APPENDIX

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

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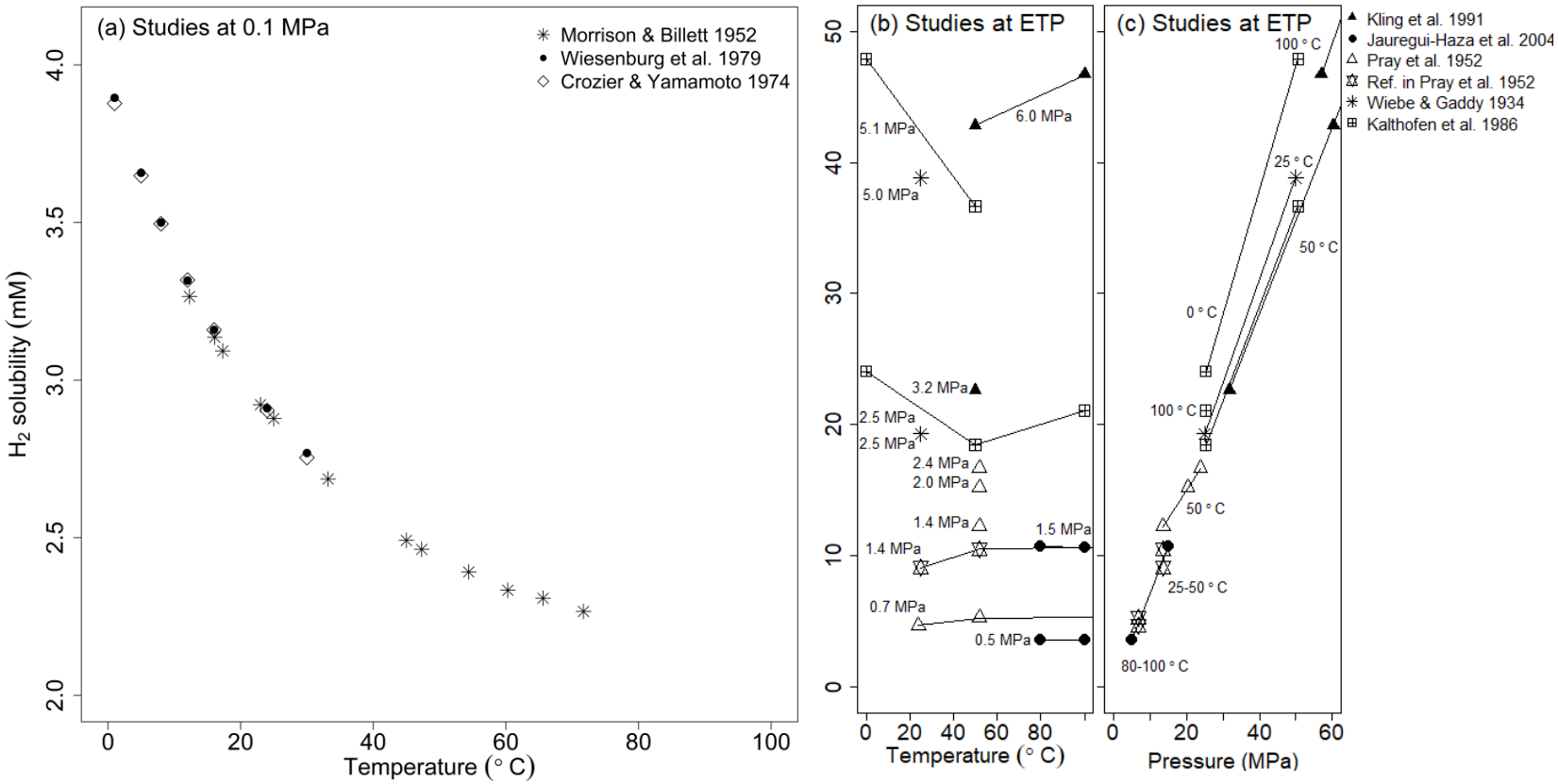

Figure. A.1. H_2 solubility as a function of temperature at 0.1 MPa **(a)** and of temperature and pressure **(b)** and **(c)**.

Table A.1. Laboratory studies investigating homoacetogenesis. Studies in bold were used for the calculation of H_2 consumption in the depleted oil and gas fields. ❃ Rate constant for H_2 turnover; ◊ Km = Michaelis Menten kinetics, Ks = Monod kinetics, V_{max}= maximum reaction rate.

