This manuscript is submitted for publication in Renewable and Sustainable Energy Reviews. Please note that, this manuscript has not undergone peer-review and is not yet formally accepted for publication. Subsequent versions of this manuscript may have slightly different content. If accepted, the final version of this manuscript will be available via the ‘Peer-reviewed Publication DOI’ link on the right-hand side of this webpage.

Manuscript Title: Estimating Microbial Hydrogen Consumption in Hydrogen Storage in Porous Media as a Basis for Site Selection

Authors
Eike Marie Thaysen*: eike.thaysen@ed.ac.uk
Sean McMahon*: b: smcmahon@staffmail.ed.ac.uk
Gion Strobel*: gion.joel.strobel@tu-clausthal.de
Ian Butler*: ian.butler@ed.ac.uk
Bryne Ngwenya*: Bryne.Ngwenya@ed.ac.uk
Niklas Heinemann*: N.Heinemann@ed.ac.uk
Mark Wilkinson*: Mark.Wilkinson@ed.ac.uk
Aliakbar Hassanpouryouzband*: hssnpr@ed.ac.uk
Chris McDermott*: christopher.mcdermott@ed.ac.uk
Katriona Edlmann*: Katriona.Edlmann@ed.ac.uk

Affiliations:
a) School of Geosciences, University of Edinburgh, Grant Institute, West Main Road, Edinburgh, EH9 3JW, UK
b) School of Physics and Astronomy, James Clerk Maxwell Building, University of Edinburgh, EH9 3FD, United Kingdom
c) Department of Petroleum Engineering, Clausthal University of Technology, Germany
Estimating Microbial Hydrogen Consumption in Hydrogen Storage in Porous Media as a Basis for Site Selection

Eike M. Thaysen1*, Sean McMahon1,2, Gion Strobel2, Ian Butler1, Bryne Ngwenya1, Niklas Heinemann1, Mark Wilkinson1, Ali Hassanpouryouzband1, Chris McDermott1, Katriona Edlmann1

1School of Geoscience, Grant Institute, The King's Buildings, The University of Edinburgh, James Hutton Road, Edinburgh, EH9 3FE, United Kingdom

2Department of Petroleum Engineering, Clausthal University of Technology, Germany

3School of Physics and Astronomy, James Clerk Maxwell Building, University of Edinburgh, EH9 3FD, United Kingdom

*corresponding author email, phone number: eike.thaysen@ed.ac.uk.
Subsurface storage of hydrogen, e.g. in depleted gas or oil fields (DOGF), is suggested as a means to overcome imbalances between supply and demand in the renewable energy sector. However, hydrogen is an electron donor for subsurface microbial processes, which may have important implications for hydrogen recovery, gas injectivity and corrosion. Here, we review the controls on the three major hydrogen consuming processes in the subsurface, methanogenesis, homoacetogenesis, and sulfate reduction, as a basis to develop a hydrogen storage site selection tool. Testing our tool on 42 DOGF showed that seven of the fields may be considered sterile with respect to hydrogen-consuming microbiota due to either temperatures >122 °C or salinities >5 M NaCl. Only three fields can sustain all of the hydrogen consuming processes, due to either temperature, salinity or pressure constraints in the remaining fields. We calculated a potential microbial growth in the order of $1-17 \times 10^7$ cells ml$^{-1}$ for these fields. The associated hydrogen consumption is negligible to small (<0.01-3.2 % of the stored hydrogen). Our results can help inform decisions about where hydrogen will be stored in the future.

**Keywords:** Hydrogen, underground storage, microbial hydrogen consumption, homoacetogens, methanogens, sulfate reducers

**Highlights**
- Review of the most important hydrogen-utilizing microorganisms in the underground.
- Elucidation of the growth criteria for 480 strains of the mayor hydrogen-utilizers.
• Development of a site selection tool for sterile hydrogen storage.
• Evaluation of the site selection tool on 42 depleted oil and gas fields (DOGF).
• Calculation of the microbial growth and hydrogen consumption in DOGF.

Abbreviations and units

SSR Sulfur species reduction
SSRM Sulfur species reducing microorganisms
DOGF Depleted oil and gas fields
EPS Extracellular polymeric substances
M Molarity (mol L\(^{-1}\))
MPa Megapascal

1. Introduction

Zero-carbon energy generation from renewable sources can help mitigate carbon emissions and abate climate change [1-3]. One of the most significant challenges for renewable energy is the imbalance between supply and demand [3, 4]. The generation of hydrogen (H\(_2\)) via electrolysis of water during periods of renewable energy oversupply and subsequent H\(_2\) storage is one way of overcoming this imbalance, as H\(_2\) can be recovered and used for electricity generation during periods of renewable energy shortage [1, 5]. Subsurface storage of H\(_2\) in salt caverns, depleted gas or oil fields or saline aquifers is being considered as an alternative to expensive purpose-built storage containers [6]. However, the artificial elevation of the H\(_2\) concentration in the subsurface may stimulate the growth of H\(_2\)-oxidizing (hydrogenotrophic) bacteria and archaea, here collectively referred to as microorganisms, with possible adverse implications for gas
injectivity and withdrawal via permeability reduction, H₂ volume loss and corrosion of metal infrastructure [4, 7]. Understanding the controls on microbial H₂ metabolism is therefore highly important.

Much of the subsurface is characterized by combinations of elevated temperature [7], high salt concentrations and high pressure [3], reduced void space [8], limited nutrient availability [9] and typically highly reducing conditions [9-11]. The evidence for microbial life at depth is plentiful (e.g. [12-16]). Most microorganisms in nature grow in biofilms attached to surfaces (communities of aggregated microbial cells embedded in a secreted matrix of extracellular polymeric substances (EPS)) [17, 18]. Even small amounts of biofilm can reduce pore throat sizes and increase the flow-path tortuosity, resulting in dramatic decreases in permeability [19].

It has been postulated that biofilms may not form in the nutrient-limited underground when the groundwater flow is low [19]. However, subsurface biofilms are commonly encountered during geoenergy activities such as fracturing, hydrocarbon recovery or in geothermal plants [20-25]. Biofilm formation may actually be enhanced under the harsh subsurface conditions as the EPS layer acts as a protective clothing which ensures the normal reproduction and metabolism of microorganisms [18].

Hydrogen plays a central role in the energy metabolism of subsurface life [9]. Yet, a quantitative assessment of the consumption of H₂ by deep microbial communities in the context of the global H₂ cycle is lacking [26]. In underground gas storage sites and oil reservoirs the most abundant H₂-oxidizers are hydrogenotrophic sulfate reducers, that couple H₂-oxidation to sulfate reduction to produce hydrogen sulfide (H₂S); hydrogenotrophic methanogens that reduce carbon dioxide (CO₂) to methane (CH₄) by oxidizing H₂; and homoacetogens that couple H₂ oxidation to carbon dioxide (CO₂) reduction producing acetate [7, 24, 27]. These
three groups of microorganisms are, amongst others, implicated in causing subsurface corrosion [7, 27, 28].

A recent review addressed the many possible abiotic and biotic H₂-producing and H₂-consuming processes in the subsurface [7]. However, it lacked a quantitative assessment of the processes of microbial growth and H₂ consumption relevant for H₂ storage. Strobel et al. [29] summarized the concept and potential of underground methanation using experimental data from the Sun Storage project. These authors highlighted controls on the growth of methanogens and changes in gas composition due to methanogenesis, but did not quantify microbial growth. Many studies report changes in gas composition, biofilm growth and clogging near injection wells but hardly any studies report quantitative figures on microbial growth or on permeability changes [30].

To date it remains unclear how subsurface microorganisms might react to elevated H₂ concentrations [7] and hence whether microbial growth is a concern for H₂ storage. Even in natural, non-engineered subsurface environments, there is little information on microbial H₂ turnover rates [31] and the behavior and population kinetics of microorganisms are not fully understood [29]. The majority of the available data on microbial H₂ turnover rates come from batch cultures at optimal growth conditions where the kinetics [31], the pace of life [32, 33], the physiological states and the prominent organisms may differ widely from the subsurface environment [7, 32]. A further complication arises from the fact that many microorganisms in the deep subsurface are not culturable with modern enrichment techniques [12, 34].

In this work, we review the state-of-the-art understanding of the controls of temperature, salinity, pH, pressure and nutrients and water on microbial growth on H₂ in the subsurface, with emphasis on the three major H₂-consuming processes methanogenesis, sulfate reduction and homoacetogenesis, to determine what reservoir conditions will be unfavorable to microbial
activity and as such more suitable sites for long term gas storage operations of 30 years or longer, such as the UK Rough gas storage site.

Physicochemical data from 42 depleted or close to depleted oil and gas fields (DOGF) of the British and Norwegian North Sea and the Irish Sea as well as five H₂ storage test sites provide the base for an evaluation of the number of sites where microbial growth of methanogens, sulfate reducers and homoacetogens can be expected. Using average nutrient contents of the microbial cells and site-specific dissolved ion concentrations, we calculate significant growth and a small H₂ consumption for growth-permitting DOGF.

2. State of the art understanding

2.1 Likely microbial hydrogen oxidation in hydrogen storage systems

Hydrogen oxidizing processes may be ranked according to the magnitude of their H₂ threshold and their standard free energy change (ΔG₀'), two useful metrics to compare the likelihood of reactions to take place and the order at which they proceed (Table 1). The H₂ threshold defines the concentration of H₂ below which it is no longer consumed. Given all other factors are at optimum, the microbial population with the lowest H₂ threshold value is expected to be the most successful population in competing for H₂ [35].

