility of H_2S, CO_2, corrosion, etc.) must be considered beforehand (Dieterich et al., 2011).

An interesting aspect could be the microbially induced increase in pH due to hydrogen consumption, which has been observed in some experiments by DOPFFEL et al. (2023). Increasing the pH above 9.0 in these experiments inhibited further microbial activity. These processes should be investigated in more detail in further experiments and could play an important role in the risk assessment of pure hydrogen storage.

The mutually reinforcing effect of temperature and salinity has already been discussed on above 55 °C and salinities above 1.7 M. The temperature of a reservoir cannot be changed on a large scale by technical means. However, secondary intensifying measures, such as water flooding, are already being used to introduce certain substances into reservoirs. Similar processes are conceivable to influence the chemical composition of the formation water.

Other processes exploit the biocidal effect of certain ions in solutions. PETTER (1931) already noted an increasing toxicity for the cations $Na^+ < K^+$, $< Mg^{2+} < Li^+ < Ca^{2+} < Ba^{2+}$ and for the anions $Cl^- < NO_3^{2-} < Br^- < B_4O_7^{2-} < I^-$. In general, Na^+ and K^+ are well interchangeable, and there is also relatively good adaptation to $MgCl_2$. However, $CaCl_2$ has a much stronger toxic effect. Here, significant reductions in activity already occur above 3 %. This effect increases significantly with increasing mineralisation. A specific effectiveness of $CaCl_2$ against sulfate reducing prokaryotes was found. (Wagner, 1976)

3.2. Application of biocides for the control of microbial activity

A common method of controlling microbial activity in underground structures is the application of biocides. It is important to ensure that the concentration does not fall below the effective threshold, for example by dilution in an open porous system, as the biocide becomes ineffective and may even act as a nutrient for microorganisms. This dilution effect is inevitable in porous reservoirs at some distance from the injection well. Each biocidal substance has a minimum effective concentration that can be determined in the laboratory using test cultures. A distinction must be made between killing and inhibiting effects.

Three EU registered biocides were initially selected for the biocide tests to be carried out. In addition, an in-house biocidal substance has been tested. Using the enriched cultures from WP3.1, various biocide tests were carried out to determine the effective concentrations for each enrichment culture. For this purpose, dilution series of the biocides were prepared and each dilution level, as well as a biocide-free control, was inoculated with a sample of the previously activated microbial culture. The tests were used to determine the inhibitory effect of each preparation, whereby the ability of bacteria to multiply is suppressed in the constant

presence of a defined concentration of biocide. If the concentration falls below this level, bacterial development usually resumes, as many bacterial cells are only inactivated, not killed. The biocide series were incubated for up to 12 weeks and analyzed for growth or microbial activity (e.g. FeS precipitation), indicating an ineffective biocide concentration. With these tests, the lowest effective biocide concentration could be determined very precisely.

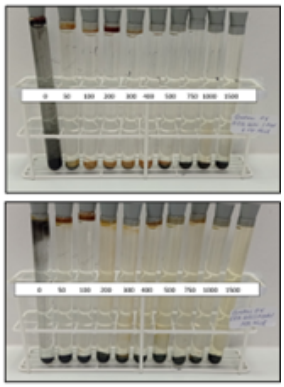
Exemplary, Figure 7 shows triplicate biocide tests with cultures of sulfate reducing microorganisms after a cultivation for up to 12 weeks with increasing biocide concentrations. At 0.1 % and 15 % salinity, the activity of all test cultures was completely inhibited by a biocide concentration of 50 ppm. In saturated brine, however, two biocides required concentrations of 200 ppm to inhibit microbial activity. It is supposed that the biocidal efficacy is limited due to high salinity. The in-house agent, on the other hand, is particularly effective at high salinity.

0.1% salinity

	Test concentration (ppm)											
Biocide:	0	50	100	200	300	400	500	750	1000	1500	2000	control
XC82681	+++	+-	-	-	-	-	-	-	-	-	-	-
XC82205	+++	-	-	-	-	-	-	-	-	-	-	-
Grotan OX	+++	-	-	-	-	-	-	-	-	-	-	-

15% salinity

	Test concentration (ppm)											
Biocide:	0	50	100	200	300	400	500	750	1000	1500	2000	control
XC82681	+	-	-	-	-	-	-	-	-	-	-	-
XC82205	+	-	-	-	-	-	-	-	-	-	-	-
Grotan OX	+	-	-	-	-	-	-	-	-	-	-	-



32% salinity

	Test concentration (ppm)															
Biocide	0	10	20	30	40	50	75	100	200	300	400	500	750	1000	1500	control
XC82681	++	+-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XC82205	++	++	++	++	++	++	++	++	-	-	-	-	-	-	-	-
Grotan OX	++	++	++	++	++	++	++	++	-	-	-	-	-	-	-	-
In-house agent	++	++	-	-	-	-	-	-	-	-	-	-		-	-	-

Figure 7: Results of biocide tests with an active culture of sulfate reducing microorganisms

Biocide application can be an effective measure to control locally occurring microorganisms. In any case, before biocide application, it should be checked to what extent other plant parts or compartments represent possible sources of bacterial contamination. For the use of biocides to condition reservoir or storage water, it must be taken into account that flow processes can lead to considerable dilution effects and consequently to a decrease in effectiveness. Additionally, fluids in porous structures often do not disperse as expected and it is difficult to ensure an equal, effective biocide concentration. Especially in the case of transport of existing bacteria, the effective use of biocides in the planned scope must be fundamentally questioned. Various preparations, especially glutaraldehyde, are biodegradable when the inhibitory concentration is not reached and can then even serve as a substrate for bacterial growth.

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Hystories project consortium

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MONTAN UNIVERSITÄT
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Microbiological Laboratories

FUNDACIÓN PARA EL DESARROLLO DE LAS NUEVAS TECNOLOGÍAS DEL HIDRÓGENO EN ARAGÓN

Mineral and Energy Economy Research Institute
Polish Academy of Sciences

Acknowledgment

This project has received funding from the Fuel Cells and Hydrogen 2 Joint Undertaking (now Clean Hydrogen Partnership) under grant agreement No 101007176.

This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and Hydrogen Europe and Hydrogen Europe Research

The Project is co-founded by European Union

Clean Hydrogen Partnership