H_2-consuming process: homoacetogenesis											
Species of bacteria/ archaea (growth stage)	**Time**	**pH**	**Temp. (°C)**	**Exposure [H_2] in air (%) (pressure; Mpa)**	**Exposure [H_2] in water (µg L^{-1})**	**H_2 consumption (nm H_2 h^{-1})**	**Biomass growth)**	**k ❃ (h^{-1})**	**Km or Ks◊ (µM)**	**Vmax (h^{-1})**	**Ref.**
NA (steady state)	**242 d**	**inlet: 8.7 outlet: 9.6-11.8**	**20 ± 2**	**200-1500 (0.2-1.5 MPa, with H_2 replenishment)**	**~2000-10000**	**~1.7-1.9*10^5**	**0**	**0.03±.006**	**0.01 Ks**	**0.5±0.2**	**[1]**
***Butyribacterium methylotrophicum* (growing)**	**2 d**	**7.2-7.4**	**37**	**64 (0.1 MPa, without H_2 replenishment)**	**NA**	**2.03-5.01*10^5**	**1.55-1.85 g mol^{-1} H_2**	**0.02-0.037**	**NA**	**NA**	**[2]**
***Sporomusa termida* (growing)**	2.4 d (1 day lag phase)	7.2	30	80 (0.1 MPa, without H_2 replenishment)	NA	0.2-2.7*10^5	0.0039-0.0138 g protein L^{-1} day^{-1}	0.09	6 Km	NA	[3]
***Acetobacterium psammolithicum* (growing)**	**15 d**	**6.8-7.9**	**30**	**80 (0.2 MPa, no H_2 replenishment)**	**NA**	**0.7-2.1*10^5**	**NA**	**NA**	**NA**	**NA**	**[4]**
Acetobacterium woodi (steady state)	119 d	7	30	80 (0.1 MPa, with H_2 replenishment)	1100	NA	1.7 g mol^{-1} H_2	0.024	NA	NA	[5]

Table A.2. Laboratory studies investigating methanogenesis. Studies in bold were used for the calculation of H_2 consumption in the depleted oil and gas fields. ֍ Rate constant for H_2 turnover; ◊ Km= Michaelis Menten kinetics, Ks= Monod kinetics, V_{max}= maximum reaction rate; χ calculated based on reaction 3, the composition of methanogenic cells, their production of biomass and their assimilation of C [6]. Resting cells are cells that do not divide nor respire or respire and divide at reduced rate.

H_2-consuming process: methanogenesis											
Species of bacteria/ archaea (growth stage)	**Time**	**pH**	**Temp. (°C)**	**Exposure [H_2] in air (%) (pressure; MPa)**	**Exposure [H_2] in water (µg L^{-1})**	**H_2 consumption**	**Biomass growth**	**k ֍ (h^{-1})**	**Km or Ks◊ (µM)**	**V_{max}**	**Ref**
***Methanospirillum hungatei JF-1* (resting)**	**7-10 h**	**6.7**	**37**	**80 (0.25 MPa, no H_2 replenishment)**	**24-42**	**2001-2382 nM h^{-1}**	**0.16-0.24 g protein $mole^{-1}$ H_2**	**NA**	**NA**	**NA**	**[7]**
***Methanospirillum hungatei JF-1* (growing)**	**47-48 h**	**6.7**	**37**	**80 (0.25 MPa, no H_2 replenishment)**	**85-93**	**852-874 nM h^{-1}**	**NA**	**0.052-0.054**	**5.8-7.3 Ks**	**140 nmol H_2 $mg\ protein^{-1}$ min^{-1}**	**[7]**
***Methanobacterium bryantii* (growing)**	**8 d**	**7.3**	**37**	**80 (0.1 MPa, with H_2 replenishment)**	**33-105**	**1.9-7.7*10^4 nM h^{-1}**	**5.2-6.4 mg protein day^{-1}**	**0.03**	**18 Ks**	**2- 3.2 mol H_2 g^{-1} cells day^{-1}**	**[8]**
NA	**12-29 d**	**NA**	**15**	**9-10 (0.1 MPa, without H_2 replenishment)**	**NA**	**9.9*10^4 nM h^{-1}**	**NA**	**NA**	**NA**	**NA**	**[9]**
***mixed culture* (growing)**	**6 h**	**7**	**35**	**NA**	**61**	**21-58*10^4 nM h^{-1}**	**NA**	**NA**	**1.0±0.18 Ks**	**NA**	**[10]**
Methanocaldococcus jannaschii (growing)	NA	6	82	~87 (0.1 MPa, no H_2 replenishment)	80-83	4.446 mol H_2 $mole^{-1}$ CH_4χ	1.5 ± 0.1*10^{12} cells $mole^{-1}$ CH_4	NA	NA	496± 21 fmol CH_4 $cell^{-1}$ h^{-1}	[11]
Methanocaldococcus jannaschii (growing)	NA	6	82	~2 (0.1 MPa, no H_2 replenishment)	15-27	4.446 mol H_2 $mole^{-1}$ CH_4χ	2.1 ± 0.2*10^{12} cells $mole^{-1}$ CH_4	NA	NA	139± 8 fmol CH_4 $cell^{-1}$ h^{-1}	[11]
Methanocaldococcus strain JH146 (growing)	6 to 14 h	4-9	82	80 (0.2 MPa, no H_2 replenishment)	2369	4.446 mol H_2 $mole^{-1}$ CH_4χ	5.85 ± 0.31*10^{12} cells $mole^{-1}$ CH_4	NA	NA	NA	[12]