The ΔG₀' marks the thermodynamic favorability of a reaction at ambient pressure and temperature, pH 7 and 1 M of all reactants. In oligotrophic (nutrient poor) high pressure and temperature environments, the order of the ΔG₀' may be used to determine which reaction is more energetically favorable. As can be seen from Table 1, more negative ΔG₀' values (more available free energy) are generally accompanied by lower H₂ thresholds. Not included in Table 1 are the kinetics which describe the rate of the electron transfer in the redox reaction.
Table 1. Biotic H\(_2\)-consuming processes ranked according to their free energy yield (\(\Delta G^0\)) and measured H\(_2\) threshold. Not included are Vanadium, Cobalt, Technetium, Uranium and Selenium reduction, due their limited relevance for H\(_2\) storage. NA= not available.

<table>
<thead>
<tr>
<th>H(_2)- oxidizing process</th>
<th>Reaction (number)</th>
<th>H(_2) threshold (nM)</th>
<th>(\Delta G^0) (KJ mol H(_2))</th>
<th>Typical ambient [H(_2)] (nmol L(^{-1}))</th>
<th>Relevance for H(_2) storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromate reduction</td>
<td>(\frac{1}{2}H_2 + \frac{1}{3}CrO_4^{2-} + \frac{2}{3}H^+ \rightarrow \frac{1}{3}Cr^{3+} + \frac{1}{3}H_2O) (1)</td>
<td>&lt;0.1(^{[36]})</td>
<td>NA</td>
<td>NA</td>
<td>low</td>
</tr>
<tr>
<td>Aerobic hydrogen oxidation (Knallgas)</td>
<td>(H_2 + \frac{1}{2}O_2 \rightarrow H_2O) (2)</td>
<td>0.051(^{[7]})</td>
<td>-237(^{[7, 36]})</td>
<td>NA</td>
<td>low</td>
</tr>
<tr>
<td>Denitrification</td>
<td>(H_2 + \frac{2}{5}H^+ + \frac{2}{5}NO_3^- \rightarrow \frac{1}{2}N_2 + \frac{6}{5}H_2O) (3)</td>
<td>&lt;0.05-0.5(^{[7]})</td>
<td>-240.1(^{[7, 36]})</td>
<td>&lt;0.05(^{[4, 35, 36]})</td>
<td>low</td>
</tr>
<tr>
<td>Halorespiration</td>
<td>(H_2 + \text{halogenated compounds} \rightarrow \text{dehalogenated compounds} + HCl) (4)</td>
<td>0.05-0.27(^{[36]})</td>
<td>-230 to -187(^{[7]})</td>
<td>NA</td>
<td>low</td>
</tr>
<tr>
<td>Iron (III) reduction</td>
<td>(H_2 + \text{ferric(oxy)hydroxides} \rightarrow \text{ferrous iron} + H_2O) (5)</td>
<td>&lt;0.11-0.8(^{[36, 38]})</td>
<td>-228.3(^{[7, 38]})</td>
<td>0.2(^{[4, 35]})</td>
<td>intermediate</td>
</tr>
<tr>
<td>Manganese (IV) reduction</td>
<td>(2H_2 + MnO_2 \rightarrow Mn(OH)_2 + 2H_2O) (6)</td>
<td>&lt;0.05(^{[35]})</td>
<td>-182.5(^{[36]})</td>
<td>0.2-1(^{[36]})</td>
<td>low</td>
</tr>
<tr>
<td>Arsenate reduction</td>
<td>(H_2 + HAsO_4^{2-} + 2H^+ \rightarrow H_3AsO_3 + H_2O) (7)</td>
<td>0.03-0.09(^{[36]})</td>
<td>-162.4(^{[36]})</td>
<td>0.4-0.7(^{[36]})</td>
<td>low</td>
</tr>
<tr>
<td>Ammonification</td>
<td>(4H_2 + 2H^+ + NO_3^- \rightarrow NH_4^+ + 3H_2O) (8)</td>
<td>0.015- 0.025(^{[38, 39]})</td>
<td>-150(^{[4, 38]})</td>
<td>&lt;0.05(^{[4, 35]})</td>
<td>low</td>
</tr>
<tr>
<td>Fumarate reduction</td>
<td>(H_2 + \text{fumarate} \rightarrow \text{succinate}) (9)</td>
<td>0.015(^{[38, 39]})</td>
<td>-86.2(^{[38]})</td>
<td>NA</td>
<td>low</td>
</tr>
<tr>
<td>Hydrogenotrophic sulfate reduction</td>
<td>(4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O) (10)</td>
<td>1-15(^{[38, 39]})</td>
<td>-38(^{[7, 38]})</td>
<td>1-2(^{[4, 35]})</td>
<td>high</td>
</tr>
<tr>
<td>Hydrogenotrophic methanogenesis</td>
<td>(4H_2 + CO_2 \rightarrow CH_4 + 2H_2O) (11)</td>
<td>0.4-95(^{[38, 40]})</td>
<td>-34(^{[4, 38]})</td>
<td>5-10(^{[4, 35]})</td>
<td>high</td>
</tr>
<tr>
<td>Sulfur reduction</td>
<td>(H_2 + S \rightarrow HS^- + H^+) (12)</td>
<td>2500(^{[7]})</td>
<td>-33.1(^{[7]})</td>
<td>NA</td>
<td>intermediate</td>
</tr>
<tr>
<td>Homoacetogenesis</td>
<td>(4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2) (13)</td>
<td>328-3640(^{[38, 39]})</td>
<td>-26(^{[4, 38]})</td>
<td>100-117-150(^{[36]})</td>
<td>high</td>
</tr>
</tbody>
</table>
Abiotically, most of the H₂-oxidizing reactions are very slow but mediated by microbial enzymes the processes are catalyzed [37, 41].

The three main microbial processes with implications for H₂ storage, hydrogenotrophic sulfate reduction, hydrogenotrophic methanogenesis (for simplicity from now on just referred to as sulfate reduction, and methanogenesis unless otherwise specified) and homoacetogenesis, require the highest threshold [H₂] and are among the processes with lowest ΔG°' (Table 1). Nevertheless, e.g. sulfate reduction is instantaneous in most geologic settings [42] possibly due to fast kinetics [37] and/or a relatively high availability of sulfate.

Because sulfate reducers may use the same substrates as sulfur reducers (i.e. sulfide and thiosulfate [43, 44]), they are here collectively referred to as sulfur species reducing microorganisms (SSRM) performing sulfur species reduction (SSR). Direct respiration of sulfur is limited by its low solubility (1.6*10⁻⁷ M) and hence requires cell attachment to the sulfur particle [45]. However, sulfur readily reacts with sulfide formed during the reduction of sulfate to form easily metabolizable polysulphides [45, 46].

Iron (III) reduction relies on the availability of iron oxides and iron-bearing minerals such as smectite and chlorite [47, 48], as well as the availability of organic carbon, since dissimilatory iron reducing bacteria (DIRB) are strict heterotrophs, i.e. synthesize cell carbon from organic compounds [49]. Iron oxides are abundant in many sediments and aquifers [47] but are typically not available in the carbon-rich oil fields because they have been reduced over millions of years and are not replenished [24]. Meanwhile, bacteria capable of reducing iron are frequently isolated from hydrocarbon-contaminated or oil-associated sites (reviewed in [50]). However, the mere observation of iron reduction by bacteria, which are given a DIRB enrichment medium in the laboratory, does not imply that these bacteria will reduce iron in nature. In addition, cell counts are often low to intermediate (10–100 cells ml⁻¹) and may
include non-hydrogenotrophs (e.g. [51, 52]). In non-engineered environments rich in Fe oxides and organic carbon, IRB may have a great advantage over SSRM, methanogens and homoacetogens, due to a very high affinity for H$_2$ [47]. We evaluate this process as of intermediate relevance for H$_2$ storage in DOGF.

Many IRB and a few SSRM can also couple H$_2$ oxidation to reduction of a variety of other trace metal oxides, e.g. MnO$_4^2$/MnO$_2$, CrO$_4^{2-}$, Co, SeO$_4^{2-}$, UO$_2^{2+}$, TcO$_4^-$, AsO$_3^-$, and VO$_4^-$ [43, 53, 54]. After Fe, the most abundant metal in sedimentary environments is Mn (~10% of Fe abundance) [47, 53]. Due to the trace content of these compounds in the environment, their reduction has low relevance for H$_2$ storage.

Oxygen and nitrate are scarce in the subsurface [11, 24, 55, 56] and aerobic hydrogen oxidation, denitrification and ammonification hence only become significant when contamination of the aquifer occurs, e.g. by drilling fluid [57-59].

Halogenated compounds are common in aquifers, and may arise from contamination or via natural processes in sediment [60, 61]. However, the concentrations of these compounds are extremely low: In aquifers of 167-1000 m depth, chlorofluorocarbons reach maximum concentrations of ≤1.1 µg L$^{-1}$ [61] and for pristine aquifers 0.003-0.007 µg L$^{-1}$ of chlorinated hydrocarbons were measured [60]. We evaluate the relevance of this process to H$_2$ storage as negligible.

Literature on the importance of anaerobic fumarate respiration using H$_2$ is scarce. Fumarate may be used as an alternative electron acceptor by SSRM [43, 62] and homoacetogens [63-66].

In the non-engineered subsurface, readily metabolizable organic matter, like fumarate, is rare [67]. Oil fields being rich in organic C compounds may contain more fumarate. Payler et al. [12] confirmed the presence of fumarate reductase, the key enzyme in fumarate reduction, in three out of five metagenomes from subsurface brines within sandstone. However, the
metagenomes belonged primarily to non-H$_2$ utilizing bacteria (*Halorubrum*) and fumarate concentrations were not reported. Acknowledging the lack of data in this field, we evaluate this process as being of low relevance for H$_2$ storage.

2.2 Factors governing microbial growth

Microbial growth and H$_2$ consumption rates vary with nutrient availability and environmental variables (e.g. [17, 68]). Each strain is adapted to an optimum set of nutrients and environmental conditions where potentially the greatest growth rates occur. Beyond the optimum conditions, organisms may grow but at reduced rate or they become dormant. In this section, we discuss the requirements for nutrients and water, and the overall impact of temperature, salinity, pH and pressure on the growth of the major microbial H$_2$–oxidizers in DOGFs, in the ranges relevant to H$_2$ storage. The specific activity of microbial strains grown at optimum conditions varies as well (reviewed in [69]) but the elucidation of differences between strains is beyond the scope of this review.

2.2.1 Nutrients

Apart from water of sufficient thermodynamic activity (see Section 2.2.4), hydrogenotrophs require H$_2$ as a source of electrons (energy), an electron acceptor and a carbon source for cell division, together with a set of macro and trace elements as well as various organic nutrients [70]. Microorganisms can only access H$_2$(aq) and hence the solubility of H$_2$(g) is of direct relevance for all H$_2$-consuming reactions. Given a gas phase of ~100 % H$_2$ in an H$_2$ storage system, the equilibrium solubility of H$_2$ exceeds the highest threshold value of an H$_2$-consuming microorganism of 3.6 µM (Table 1) by ~3 orders of magnitude at ambient pressure and temperature and under static conditions (Fig. A.1a), with further increase at higher pressures (Fig. A.1b). While under non-static conditions hydrogenotrophs will consume part
of the H₂, these figures suggest no limitation by the H₂ solubility on microbial growth under H₂ storage conditions.

Elemental requirements include the macro elements C, N, H, P, Ca, Mg, S and Fe (>95 % of the microbial cell dry weight), and the trace elements Co, Mn, Ni, Mo, Cu, Zn, W as well as Se for some metabolic groups [71, 72]. For optimum growth, many microorganisms additionally require different vitamins (e.g. lipoic acid, biotin, riboflavin, folic acid, thiamine, etc.), yeast extract, coenzyme M, aromatic acids and phospholipids or a combination of these (e.g. [8, 65, 73-75]).

Nutrients may be assimilated from the solution or directly from minerals (e.g., [76-79]), the latter being of particular importance in oligotrophic environments [77]. Carbon, sulfur, phosphorous and iron are amongst the key elements released by mineral weathering [77]. The extent to which subsurface microbial communities depend on mineral weathering is unknown [77]. For soils, Huang et al. [80] analyzed that >50 % of the 1100 microbial strains were capable of mineral weathering, as tested by their ability to mineralize biotite.

Microbial cell carbon may be assimilated from CO₂ alone (autotrophy) or from organic carbon compounds (heterotrophy) [81]. Methanogens and homoacetogens can grow autotrophically or heterotrophically, and several can grow mixotrophically (e.g. [66, 82, 83]). SSRM typically grow heterotrophically but some grow autotrophically or mixotrophically [84, 85]. Nitrogen may be assimilated from ammonia and nitrate or by nitrogen-fixation (diazotrophy). Diazotrophy is common amongst SSRM, methanogens and homoacetogens [86-89], though homoacetogens often inhabit ammonia-rich environments [88].

Little is known about the differences in the nutrient requirements on the level of functional groups and the variation in nutrient requirement within a functional group. SSRM have a higher requirement for iron (1.8*10⁻⁶ M) than is usually observed for microorganisms [90] while
methanogens have a higher requirement for sulfur with optimal levels ranging from 0.03 to 0.79 mM (reviewed in [91]).

Literature on when nutrients become limiting is also scarce. Sulfate reducing SSRM require a minimum sulfate and phosphorus concentrations of ~3 mM and ~3.2-320*10^{-5} mM, respectively, for growth [92, 93]. Methanogens of the order Methanosarcinae require 29.6 mM Mg for optimum growth and growth ceases at 15.8 mM (reviewed in [91]). When grown under optimum conditions, the growth rate of autotrophs may be limited by the rate of transfer of H₂ and CO₂ from gas to liquid, as was shown for the methanogen Methanobacterium thermoautotrophicum [94] and for the sulfate reducers within Desulfotomaculum sp [95].

Carbon is unlikely to be limiting in the hydrocarbon-rich DOGF [56, 96, 97] but this is not a given in saline aquifers with no history of oil or gas. Sulfate is present in significant concentrations in most DOGF (Table 2) but H₂ injection can cause sulfate depletion due to accelerated growth of SSRM [98]. Nitrogen in the form of ammonia may be limiting in DOGF [51, 56, 90] but nitrate levels may be elevated [51], often due to contamination by drilling fluid [57-59].

**2.2.2 Temperature**

Temperatures of 22.5–80 °C or 20–100 °C have been suggested for H₂ storage based on a recommended depth range of 500- 2000 m for H₂ storage in DOGF and saline aquifers [99-101]. Microorganisms are classified according to their preferred growth temperature: psychrophiles grow optimally below 20 °C, psychotrophs grow optimally at or above 20 °C and may tolerate temperatures below 5 °C, mesophiles grow between 20 and 45 °C, thermophiles grow above 45-50 °C, and hyperthermophiles show optimal growth at temperatures of 80 °C or above [102, 103].
High temperatures alter the energetic properties (e.g., vibrational modes) of biomolecules in their aqueous solvent, change the substrate solubility or viscosity and the ionization of the aqueous medium [104]. Adverse effects of high temperature include DNA denaturing or damage, decreased protein stability, hydrolysis of ATP and ADP, amongst others [104, 105]. The metabolic strategies of thermophiles are highly diverse. For a discussion, the reader is referred to [106].

Thermophiles and hyperthermophiles are challenged by increased reaction rates at elevated temperature which can imply that abiotic reaction rates are so fast that there is no benefit to the microorganism if it catalyzes the reaction [41]. High-temperature-adapted microorganisms are therefore thought to produce enzymes with faster reaction rates [107].

Most cultivated hydrogenotrophic methanogens are mesophiles but known optimal growth temperatures for methanogens range from 15 to 98 °C (Fig. 1a). A considerable number of methanogens favor temperatures above 60 °C (Fig. 1a). The highest temperature that a methanogen was found to grow under is 122 °C (Methanopyrus kandleri) (Fig. 1b) [108].

Cultivated SSRM typically have optimum growth temperatures of 20-30 °C or 50-70 °C where sulfur reducing archaea have higher optimum growth temperatures than sulfur and sulfate reducing bacteria. The full range for optimum growth of SSRM spans 10-106 °C (Fig. 1i). The critical temperature for growth of cultivated SSRM is 113 °C (Pyrolobus fumarii) [109].

Homoacetogens typically have optimum growth temperatures between 20-30 °C (85 % of the here gathered cultivated strains; Fig. 1e). Thermophilic growth temperatures ≥60 °C have been reported for eight strains, only (e.g. Moorella mulderi, Thermoanaerobacter kivui, Acetogenium kivui) [110-112]. Corresponding upper limits for growth are 70-72 °C (Fig. 1f) [110-112].
**Figure 1.** Distribution of optimum growth temperature, critical growth temperature, optimum pH values and critical salinity for 123-140 methanogens (a-d), 21-91 homoacetogens (e-h) and 151-255 sulfur species reducing microorganisms (SSRM) (i-l). Distributed between the graphs for the different groups of H₂-oxidizers are the temperatures, pH values and salinities of 42 depleted oil and gas fields (DOGF) and five test sites for H₂ injection. Where ranges of a parameter were given (see Table A.4), the lower end value was plotted.
2.2.3 Salinity

The relevant salt concentration range for H₂ storage is 0-5 M NaCl [100], at which highly diverse prokaryote communities can be found [113]. Microorganisms are classified according to their salt tolerance: Non-halophilic microorganisms grow up to 0.2 M NaCl, slight halophile grow at 0.2–0.5 M NaCl, moderate halophile between 0.5–2.5 M NaCl, and extreme halophile that grow best in hypersaline media containing 2.5–5.2 M NaCl [113].

High salt concentrations exert osmotic stress [114], requiring any microorganism living at high salt concentrations to maintain its intracellular environment at least isosmotic with the environment [113]. Commonly, salt tolerance/requirement is enhanced at increased temperatures [113] but there are many examples of mesophilic halophiles.

Most hydrogenotrophic methanogens favor salt concentrations up to 0.77 M NaCl (the approximate salinity for seawater) but 16 known strains survive under more halophilic conditions. Two extremely halophilic mesophilic hydrogenotrophic methanogens, will tolerate salt concentrations of ~3.3–3.4 M, *Methanocalculus halotolerans FR1T* [115] and *Methanocalculus natronophilus* [116] (Fig. 1d).

The large majority of SSRM grow optimally at low salinities between >0-0.4 M. However, fourteen SSRM (all mesophiles) have upper salinity limits for growth of ≥ 1.7 M NaCl (Fig. 1h). *Desulfovibrio oxyclinae, Thiobacillus halophilus, Desulfohalobium utahense* and *Desulfohalobium retbaense*, have the highest upper salinity limits for growth of 4.0 to 4.2 M NaCl [117-120] (Fig. 1h).