Table A.3. Laboratory studies investigating SSR. Studies in bold were used for the calculation of H_2 consumption in the depleted oil and gas fields. ֍ Rate constant for H_2 turnover; ◊ Km= Michaelis Menten kinetics, Ks= Monod kinetic, V_{max}= maximum reaction rate. Resting cells are cells that do not divide nor respire or respire and divide at reduced rate.

Species of bacteria/ archaea (growth stage)	Time	pH	Temp. (°C)	Exposure [H_2] in air (%) (pressure; MPa)	Exposure [H_2] in water (µg L^{-1})	H_2 consumption (nM h^{-1})	SO_4^{2-} or S (mM)	Biomass growth	k ֍ (h^{-1})	Km or Ks◊ (µM)	Vmax	Ref
H_2-consuming process: sulfate reduction												
NA (steady state)	**242 d**	**inlet: 8.7 outlet: 9.6-11.8**	**20 ± 2**	**200-1500 (0.2-1.5 MPa, with H_2 replenishment)**	**~2000-10000**	**0.9-2.6*10^4**	**0.3**	**0**	**NA**	**(H_2): 0.001 Ks (SO_4^{2-}): 1000 Ks**	**0.07± 0.04 h^{-1}**	**[1]**
***Desulfovibrio G11* (resting)**	**11.5-15 h**	**6.7**	**37**	**80 (0.25 MPa, no H_2 replenishment)**	**14.1-16.1**	**507-578**	**23.8**	**0.71-0.99 g protein $mole^{-1}$ H_2**	**NA**	**NA**	**NA**	**[7]**
***Desulfovibrio G11* (growing)**	**13.3 h**	**6.7**	**37**	**80 (0.25 MPa, no H_2 replenishment)**	**NA**	**6.7*10^4**	**23.8**	**NA**	**4.9-6.5*10^-2*5.7**	**2.4-4.2 Ks**	**110 nmol mg $protein^{-1}$ min^{-1}**	**[7]**
***Desulfomicrobium hypogeium* (growing)**	**15 d**	**6.8-7.9**	**30**	**50-80 (0.1-0.2 MPa, no H_2 replenishment)**	**NA**	**0.5-2.5*10^5**	**10.0**	**NA**	**NA**	**NA**	**NA**	**[4]**
***Desulfotomaculum sp* (growing)**	60 d	6.5-7.0	55	80 (0.1 MPa, no H_2 replenishment)	NA	0.7-1.3*10^7	25.9	NA	NA	NA	NA	[13]
H_2-consuming process: sulfur reduction												
***Pyrobaculum islandicum* (growing)**	45 h	6	100	80 (0.1 MPa, no replenishment)	NA	2.5-11.1*10^5	6.3	0.3-10*10^6 cells ml^{-1} h^{-1}	NA	NA	NA	[14]

Table A.4. Reservoir conditions for 42 DOGF and five H_2 storage test sites. Except where indicated otherwise, data are from [15]. ω= reference [16]. ♣= data from reference [17] where the salinity was calculated from the major ions in solution or from the [Cl^-]. ❀= reference [18]. NA= not available. Values in red and green show unfavorable and tolerable conditions, respectively, for the growth of the major of H_2-utilizing microorganisms.

Field name	Field area (Km^2)	P (MPa)	Temp (°C)	pH	Salinity (M)
Frigg	100	19.5	61	6.5-7.4	1.08ω;0.07-0.53♣
Boa	NA	20.4	73	NA	NA
Rhum	25	83.5	150	NA	NA
Fulmar	11	38.8	140	NA	2.36
Brent	78	39.4	96	NA	0.43
Britania	246	38.0	129-145	NA	0.29-1.71
Miller	45	49.3	121	7.2	1.61♣
Beryl	49	36.0	101	6.1	1.11-1.54
Judy (Andrew 1)	NA	46.9	137	7.4	0.14-0.15♣
Judy/Joanne	NA	48.2	146	NA	NA
Ravenspurn North	24	30.9	103	NA	NA
Ravenspurn South	36	30.5	93	NA	NA
Amethyst	97	27.9	88	5.6	4.45♣
Murdoch	NA	41.8	112	4.3	4.45♣
Boulton B	NA	44.1	116	NA	3.42
Schooner	55	44.0	110	NA	1.61
Clipper	49	26.2	79	NA	3.42
Leman	253	20.5	52	8.5	5.9♣
North Sean	5	27.0	94	NA	3.85
South Sean	10	27.0	89	NA	3.85
East Sean	4	26.4	97	NA	3.85
Barque	36	26.0	79	4.7	3.42
Hamilton	15	9.6	30	5.8	1.59-4.18♣
Hamilton North	8	10.5	30	7.9	2.93♣
Lennox	9	11.1	30	NA	4.62
North Morecambe	24	12.3	33	NA	5.13
South Morecambe	84	12.7	33	NA	5.13
Rhyl	NA	14.9	36	5.5	5.80♣
Dalton	NA	28.8	91	5	0.26
Beaufort	1.7	27.6	91	NA	3.35
Bessemer	NA	18.2	91	NA	3.35
Brown	1.5	27.4	89	NA	3.87
Camelot	8.9	19.3	60	NA	3.08
Corvette	3.2	28.6	79	NA	3.42
Davy	6	28.2	88	6.8	1.25♣
Gawain	11.1	28.4	80	NA	3.42
Indefatigable	155	28.4	91	NA	3.35
Viking	NA	28.6-37.2	65-80	NA	3.76
The V fields gas complex	127	23.9-26.4	61-81	NA	3.25-4.96
Agat	NA	35.1❀	101❀	NA	NA
Veslefrikk	NA	29.8-35.0❀	67-114❀	6.5❀	0.27-0.72❀
Heidrun North	NA	23.4❀	76❀	NA	0.86❀
Emsland	NA	NA	120-130	NA	5.46
Altmark	NA	20	80	NA	7.18
Ketzin	NA	6	35	6	4.02
Lehen	NA	4.7	40	8.2	0.24-0.31
Lobodice	NA	4	20-45	6.7	0.03

Text A.1. Elemental Cell Composition for Major Hydrogen Oxidizing Microorganisms (A) and Calculation of the Number SSRM Cells that could grow based on the Nitrogen Content in the Frigg Reservoir (B)

A. For hydrogenothrophic methanogens grown under optimal conditions the cell composition is: C (37.1-42.6 %), H (5.5-6.4 %), N (9.5-10.1 %), Na (0.4-1.6 %), K (1.1-5.5 %), S (0.6-1.0 %), P (1.9-2.8 %), Ca (0.009-0.06 %), Mg (0.09-0.4 %), Fe (0.07-0.28 %) [19]. From these figures, an oxygen content of 29-44 % can be estimated by difference. A similar complete dataset could not be retrieved for the composition of other H_2-consuming bacteria. The homoacetogen *Acetobacterium sp. strain 69* has the cell composition C (45 %), H (6 %), N (10 %); O (29 %) and 7% other, not specified elements [20]. Cells of the model SSRM *Desulfovibrio desulfuricans* have the elemental formula $CH_{1.4}O_{0.4}N_{0.2}$ [21]. Assuming an average C content of 46 % for bacteria residing in 6 different aquatic ecosystems [22] the remaining elemental composition of *D. desulfuricans* is H (5 %), N (11 %); O (25 %) and 13 % other. Contents of P, Na, S, K, Ca, Mg and Fe in homoacetogens and SSRM were assumed to be as for methanogens.

B. The N content per cell, N_{cell}, was calculated to $8.6*10^{-11}$ mg N $cell^{-1}$ according to Eq. A.1

$$N_{cell} = \frac{P.N_{cell}}{100} \times m_{cell} \quad \text{(A.1)}$$

where $P.N_{cell}$ is the percentage of N in the SSRM cells, 11 %, and m_{cell} is the mass of the SSRM cells, $8.6*10^{-10}$ mg $cell^{-1}$.