The salt tolerance of homoacetogens is poorly investigated. The majority of homoacetogens have low optimum salinities of >0-0.4 M NaCl. However, a few strains, i.e. *Natroincola histidinovorans, Sporohalobacter lortii* and *Halanaerobium praevalens*, grow optimally at >1.4 M NaCl and will tolerate salinities up to 2.6-5.1 M (Fig. 1h) [121-123].
2.2.4 Brine complexity

Natural brines contain dissolved ions whose interaction is extremely complex and may cause physicochemical stressors to brine habitability such as low water activity \( (a_w) \), high ionic strength, chaotropy (ability to disrupt the network of \( \text{H}_2 \)-bonds between water molecules) or a combination of these [12]. Most bacteria grow well at an \( a_w \) around 0.98 (the approx. \( a_w \) for sea water) but relatively few species can grow at \( a_w \) of 0.96 or lower [124]. Halophilic microorganisms, including halophilic methanogens are one exception; several can grow at \( a_w \) as low as 0.75 [125] in [124]; [126]. Steinle et al. [127] challenged these limits by detecting SSR in a nearly \( \text{MgCl}_2 \) saturated brine with \( a_w \) of ~0.4.

There are indications of a more important role of chaotropy over \( a_w \) in limiting microbial life [126]. Chaotropic agents include \( \text{MgCl}_2, \text{CaCl}_2, \text{FeCl}_3, \text{KI, LiBr, LiCl} \) while examples of kosmotropic agents are \( \text{NaCl, KCl, Na}_2\text{SO}_4, \text{MgSO}_4, \text{K}_2\text{SO}_4, \text{FeSO}_4 \) [128]. As such one may speculate that most subsurface brines due to their dominance of \( \text{NaCl} \) and richness in sulfate are kosmotropic and albeit also stress-inducing, more permissive of microbial growth [12, 126]. Meanwhile, the interactions between chaotro- or kosmotropic agents, \( a_w \) and other physicochemical properties of brines may be very complex and hitherto not understood [12]. The further elucidation of this topic is subject to more research and beyond the scope of this paper.

2.2.5 pH

The brine pH may affect the growth of microorganisms via 1) a direct effect on the growth metabolism, and 2) an effect on the redox reaction. With respect to the former, most methanogens, homoacetogens and SSRM are adopted to a pH of 6.5-7.5 (Fig. 1c, g, k). Most methanogens and SSRM cannot grow outside the pH range 4–9.5 [29, 129, 130] (Fig. 1c, k). Ten known methanogens can endure a critical pH-value of 10 (e.g. the \textit{Methanosalsum}
natronophilum and zhilinae, and the Methanocalculus natronophilus and alkaliphilus [131].

At the other end of the spectrum, ten known methanogens can endure acidic conditions of pH 4, e.g. the Methanoregula boonei, the Methanothermococcus okinawensis, the Methanosarcina spelaei and the Methanocaldococcus bathoardescens [132-135].

Eighteen known SSRM are adapted to highly alkaline environment >pH 10, e.g. the Desulfonatronovibrio hydrogenovorans, the Desulfospira natronophilica and the Desulfovibrio vietnamensis [136-138]. Thirteen known SSRM grow down to a pH of 4. Nine known SSRM, all of them sulfur reducers, grow down to a pH of 1, e.g. the Thiobacillus caldus, the Sulfolobus acidocaldarius, the Acidianus infernus and brierleyi, and the Stygiolobus azoricus [139-142].

Seven known homoacetogenic strains have high critical pH values up to 10.0-10.7, e.g. Clostridium ultunense, Natronella acetigena, Fuchsiella alkaliacetigena and ferrireducens, Natronoincola histidinovorans, Peptostreptococcus productus B-52 and Moorella sp HUC22-1 [121, 122, 143-146]. The Clostridium drakai, ljundahlit, scatologenes, coccoides and termoaautrophicum are the most acidophilic known strains; they can tolerate pH as low as 3.6-4.5 [147-151].

2.2.6 Pressure

Pressure ranges for H₂ storage of 5-20 MPa [99] or 1-50 MPa [100] have been reported. Life at high pressure requires homeostatic changes [103]. The high pressures encountered in pore spaces in the crust are generally less inhibitory to microbial cellular activity than the high temperatures, partly because of the relatively high osmotic pressure of cytoplasm [102], in particular in thermophiles and hyperthermophiles [41]. DNA synthesis and protein synthesis are among the most pressure-sensitive cellular processes [103, 152]. Protection against pressure includes biofilm [18] or spore formation [103].
At 30-50 MPa, the growth of various mesophilic, atmospheric-pressure-adapted microorganisms is inhibited [152] whereas pressure effects are generally favorable for the growth of hyperthermophiles; above 100 °C, elevated pressures are required to maintain a liquid environment [105]. Microorganisms that grow optimally at 10 MPa or above are obligate and facultative piezophiles, where the former do not tolerate ambient pressure and the latter do [103]. A recent publication listed all identified piezophiles and grouped them according to their growth temperature optimum [103]. The list of species is rather short (and as we find incomplete despite being published in 2020), possibly due to the fact that, to date, it has not been possible to isolate genes associated with piezophily, so the effects of pressure on any particular organism can only be determined empirically [103]. Empirical efforts however, do not commonly include pressure tolerance in the description of the environmental growth criteria of a microorganism. In addition, most mesophiles and thermophiles from habitats with pressures of <50 MPa will grow in enrichment cultures incubated at atmospheric pressure [34].

The large majority of identified cultivated piezophiles are psychrophiles (27 strains) [103], the relevance of which is low to our study. Only four mesophilic strains were reported, three of them hydrogenotrophic sulfate reducers (the Desulfovibrio profundus, piezophilus, and hydrothermalis), growing optimally at 10-40 MPa [103]. Eight thermophiles were identified, including one hydrogenotrophic methanogen, Methanococcus thermolithrophicus, growing optimally at 50 MPa. The hyperthermophilic group hosts the hydrogenotrophic Methanopyrus kandleri and Methanocaldococcus jannaschii growing optimally at 20 to 75 MPa, respectively. Examples of hydrogenotrophic piezophiles that are not included in [103] are the mesophilic SSRM Parococcus pantrophus and Pseudodesulfovibrio indicus which growth optimally at 30 and 10 MPa, respectively [153, 154], and the thermophilic SSRM Piezobacter thermophiles and Archaeoglobus fulgidus TF2 which grow optimally at 30 and 42 MPa, respectively [75, 155].
A temperature dependence of the pressure response was reported for the SSRM Desulfovibrio indonesiensis which has similar growth rates at high and ambient pressure 45 °C but reduces its growth rate at 20 °C and 30 MPa relative to at 0.1 MPa [156]. Elevated pressure may increase the maximum growth temperature by 2-12 °C relative to lower pressure (0.1-3 MPa) [105, 108, 157].

2.2.7 Inhibitors

Exposure to hydrogen sulfide, H₂S, and its bisulfide ion, HS⁻, causes damage to microbial proteins and coenzymes [91, 158]. It remains unclear whether H₂S or HS⁻ is responsible for the toxicity effect but there is general consensus that H₂S can penetrate the microbial cell membrane more easily than HS⁻ [158]. Hydrogen sulfide dissociates with a pKₐ of 6.99 at 10 MPa and 25 °C to form >99 % HS⁻ at pH 8.5 [159].

Growth of SSRM and methanogens is adversely affected at concentrations of H₂S >3.8-4.0 mM [160-162]. At 5.0-6.3 mM H₂S growth is completely inhibited for SSRM [160, 162], without however stopping all metabolic activity [160]. For methanogens and homoacetogens 3.8-7.5 mM H₂S and total sulfide concentrations of 3.3 mM, respectively, stop the growth [158, 162]. In systems with circumneutral pH and ferric ion concentrations above 1 mM, the concentrations of H₂S are predicted to be kept below toxic levels due to its precipitation in makinawite [46].

Carbon dioxide pressure above 1 bar can be toxic for microorganisms as shown for the SSRM Desulfotomaculum geothermicum and the methanogen Methanothermococcus thermolithotrophicus [163]. For many anaerobes like methanogens and homoacetogens, oxygen is toxic too [64, 102].

Nitrate inhibits homoacetogenesis [164], and ammonium [165] and sulfate inhibit methanogenesis (reviewed in [166]), with minimum inhibitory concentrations varying
depending on the environment [162, 165]. For instance, sulfate concentrations as low as $2 \times 10^{-4}$ M were shown to inhibit methanogenesis for 10 hours in lake sediments, possibly by competition with SSRM for available $H_2$ and C-substrate [161] (see section 2.3.9). Under $H_2$ storage conditions however, sulfate is likely not to affect methanogenesis, because sulfate inhibition was shown to be reversed by addition of $H_2$ [161]. For a discussion of an inhibitory effect of $H_2$, see section 2.5.

### 2.2.8 Summary of environmental growth constraints

Acknowledging the lack of data for the pressure sensitivity of many microorganisms [103], and considering a general abundance of nutrients in DOGF (Table 2), we evaluate temperature and salinity as the most crucial environmental factors constraining the growth of homoacetogens, methanogens and SSRM in DOGF. Pressures encountered in the crust are documented to have less effect than temperature on microbial cellular activity, particularly in thermophiles and hyperthermophiles [41, 102]. The pH does not pose a similar constraint to the growth of homoacetogens, methanogens and SSRM because the pH ranges for growth typically span two to three pH units (not shown) and for most species they comprise the typical aquifer pH values of 6-7 [167] (Table A.4). Brine complexity and inhibitors were not included in this analysis due a lack of information on the brine composition of DOGF beyond a limited set of dissolved ions.