The calculation of the dissolved N_2 concentration, C_{N2}, was based on the partial pressure of N_2 in the aquifer, pN_2, of 0.99 atm, and used Eq. A.2.

$$C_{N2} = pN_2 \times M \times K_{HN2} \quad \text{(A.2)}$$

where M is the molar mass of N of 14 g mol^{-1} and K_{HN2} is the Henrys law's constant for N_2 at the 334.15 K of the Frigg reservoir. The latter was calculated to $3.8*10^{-4}$ mol L^{-1} atm^{-1}using Eq. A.3

$$K_{HN2} = K_H^0 \times e^{\left(F*\left(\frac{1}{T}-\frac{1}{T^0}\right)\right)} \quad (A.3)$$

where K_H^0 is Henry's laws constant for N_2 at 298.15 K, 0.00061 mol L^{-1} atm^{-1}, F is the Van't Hoff coefficient for N_2, 1300, T is the actual temperature in K and T^0 is the reference temperature, 298.15 K. Inserting the K_{HN2} into Eq. A.2 yielded a C_{N2} of 5.3 mg L^{-1}.

Finally, the potential growth of SSRM, G, in the Frigg aquifer was calculated to $6.2*10^{10}$ cells L^{-1} by dividing the C_{N2} with N_{cell} (Eq. A.4).

$$G = \frac{C_{N2}}{N_{cell}} \quad (A.4)$$

Text A.2. Example Calculation of the H_2 Consumption per Synthesized Cell for Methanogens, Exemplified by *Methanobacterium bryantii*.

The mass of protein, $m_{prot,}$ for a living, i.e. wet, *Methanobacterium bryantii* cell was calculated to be $1.4*10^{-13}$ g according to Eq. A.5

$$m_{prot} = \frac{prot_{cell}}{100} \times m_{cell} \times \chi \quad \text{(A.5)}$$

where $prot_{cell}$ is the cell protein content of 50 % for dry cells of *Methanobacterium bryantii* [8], m_{cell} is wet cell mass, of $1.77*10^{-12}$ g for methanogens [6], and χ is the dry weight to wet weight ratio of 0.4 for bacteria in general [23].

The daily growth rate, GR_{cell}, was 9.9-52*10^9 cells L^{-1} day^{-1}, according to Eq. A.6

$$GR_{cell} = \frac{GR_{prot}}{m_{prot}} \quad \text{(A.6)}$$

where GR_{prot} is the daily growth rate of 0.0014-0.0074 g protein L^{-1} day^{-1} [8]. Finally, Eq. A.7 allowed the calculation of the H_2 consumption per synthesized cell, H_2usage_{cell} to 3.5-4.6*10^{-5} nM $cell^{-1}$ using a microbial H_2 consumption, ΔH_2, of 4.5-18*10^{-4} [8].

$$H_2usage_{cell} = \frac{\Delta H_2}{GR_{cell}} \quad \text{(A.7)}$$

The time, t, for when the cell count is reached was calculated from the daily growth rate, GR_{cell} and the estimated microbial counts, G, according to Eq. A.8

$$t = \frac{G}{GR_{cell}} \quad \text{(A.8)}$$

Text A.3. Calculation of the Hydrogen Consumption in a Hydrogen Storage System

We calculated the potential H_2 consumption, ΔH_{2pot}, to 7.8 -3117*10^4 nM L^{-1} for methanogens in the Frigg reservoir by dividing H_2usage_{cell} (SI Text 3) with G (SI Text 2) (A.9).

$$\Delta H_{2_{pot}} = \frac{H_2usage_{cell}}{G} \quad (A.9)$$

The Frigg field holds an aquifer volume, V_{field}, of 4.8 km^3. The moles of H_2 the in aquifer, n_{H2}, were calculated anticipating equal volumes of H_2 and water and using the ideal gas law (Eq. A.10).

$$n_{H2} = \frac{P \times V}{R \times T} \quad (A.10)$$

where P is the aquifer pressure of 19.5 MPa, V is the volume of H_2 (or brine) of 2.4*10^{15} cm^3 resulting from V_{field} x 0.5, R is the gas constant of 8.314 cm^3 MPa mol^{-1} K^{-1} and T is the aquifer temperature. The Frigg aquifer holds 1.7*10^{13} moles H_2.

Finally, the percentage of H_2 consumed as a function of growing and resting methanogen cells, H_2usage, was calculated to <0.01-1.3 % according to Eq. A.11

$$H_2usage = 100 * \frac{\Delta H_{2_{pot}}}{C_{H2}} \quad (A.11)$$

where c_{H2} is the concentration of H_2 in the aquifer of 7.13*10^9 nM L^{-1} resulting from dividing n_{H2} with V.

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