Figure 2 shows the critical temperature versus critical salinity for 269 cultivated strains and reveals that salt tolerances up to 1-1.7 M are widely distributed over the entire temperature range while salt tolerances >1.7 M are mainly found at a critical temperature tolerances of 40-50 °C. Hence, from the point of view of minimizing microbial impacts on $H_2$ storage, sites with temperatures >50 °C and salinities >1.7 M are preferred.
Table 2. Reservoir conditions for selected depleted, or soon to be depleted oil and gas fields. Except where otherwise indicated, the data are from [168]. ‡= reference [167]. The salinity was calculated from the chloride concentration and the concentrations of dissolved $\text{N}_2$ was estimated from the mol percentage in the gas phase, neglecting any effect of salinity. NA= not analyzed. See Table A.4 for extended data.

<table>
<thead>
<tr>
<th>Field name</th>
<th>Area (Km²)</th>
<th>P (MPa)</th>
<th>Temp (°C)</th>
<th>Salinity (M)</th>
<th>pH</th>
<th>HCO$_3^-$ (mM)</th>
<th>$\text{N}_2$ (mM)</th>
<th>SO$_4^{2-}$ (mM)</th>
<th>$\text{K}^+$ (mM)</th>
<th>$\text{Ca}^{2+}$ (mM)</th>
<th>Mg$^{2+}$ (mM)</th>
<th>P (mM)</th>
<th>Na$^+$ (mM)</th>
<th>Cl$^-$ (mM)</th>
<th>Fe$^{2+}$ (mM)</th>
<th>Organic acids (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frigg</td>
<td>100</td>
<td>19.5</td>
<td>61</td>
<td>0.07-0.53</td>
<td>6.5-</td>
<td>16.3</td>
<td>0.4</td>
<td>NA</td>
<td>26.3-</td>
<td>0.4-2.0</td>
<td>1.9-7.1</td>
<td>NA</td>
<td>75.2-</td>
<td>534.8</td>
<td>0.04-</td>
<td>NA</td>
</tr>
<tr>
<td>Hamilton</td>
<td>15</td>
<td>9.6</td>
<td>30</td>
<td>1.59-4.18</td>
<td>5.8</td>
<td>4.8</td>
<td>2.1</td>
<td>0.6-</td>
<td>8.4-</td>
<td>195.5</td>
<td>0.012-</td>
<td>1354.8</td>
<td>58.7-</td>
<td>490.3</td>
<td>0.27</td>
<td>NA</td>
</tr>
<tr>
<td>Barque</td>
<td>36</td>
<td>26.0</td>
<td>79</td>
<td>4.83</td>
<td>4.7</td>
<td>0.3</td>
<td>0.8</td>
<td>3.5</td>
<td>42.2</td>
<td>156.8</td>
<td>NA</td>
<td>2920.4</td>
<td>4405.4</td>
<td>2.15</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hamilton</td>
<td>8</td>
<td>10.5</td>
<td>30</td>
<td>2.93</td>
<td>7.9</td>
<td>11.0</td>
<td>2.3</td>
<td>23.1</td>
<td>18.8</td>
<td>13.6</td>
<td>13.6</td>
<td>2640.9</td>
<td>2662.9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>North</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Miller</td>
<td>45</td>
<td>49.3</td>
<td>121</td>
<td>1.61</td>
<td>7.2</td>
<td>NA</td>
<td>0.6</td>
<td>41.6</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>1358.7</td>
<td>1471.9</td>
<td>0.02</td>
<td>0.002</td>
<td>NA</td>
</tr>
<tr>
<td>Beryl</td>
<td>49</td>
<td>36.0</td>
<td>101</td>
<td>1.88</td>
<td>6.1</td>
<td>5.6</td>
<td>0.4</td>
<td>20.8</td>
<td>90</td>
<td>NA</td>
<td>NA</td>
<td>1469.6</td>
<td>1717.9</td>
<td>0.05</td>
<td>0.002</td>
<td>117.4</td>
</tr>
<tr>
<td>Judy</td>
<td>NA</td>
<td>46.9</td>
<td>137</td>
<td>0.14-0.15</td>
<td>6.8</td>
<td>8.4</td>
<td>0.6</td>
<td>6.4</td>
<td>2.9</td>
<td>4.5</td>
<td>NA</td>
<td>117.4</td>
<td>131.7</td>
<td>0.11</td>
<td>0.002</td>
<td>NA</td>
</tr>
<tr>
<td>(Andrew 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Amethyst</td>
<td>97</td>
<td>27.9</td>
<td>88</td>
<td>4.45</td>
<td>5.6</td>
<td>1.0</td>
<td>1.6</td>
<td>3.7</td>
<td>33.2</td>
<td>521.5</td>
<td>148.5</td>
<td>2673.9</td>
<td>4064.6</td>
<td>2.51</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Rhyf</td>
<td>NA</td>
<td>14.9</td>
<td>36</td>
<td>5.80</td>
<td>5.5</td>
<td>13.5</td>
<td>2.8</td>
<td>14.0</td>
<td>62.4</td>
<td>147.0</td>
<td>21.2</td>
<td>4777.0</td>
<td>5297.9</td>
<td>0.81</td>
<td>&gt;1.2</td>
<td>NA</td>
</tr>
<tr>
<td>Dalton</td>
<td>NA</td>
<td>28.8</td>
<td>91</td>
<td>0.26</td>
<td>5</td>
<td>1.0</td>
<td>5.9</td>
<td>0.9</td>
<td>1.0</td>
<td>1.8</td>
<td>5.5</td>
<td>189.1</td>
<td>237.0</td>
<td>0.00</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Davy</td>
<td>6</td>
<td>28.2</td>
<td>88</td>
<td>3.87</td>
<td>6.8</td>
<td>6.5</td>
<td>NA</td>
<td>7.0</td>
<td>219.2</td>
<td>15.6</td>
<td>10.7</td>
<td>818.3</td>
<td>1142.7</td>
<td>0.66</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Veslefrikk*</td>
<td>NA</td>
<td>29.8</td>
<td>67-114</td>
<td>0.29-0.72</td>
<td>6.5</td>
<td>8.4</td>
<td>NA</td>
<td>0.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>298.0</td>
<td>281.0</td>
<td>NA</td>
<td>2.2-8.1</td>
<td>NA</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.9</td>
<td>1.1</td>
<td>5.2</td>
<td>44.7</td>
<td>166.8</td>
</tr>
</tbody>
</table>

Average: 7.9 1.1 5.2 44.7 166.8 42.2 0.113 1473.7 1857.4 0.967 3.3
Growth of all investigated microbial groups occurs up to 72 °C (Fig. 1). Above 72°C, known homoacetogens will not grow, and at 80-94°C sulfate reducers cease to grow. Thirty-six cultivated SSRM and eleven methanogens have optimum growth temperature of ≥80 °C (Fig. 1a and g) and will still grow, albeit at reduced rate, beyond their optimum temperatures. The maximum growth temperature for known methanogens and sulfur reducers is 122 °C and 113 °C, respectively. The upper salinity limit that allows growth of all the major groups of investigated H₂-oxidizing microorganisms is 3 M NaCl. The upper pH limit is 9.5 and the upper pressure limit for most mesophiles is 30-50 MPa.

Figure. 2. Critical temperature (without salinity stress) versus critical salinity (without temperature stress) for methanogens, homoacetogens and SSRM.

2.3 Microbial growth regulation by competition and syntrophy
Homoacetogenic bacteria are ubiquitous in anaerobic sediments [65, 169] and often co-exist with SSRM and methanogens [15, 170], as revealed by a combination of molecular (16S RNA
gene sequences) and culturing (e.g. metabolites, radiotracer) techniques. Few habitats have been identified in which homoacetogens compete with other H\(_2\)-consumers (culturing studies) [169, 171]. Exceptions include a low-temperature and low-salinity petroleum reservoir where homoacetogens dominated over methanogens and SSRM (molecular study) [62], a granite groundwater at 400 m depth where cell numbers of methanogens and homoacetogens were balanced (molecular study) [83], and subsurface marine sediments where mixotrophic homoacetogenesis outperformed methanogenesis (culturing study) [82].

Kinetic advantages of SSRM and methanogens (i.e. a higher affinity for H\(_2\), expressed as a low Michaelis-Menten constant, \(K_M\), or Monod half saturation constant, \(K_S\) (H\(_2\) concentration at which growth rate reaches half maximum growth rate), and a higher maximum growth/reaction rate, \(V_{\text{max}}\) or \(\mu_{\text{max}}\) for Michaelis-Menten kinetics and Monod kinetics, respectively) were proposed as the underlying cause for the few examples of the poor competitiveness of homoacetogens [172]. Very limited information on the H\(_2\) consumption kinetics of homoacetogenic bacteria is available in literature [173]. The available data show that \(\mu_{\text{max}}\) differs by one order of magnitude between strains (0.02-0.5 h\(^{-1}\)) [4, 173]. This may or may not be lower than the \(\mu_{\text{max}}\) for SSRM 0.057-5.5 h\(^{-1}\) [4, 40, 174] and methanogens 0.032-1.4 h\(^{-1}\) [40, 174]. Krumholz et al. [172] showed that homoacetogens were not able to compete effectively for H\(_2\) in the presence of SSRM in a subsurface sandstone ecosystem at 30 °C regardless of \(pH_2\), and despite significant homoacetogenesis at excess H\(_2\). Findings by Berta et al. [4] for a groundwater sediment held under excess \(pH_2\) and 20 °C contrasts this as homoacetogenesis rates were up to 21 times higher than SSR.

Environmental conditions may be a crucial determinant for the competitiveness of homoacetogens, as low temperatures (~15 °C) [175, 176] and low pH values [64, 93] favor their growth over methanogens. Under excess \(pH_2\), homoacetogenic strains with high \(\mu_{\text{max}}\) such...
as *Acetobacterium bakii* will outcompete methanogens [176]. The outstanding metabolic
flexibility of homoacetogens for utilizing a vast variety of substrates may additionally explain
why homoacetogens can compete with more specialized microorganisms like SSRM or
methanogens [65, 176, 177].

As for the competitiveness of methanogens and SSRM, the H$_2$ thresholds of methanogens
may be comparable (1-15 nM) or higher (>15-95) than for sulfate reducers and significantly
lower than for sulfur reducers (≪2500 nM; Table 1), indicating an advantage of sulfate
reducers over methanogens and sulfur reducers in most non-engineered, low pH$_2$
environments. In line with this, Lackner [178] recently reviewed that sulfate reducers
outcompete methanogens for H$_2$ in most studies. However, at excess H$_2$, methanogens and
sulfate reducers would be expected to process equal shares of the in situ H$_2$ pool [174]. Also,
since concentrations of sulfate are much lower than bicarbonate in non-marine natural
environments [40] (Table 2), the growth of sulfate reducers at excess H$_2$ will be limited by
availability of their electron acceptor, making it possible for methanogens to compete [40]. As
a general rule pH values below 7 favor the growth of methanogens over sulfate reducers [130].
Above pH 7.5, sulfate reducers grow faster than methanogens and would be expected to
outcompete them [130].

Syntrophic relationships between different functional groups have been documented frequently
(wherby the metabolic products of one group serve as substrates for the other). For example,
SSRM and homoacetogens were shown to participate cooperatively in microbial induced
corrosion of steel where SSRM grew on acetate produced by homoacetogenesis [70]. Substrate
 provision by the co-culturing *Desulfovibrio vulgaris* enhanced growth of the dehalogen
*Dehalococcoides ethenogenes* 195 by 24 % and caused three times higher dechlorination rates
[179]. Syntrophy may also explain the detection of a combination of the SSRMs *Desulfovibrio*
and the homoacetogens *Acetobacterium* in petroleum and subsurface CO$_2$ reservoirs [62, 180],
and the presence of H$_2$-producing heterotrophs along with methanogens in petroleum reservoirs
where the latter rely on H$_2$-transfer by the former [181].

### 2.4 Microbial ecology in natural gas and petroleum reservoirs

Recent years have seen a considerable effort in describing deep subsurface microbial
communities, including those from gas and petroleum reservoirs. Isolated hydrogenotrophic
microbes from these habitats are from the SSRM families *Archaeoglobaceae* [182],
*Desulfomicrobiaceae*, *Desulfobulbaceae*, *Peptococcaceae*, *Desulfo bacteriaceae*,
*Desulfovibrionaceae*, *Desulforobacteriaceae*, *Sulfurospirillaceae*, *Rhodobacteraceae*,
*Ectothiorhodospiraceae*, *Hydrogenothermaceae* [27, 56, 97, 98, 183-187], the
*Eubacteriaceae* and *Sporomusaceae* families which host homoacetogenic strains [97, 186,
188], and the methanogen families *Methanosarcinaceae*, *Methanobacteriaceae*,
*Methanomicrobiaceae*, *Methanopyraceae*, *Methanococcaceae*, *Methanocalculateae* and
*Methanosetaeae* [98, 115, 186] in addition to uncultured microbial taxa [56, 184, 185, 189].

Our collection of hydrogenotrophs (Fig. 1) lists many examples of the above microbial
families, including the strain that holds the highest critical temperature for a methanogen,
*Methanopyrus kandleri*. Sulphur reducing families that define the upper temperature limits for
SSRM like *Thermoproteaceae* and *Pyrodictiaceae* were not reported. The cause for their
absence may be a predominance of mesophilic and thermophilic sites but may also reflect a
generally stronger growth of sulfate reducers over sulfur reducers in oil and gas reservoirs.
Ranchou-Peyrouse et al. [98] showed that the microbial community in 35 out of 36 subsurface
wells from seven natural gas storage sites was dominated by sulfate reducers.

### 2.5 Effect of high hydrogen concentrations on the microbial metabolism and community
structure
A range of studies investigated the metabolism of methanogens at excess H\textsubscript{2} and ambient pressure, with unambiguous results. Conrad et al. [190] demonstrated that excess H\textsubscript{2} stimulated methanogenesis and growth rates in a paddy soil (species not specified). Opposed to this, results by Topcuoglu et al. [181] and Stewart et al. [191] suggest an inhibitory effect of high partial pressures of H\textsubscript{2}, p\textsubscript{H\textsubscript{2}}, expressed as a ~10-fold drop in the growth yield (cells per mole CH\textsubscript{4}) of Methanocaldococcus jannaschii and a slight drop of ~0.1-0.7 h\textsuperscript{-1} in the growth rate. Similar observations were made for Methanothermobacter thermoautotrophicus [192]. However, within the excess H\textsubscript{2} experiment, higher H\textsubscript{2} concentrations stimulated growth [181], suggesting a complex influence of pH\textsubscript{2}. Methanogens seem to express a pH\textsubscript{2}-dependent change in their ecological strategy, i.e. maximum growth rate vs. maximum growth yield, as a means to cope with different environmental conditions [181]. Indeed, M. jannaschii is capable of sensing subtle changes in dissolved H\textsubscript{2} concentration and restraining the energy-intensive growth of flagella to H\textsubscript{2}-limiting conditions whereas at excess H\textsubscript{2} cells are mostly flagella devoid [193].

Only few studies investigated microbial H\textsubscript{2} turnover at high pH\textsubscript{2} of up to 1.5-24.8 MPa [4, 194, 195]. Methanogens (M. jannaschii) showed a strong inhibitory effect at high pH\textsubscript{2} [194]. However, the authors added CO\textsubscript{2} at a pressure of at least 0.2 MPa to the hydrogen gas mixture which at pCO\textsubscript{2} >0.1 MPa can be toxic methanogens [163]. Hence it is not clear whether H\textsubscript{2} or CO\textsubscript{2} performed the toxic action. For homoacetogens and SSRM, the H\textsubscript{2} consumption was shown not to change in response to different pH\textsubscript{2} of 0.1-3.5 MPa [4, 195], indicating neither stimulation nor toxicity at different levels of excess H\textsubscript{2}. The comparison to limiting H\textsubscript{2} conditions was not made.

Apart from microbial metabolism, the microbial community may also change in response to high pH\textsubscript{2}. Given a perturbation by H\textsubscript{2} injection it can be anticipated that other types of
microorganisms, e.g. the in hydrocarbon reservoirs, common fermenters [24, 96, 98, 183] will
decrease in abundance while hydrogenotrophs will increase [7], in line with the Baas Becking
principle [196]. An increase in hydrogenotrophs in response to H$_2$ addition was recently
confirmed for soils, however H$_2$ consumption increased in only one of the investigated soils,
suggesting a pronounced influence of the indigenous microbial community [197]. Bioreactor
experiments support a decrease in microbial diversity in response to high pH$_2$ as well [198,
199]. Puente-Sanchez et al. [200] were the first to report differences in the subsurface H$_2$-
consuming community in response to varying pH$_2$ within the Iberian Pyrite Belt. Ranchou-
Peyruse et al. [98] showed that town gas storage with more than 50 \% H$_2$ changed the microbial
community from a predominantly sulfate reducing community to a dominance of methanogens,
and this balance was active even decades after injection stopped, possibly via H$_2$ trapping in
the microporous system [98]. It was suspected that all sulfate was initially used up by SSRM
following increased growth of methanogens [98].

3. Evaluating the potential hydrogen consumption in DOGFs

3.1 Calculation of the microbial growth

We screened 42 DOGF in the North Sea and the Irish Sea and five H$_2$ storage test sites for
temperature, salinity, pH and pressure data (Fig. 1, Table A.4). We discovered significant
differences in the salinity for the DOGF reported by sources [201] and [168]. Because we relied
on the solution compositions for the calculation of the potential microbial growth in the fields,
which are available from [168], we chose to use the salinity data from the same source.
The environmental data from the DOGF and H$_2$ storage test sites were aligned with the
constraints for growth of methanogens, homoacetogens and SSRM (Fig. 1-2) to select in which
fields growth can be expected. For the few fields that fulfil the growth constraints of all
investigated microorganisms, we calculated a first-order estimate of the microbial growth using the elemental cell composition as a proxy for the nutrient requirement [202, 203] (Text A.1).

Our calculations assumed that the supply of N and C are covered by diazotrophic and autotrophic growth, respectively. Requirements for trace elements were neglected in the calculation due to a lack of information on the relevant trace element contents in the reservoirs. Where a nutrient for a specific field was not available we used the average value from the fields given in Table 2. Any effect of the pH$_2$ on microbial growth was neglected. We assumed that cells neither die nor are removed, and that nutrients are not replenished by inflow, re-mineralization from decaying biomass or mineral dissolution. Simultaneous growth by different microorganisms was not considered.

Percentages of nutrients in the cells (Text A.1) were converted to mass using a wet cell mass of 1.77*10^{-12} g for methanogens [204], 3.2-6.2*10^{-13} g for homoacetogens and 7.81*10^{-13} g for SSRM. The cell wet weight of homoacetogens was calculated by dividing the cell volume of 1.62-3.14 µm$^3$ for the subsurface mixotrophic homoacetogen Acetobacterium psammolitichum [172] with an assumed bacterial density of 1*10^{-12} g µm$^{-3}$ [205]. The cell wet weight of SSRMs was calculated using a cell dry weight of 3.125*10^{-13} g for Desulfovibrio desulfuricans [206] and dividing this with a general bacterial dry weight to wet weight ratio of 0.4 [207]. Subsequently, the concentrations of C, H, O, Ca, K, Na, S, Mg, P and Fe in the DOGF (Table 2) were divided by the mass of the respective cell nutrients per microbial cell calculated above. This resulted in the maximum cell count within each microbial group, $G$, that could potentially be created based on a single nutrient, where the lowest $G$ indicated the limiting nutrient for cell growth. For an example of those calculations, see Text A.1.

3.2 Estimation of the cell-specific hydrogen consumption
Hydrogen may be consumed at rates of 0.02-5.0*10^5 nM h^-1 for homoacetogens, 0.02-5.8*10^5 nM h^-1 for methanogens and 0.005-130*10^5 nM h^-1 for SSRM (Tables A.1-A.3), the latter considering sulfate concentrations in the range of 0-2.3*10^2 M in the DOGF (Table 2). In a few studies, the microbial H_2 consumption was related to growth (Tables A.1-A.3), enabling the calculation of the H_2 consumption per synthesized cell and the time for when the microbial cell count G would be reached (Text A.2).

3.3 Calculation of the hydrogen consumption in a hydrogen storage system

We calculated the minimum H_2 consumption for the DOGF Frigg and Hamilton by dividing the H_2 consumption per synthesized cell with the microbial cell count. The calculation of the moles of H_2 the in aquifer anticipated equal volumes of H_2 and water and used the ideal gas law and the field size, temperature and pressure data in Table 2 and Table A.3. The percentage of H_2 that was consumed as a function of growing and resting microbial cells was calculated by dividing the potential H_2 consumption with the H_2 concentration in the reservoir. Text A.3 shows our calculations for the Frigg reservoir and methanogens.

4. Results and discussion

4.1 Characterization of the likelihood for growth in 42 DOGF

Using the environmental limits constraining microbial growth on H_2, we analyzed the physicochemical parameters for 42 DOGF in the British and Norwegian North Sea and the Irish Sea and five H_2 storage test sites (Fig. 1, Table A.4). Of the 47 fields, five fields have a temperature of 122 °C or higher and may be considered sterile with respect to H_2-consuming microorganisms. Thirty-two fields have a temperature >72 °C, implying that homoacetogenesis cannot take place. Twenty fields have a temperature ≥90 °C implying that homoacetogenesis and sulfate reduction cannot take place. Fourteen DOGF have a temperature >90 °C and <122 °C and pressures of 18.2-44 MPa where (piezophile) methanogens and SSRM will grow.
Of the fifteen sites with temperatures <72 °C where all investigated groups of microorganisms will grow, only six fields (Frigg, Hamilton, Veslefrikk, Ketzin, Lehen and Lobodice) fulfill the remaining pressure and salinity requirements for growth. Two fields, Leman and Rhyl, have salinities ≥5.8 M where no significant microbial growth can be expected. This finding is supported by stable gas compositions at the similarly saline H2-storage test sites of the H2STORE project, Emsland and Altmark (Fig. 1, Table A.4), though a low microbial population of ~10^2 cells ml^-1 was present [208]. Lennox, North Morecambe and South Morecambe with temperatures of 30-33 °C and salinities of 4.6-5.1 M, could permit the growth of the halophile homoacetogenic strain *Halanaerobium praevalens*, only. Hamilton North, Camelot and The V gas field complex with salinities of 2.9-5.0 M may permit the growth of sulfate reducers and *Halanaerobium praevalens*. The Viking field has temperature of 65-80 °C and a salinity of 3.8 M and so is likely to host only mesophilic SSRM, although pressures >30 MPa that could become growth inhibiting. The H2-storage test site Ketzin has similar salinity to the Viking field but a lower pressure (4.0 M NaCl, 35 °C, 6 MPa). Here SSRM were suspected to cause a 2-4 % decrease in H2 and a reduction in the concentration of sulfate from 22 to 8*10^3 M [208].

4.2 Microbial growth estimates for three low-temperature and low-salinity DOGF

Our first order approach for calculating microbial growth in the Veslefrikk reservoir yielded a maximum 1*10^8 methanogenic cells mL^-1, 2*10^8 SSRM cells mL^-1 or 5*10^8 homoacetogenic cells mL^-1. The Frigg reservoir a maximum of 1*10^8 methanogenic cells mL^-1, 1*10^8 SSRM cells mL^-1 or 2*10^8 homoacetogenic cells mL^-1. The Hamilton reservoir could host a maximum of 1*10^7 methanogenic cells mL^-1, 2*10^7 SRCM cells mL^-1 or 6*10^7 homoacetogenic cells mL^-1. These cell counts describe a maximum cell growth for each hydrogenotrophic group because simultaneous growth of hydrogenotrophs was not considered. The higher growth of homoacetogens over SSRM and methanogens results from a lower wet cell mass that causes a
lower nutrient demand per cell (see Text A.1). Our calculations are in line with total cell concentrations of $10^5$-$10^{15}$ cells/mL in oil reservoirs [209], and equal to or up to four order of magnitudes higher than cell counts from gas reservoirs ($0.001$-$1.2*10^7$ cells mL$^{-1}$)[51, 97, 185]. The range of the literature data reflects that our simple methodology to calculate microbial numbers may serve as a first approximation to estimating microbial growth in the subsurface, only.

Acknowledging that trace elements were not accounted for in our calculation, N and P are the first limiting nutrients in the reservoirs Frigg, Hamilton and Veslefrikk. However, this does not imply that microbial growth is N and P limited, as many microorganisms may use of ammonium (not measured) as N-source, and in the Hamilton reservoir the C:P ratio was between 59:1 and 158:1, whereas the limiting C:P ratio for microbial growth is in the range of 400:1 to 800:1 (reported for the SSRM D. desulfuricans) [92]. At moderately acidic pH values such as the pH of 5.8 in the Hamilton reservoir, P may further be continuously replenished by mineral buffering with apatite.

4.3 Hydrogen consumption in three low-temperature and low-salinity DOGF

The H$_2$ consumption in the Frigg reservoir by homoacetogens constitutes $0.01$-$3.2\%$ of the H$_2$ in the aquifer, $0.01$-$1.3\%$ for methanogens and $0.01$-$1.3\%$ for SSRM. In the Hamilton reservoir, the rates are $0.01$-$2.0\%$, $0.01$-$2.3\%$ and $0.01$-$0.5\%$ for homoacetogens, methanogens and SSRM, respectively. For actively growing cells these consumption rates may be reached after only 0.1-19.1 days, which is the time it takes for the microorganisms to grow up to their maximum cell counts, based on the dissolved nutrient concentrations. Resting cells, i.e. cells that undergo no or only very little cell division, need 2.5-3.5 months (SSRM) or up to 3.6-6.6 years (methanogens) to reach the maximum cell count and consume the given percentage H$_2$. 
In a real aquifer system, nutrients are likely to at least partly be replenished by decaying cells, mineral weathering and inflowing brine, and cells will continue to consume H$_2$ beyond the time it takes to reach the maximum cell count (maintenance). As such our H$_2$ consumption estimates may be regarded as minima. On the other hand, considering that, with the exception of one study (Berta et al. [4]), our calculations employ laboratory H$_2$ consumption rates at optimal nutrient supply and optimal physicochemical conditions (Tables A.1-A.3), the H$_2$ consumption in the oligotrophic subsurface is likely overpredicted. Comparing the employed laboratory H$_2$ consumption rates to H$_2$ consumption rates by SSR and methanogenesis in oil and natural gas reservoirs of ~0.4-330 nM h$^{-1}$ and 0.02-1205 nM h$^{-1}$, respectively (SO$_4^{2-}$: 8.3-805*10$^{-5}$ M; HCO$_3^{-}$: 3.5-246*10$^{-4}$ M) [51, 183], shows that the field H$_2$ consumption by SSR is 1.5 times to five orders of magnitude lower, and 1.4 times to 7 orders of magnitude lower for methanogenesis. Within the operation and injection wells of a natural gas reservoir, H$_2$ consumption rates by SSR and methanogenesis were 2393 and 4475 nM h$^{-1}$, respectively, [51], which falls within the lower range of the values reported from laboratory studies. Acknowledging the unknown but presumably low pH$_2$ in above experiments, and that maintenance requirements were not included in our H$_2$-consumption calculations, we expect the actual H$_2$ consumption in a H$_2$ storage system to lie within the higher range of our calculated values.

Our lower-end results are in agreement with no H$_2$ consumption during storage operations of H$_2$-rich town gas in Beynes, France [210]. Our upper end results are in agreement with a loss of ~3 %, presumably by methanogenesis, at the H$_2$ storage (SunStorage) test site in Lehen, Austria [211]. A H$_2$ consumption of 17 % by methanogens at the Lobodice town gas storage site over a time span of seven months [210, 212] seems exceptional in the light of our calculations and the reported SSR and methanogenesis rates from the field. With a very low salinity of 0.03 M, temperatures of 20-45 °C, a pH of 6.7 and 4 MPa pressure, Lobodice is
among the few sites which has highly favorable conditions for microbial growth considering all of these parameters (Table A.4). The high H₂ consumption at Lobodice highlights the importance of our site selection tool, as H₂ storage may face serious economical and technical problems if a site with growth-favoring conditions is selected.

As mentioned, Berta et al. [4] measured high H₂ consumption rates under excess H₂ and oligotrophic conditions (P< 9.7*10⁻⁷ M; SO₄²⁻≤ 9.5*10⁻⁴ M; DOC= 2.6*10⁻⁴ M), indicating that nutrient scarcity does not imply low H₂ consumption. A comparison to the nutrient concentrations in the DOGF reveals that many of them have a higher nutrient status (P= 0.002-0.452*10⁻³ M; SO₄²⁻= up to 23.1*10⁻³ M; organic acids= 1.2-8.1*10⁻³ M, Table 2), implying that H₂ consumption in DOGF under excess H₂ conditions may be even higher than reported in [4]. The experiment by Berta et al. [4] is further highly relevant because cells were at steady state, i.e. at the predominant growth stage in nature, but still consumed vast amounts of H₂. Indeed the H₂ consumption of cells at steady state or resting may be just as high or higher than for growing cells but growth is low or absent (Tables A.1-A.3).

4.4 Knowledge gaps and future research

More work is needed to predict the magnitude of microbial growth, H₂ consumption rates, and (not least) the mutual interaction of the microbial processes in DOGFs. The list of unknowns and uncertainties is long. To begin with are the poorly elucidated nutrient requirements of the microorganisms, especially in mixed cultures (e.g., [71]). Adding to this are the missing or incomplete datasets on the physical environment of certain reservoirs along with their gas phase and brine compositions, including chaotropy and kosmotropy characteristics. A better elucidation of the latter would allow the calculation of the dominating microbial processes via their free energies of the reaction. Combined with an analysis of the microbial community and
metabolism this could give new insights into whether or not we can theoretically predict which
microbial processes occur in DOGF and to which extent.

A further complication is the non-cultivability of many microorganisms in the deep subsurface,
including DOGF [12, 34, 56, 98, 183]. Considering tiny culturabilities of \( \leq 0.1\% \) of the total
viable cell count in many subsurface environments [34], any attempts to assign sterile habitats
or quantify microbial \( \text{H}_2 \) consumption via cultivated microorganisms may seem in vain. In gas
reservoirs, the percentage of cultured bacteria may be higher, ranging between 86-95\% within
each phylum [98]. Field-based metabolic activity measurements could circumvent any non-
cultivability issues observed in laboratory experiments. Field studies should also be prioritized
considering that microbial cell sizes and masses in nature are only 4-21\% of the laboratorial
cell masses [213] which reduces the nutrient requirement per cell, thereby allowing more cells
to proliferate on any given amount of nutrients.

The lack of knowledge about the changes in microbial ecology as a response to increased \( \text{H}_2 \)
concentrations beyond the level of functional groups is one of the major hurdles in our attempt
to understand of the effect of high \( \text{H}_2 \) concentrations on the subsurface microbiology. Emerging
evidence on the subject highlights species-specific responses to high \( p\text{H}_2 \) [98, 198, 200], and
that \( \text{H}_2 \) injection may leave its fingerprint on the subsurface microbial community for decades
[98]. Knowledge about the initial effect of a drastic increase in \( p\text{H}_2 \) in the subsurface is lacking.

One possibility is that more EPS will be produced as a response to the perturbation with
increased \( \text{H}_2 \), as has been shown for other types of perturbation [18, 92, 214], and considering
the toxicity of high \( p\text{H}_2 \) on methanogens [181, 192, 194], with possible adverse effects on gas
injectivity and withdrawal.

Future research should address the effect of high \( p\text{H}_2 \) on the metabolisms of different functional
groups and the EPS production in different geological settings and under changing nutritional
supply and physicochemical conditions. Mixed culture studies at low and high \( p\text{H}_2 \) can give insight into competitive and syntrophic relations under these conditions and reveal changes in the microbial community structure due to the perturbation with elevated \( \text{H}_2 \). Protocols for the careful cultivation of nutrient-deprived deep subsurface cells need to be developed. More baseline research includes determinations of the critical salinities and pressure tolerances that to date are missing for many cultivated strains, as well as the study of the brine compositional effects on the microbial community and metabolism. Research employing already cultivated species can make use of the fact that the large majority of the cultivated species isolated from subsurface environments can be found in other near-surface marine and terrestrial geothermal environments \([9, 183]\), and should employ chemostat studies that mimic the natural environment.

5. Conclusion

Here we presented a novel site selection tool for \( \text{H}_2 \) storage and demonstrated its application for 42 DOGF in the British and Norwegian North Sea and the Irish Sea and five \( \text{H}_2 \) storage test sites. Our results highlight the hard limits to the cultivable microbial life on \( \text{H}_2 \) and can –with some certainty- exclude life in several high-salinity or high-temperature, i.e. deeper reservoirs. For low-salinity and low-temperature reservoirs our calculations indicate significant microbial growth and a small but not insignificant \( \text{H}_2 \) consumption, both of which may further increase during repeated storage cycles, giving replenishment of nutrients by mineral weathering, decaying microbial cells and inflowing water. Hence, from the point of view of minimizing \( \text{H}_2 \) loss, clogging and corrosion, sites with more extreme conditions may be chosen over low-temperature and low-salinity reservoirs where the majority of microorganisms can proliferate. Yet, any storage operation will have to consider increased operational difficulties and costs.
with increased depth. Additional investigation on subsurface life on H$_2$ is encouraged to help manifest whether H$_2$ consumption in low-temperature aquifers is a threat to H$_2$ storage.

ASSOCIATED CONTENT

Appendix. Figure A.1 shows the solubility of hydrogen as a function of temperature and pressure. Laboratories studies investigating homoacetogenesis, methanogenesis and SSR are listed in Tables A.1, A.2 and A.3, respectively. Table A.4 provides the reservoir conditions for 42 DOGF and five H$_2$ storage test sites. Text A.1 holds a discussion of the importance of other hydrogen oxidizing processes for hydrogen storage. A detailed calculation of the number SSRM cells that could grow based on the N content in the Frigg reservoir can be found in Text A.1. Text A.2 and A.3 hold an example calculation of the hydrogen consumption per synthesized cell and the calculation of the potential hydrogen consumption in a hydrogen storage system, respectively.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of interest

The authors declare no competing financial interest.

Funding Sources

This work was carried out for the HYSTORPOR project and was supported by the Engineering and Physical Science Research Council (EPSRC) [grant number EP/S027815/1].

ACKNOWLEDGMENT
I would like to thank Pete M. Higgins for excellent advice and discussions on how to estimate microbial numbers from few data.

REFERENCES


Machel HG. Bacterial and thermochemical sulfate reduction in diagenetic settings: old and new insights. Sediment Geol 2001;140:143-75.


51. Ivanova AE, Borzenkov IA, Tarasov AL, Milekhina EI, Belyaev SS. A microbiological study of an underground gas storage in the process of gas extraction. Microbiology 2007;76:461-8.


Drake HL. Acetogenesis London, United Kingdom Chapman & Hall; 2012.


96. Slobodkin AI, Slobodkina GB. Thermophilic prokaryotes from deep subterranean habitat. Mikrobiologiya 2014;83(3):255-70.


103. Salwan R, Sharma V. Physiological and biotechnological aspects of extremophiles.


114. Oren A. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. Hydrobiologia 2001;466(1-3):61-72.


123. Zeikus JG, Hegge PW, Thompson TE, Phelps TJ, Langworthy TA. Isolation and
description of Haloanaerobium praevalens gen. nov. and sp. nov., an obligately anaerobic
126. Hallsworth JE, Yakimov MM, Golyshin PN, Gillion JLM, D'Auria G, de Lima ALves
F, et al. Limits of life in MgCl$_2$-containing environments: chaotropicity defines the window.
edge: active microbial communities in the Kryos MgCl$_2$- brine basin at very low water
129. Cray JA, Russell JT, Timson DJ, Singhai RS, Hallsworth JE. A universal measure of
130. Yuan H, Chen Y, Zhang H, Jiang S, Zhou Q, Gu G. Improved Bioproduction of
Short-Chain Fatty Acids (SCFAs) from Excess Sludge under Alkaline Conditions. Environ
131. O'Flatherty V, Mahony T, O'Kennedy R, Colleran E. Effect of pH on growth kinetics
and sulphide toxicity of a range of methanogenic, syntrophic and sulphate-reducing bacteria.
132. Sorokin DY, Abbas B, Merkel AY, Riipstra EIC, Sinninghe Samste JS, Sukhacheva
MV, et al. Methanosalsum natronophilum sp. nov., and Methanocalculus alkaliphilus sp.
nov.,haloalkaliphilic methanogens from hypersaline soda lakes. Int J Syst Evol Microbiol
2015;65:3739-45.


Sorokin DY, Muyzer G. Desulfurispira natronophila gen. nov. sp. nov.: an obligately anaerobic dissimilatory sulfur-reducing bacterium from soda lakes. Extremophiles 2010;14:349-55.


Hallberg KB, Lindstroem EB. Characterization of Thiobacillus caldus sp. nov., a moderately thermophilic acidophile. Microbiology 1994;140:3451-1456.


168. Oil and gas field data from the North Sea [Internet]. Oil and Gas Authority 2020 [cited 19.5.2020]. Available from: https://www.ogauthority.co.uk/data-centre/.


61


